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Latvijas Biozinātņu un tehnoloģiju universitāte

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Pārtikas un vides higiēnas institūts

Alīna Zolova

tematiski vienotu zinātnisko publikāciju kopa

KRIPTOSPORIDIOZES EPIDEMIOLOĞIJA PIENA GOVĪM LATVIJĀ
EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN DAIRY COWS IN LATVIA

zinātnes doktora grāda (Ph.D.) iegūšanai
lauksaimniecības, meža un veterinārās zinātnēs

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Promocijas darbs izstrādāts doktorantūras studiju ietvaros Latvijas Biozinātņu un tehnoloģiju universitātes Veterinārmedicīnas fakultātē laika periodā no 2015. līdz 2024. gadam. IgG līmeņa noteikšana paraugos tika veikta Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta "BIOR" Mikrobioloģijas un patoloģijas laboratorijā. Pētījums daļēji izstrādāts ar pēcdoktorantūras pētniecības atbalsta programmas Nr. 1.1.1.2/VIAA/1/16/204 "One Health" Multidisciplināra pieeja atlasītu parazītisko zoonožu epidemioloģijai un profilaksei" granta atbalstu, kas tika piešķirts Dr. biol. Gunitai Deksnei, Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta "BIOR" Parazitoloģijas laboratorijā.

The doctoral thesis was developed within the framework of doctoral studies at the Faculty of Veterinary Medicine of the Latvia University of Life Sciences and Technologies between 2015 and 2024. The determination of IgG levels in the samples was carried out at the Microbiology and Pathology Laboratory of the Institute of Food Safety, Animal Health and Environment "BIOR". The third publication was partially supported by the Postdoctoral Research Support Program Grant No. 1.1.1.2/VIAA/1/16/204 "One Health" — A Multidisciplinary Approach to the Epidemiology and Prevention of Selected Parasitic Zoonoses, awarded to Dr. biol. Gunita Deksne at the Parasitology Laboratory of the Institute of Food Safety, Animal Health and Environment "BIOR".

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Promocijas darba aizstāvēšana notiks 2026. gada 24. augustā pulksten 12:00, LBTU Veterinārmedicīnas fakultātē Jelgavā, K.Helmaņa ielā 8, A300 auditorijā.

The defense of this theses will take place at Latvia University of Life Sciences and Technologies Faculty of Veterinary Medicine, Jelgava, Kr. Helmana Street 8, auditorium No A300, on 24th of August, 2026, at 12 o'clock.

ANOTĀCIJA

Jaundzimušo teļu kriptosporidioze ir viena no nopietnākajām parazitārajām invāzijām mūsdienu lopkopībā, jo tieši vienšūņi *Cryptosporidium spp.* tiek uzskatīti par galveno etioloģisko faktoru teļu masveida saslimstībai pirmajās dzīves nedēļās. Šī patogēna aktualitāti nosaka ne tikai tā plašā izplatība ganāmpulkos, kur invāzijas ekstensitāte teļiem līdz trīs mēnešu vecumam var sasniegt pat 39,4 %, bet arī tā būtiskā loma jaundzimušo mirstības veicināšanā, radot neatgriezeniskus zarnu trakta bojājumus. Īpaši bīstama ir suga *C. parvum*, kas ne tikai izraisa smagu klīnisko gaitu dzīvniekiem, bet ir atzīta par bīstamu zoonozi, radot tiešus draudus cilvēku veselībai caur kontaminētu vidi un ūdens resursiem. Tā kā parazīta oocistas ir ārkārtīgi rezistentas pret standarta dezinfekcijas metodēm un spēj ilgstoši izdzīvot apkārtējā vidē, kriptosporidiozes ierobežošana prasa kompleksu pieeju, kas balstīta uz padziļinātu epidemioloģisko izpēti.

Promocijas darba zinātniskais nozīmīgums sakņojas visaptverošā *Cryptosporidium* ģints sugu un genotipu analīzē, kas ir pirmais šāda mēroga pētījums Latvijā pēdējo 30 gadu laikā, aptverot vairāk nekā 190 novietņu visos reģionos. Pētījuma ietvaros identificētās četras galvenās sugas (*C. parvum*, *C. bovis*, *C. andersoni* un *C. ryanae*) un to specifiskā saistība ar dzīvnieku vecuma grupām sniedz fundamentālu izpratni par invāzijas dinamiku. Zinātniski nozīmīgi ir darba secinājumi par pasīvās imunitātes mehānismiem, konstatējot, ka tradicionālais imūnglobulīna G (IgG) līmenis nav izšķirošs faktors aizsardzībā pret šo specifisko parazītu. Tas paver jaunus pētniecības virzienus par lokālo zarnu imunitāti un pirmpiena bioaktīvo savienojumu lomu, pierādot, ka mātes bioloģiskie faktori, piemēram, laktācijas reize, būtiski modulē teļu uzņēmību pret invāziju.

Tautsaimniecisko nozīmīgumu definē kriptosporīdiju radīto tiešo un netiešo ekonomisko zaudējumu mazināšana piensaimniecības nozarē. Tā kā invadētie teļi uzrāda ievērojami zemāku dzīvmasas pieaugumu un ir uzņēmīgāki pret citām infekcijām, darba rezultāti kalpo par pamatu racionālai ganāmpulka vadībai. Praktiskie priekšlikumi par pagarinātu pārejas piena izēdināšanu (vismaz divas nedēļas) un savlaicīgu pirmpiena nodrošināšanu piedāvā lauksaimniekiem izmaksu ziņā efektīvu metodi parazitārā spiediena mazināšanai. Turklāt, precizējot zoonozes riskus un oocistu intensīvas izdalīšanās periodus, darbs sekmē drošākas darba vides izveidi novietnēs un samazina vides piesārņojuma riskus tādējādi veicinot Latvijas lopkopības produktu kvalitāti un nozares kopējo stabilitāti.

ABSTRACT

Neonatal calf cryptosporidiosis is one of the most serious parasitic infections in modern livestock production, as the protozoa *Cryptosporidium* spp. are considered the primary etiological factor for mass calf morbidity during the first weeks of life. The relevance of this pathogen is determined not only by its widespread prevalence in herds, where the infection prevalence in calves up to three months of age can reach up to 39.4 %, but also by its significant role in contributing to neonatal mortality through irreversible intestinal tract damage. The species *C. parvum* is particularly dangerous; it not only causes a severe clinical course in animals but is also recognized as a hazardous zoonosis, posing a direct threat to human health through a contaminated environment and water resources. Since the parasite's oocysts are extremely resistant to standard disinfection methods and can survive in the environment for extended periods, controlling cryptosporidiosis requires a comprehensive approach based on in-depth epidemiological research.

The scientific significance of the doctoral thesis is rooted in a comprehensive analysis of *Cryptosporidium* genus species and genotypes, which is the first study of this scale in Latvia in the last 30 years, covering more than 190 holdings across all regions. The four main species identified within the study (*C. parvum*, *C. bovis*, *C. andersoni*, and *C. ryanae*) and their specific association with animal age groups provide a fundamental understanding of infection dynamics. Scientifically significant are the thesis conclusions regarding passive immunity mechanisms, establishing that traditional immunoglobulin G (IgG) levels are not a decisive factor in protection against this specific parasite. This opens new research directions regarding local intestinal immunity and the role of colostrum bioactive compounds, proving that maternal biological factors, such as lactation number, significantly modulate calf susceptibility to infection.

The economic significance is defined by the mitigation of direct and indirect economic losses caused by *Cryptosporidium* in the dairy sector. Since infected calves exhibit significantly lower body weight gain and are more susceptible to other infections, the results of the study serve as a basis for rational herd management. Practical proposals regarding extended transition milk feeding (at least two weeks) and the timely provision of colostrum offer farmers a cost-effective method to reduce parasitic pressure. Furthermore, by clarifying zoonotic risks and periods of intense oocyst shedding, the thesis contributes to the creation of a safer working environment in holdings and reduces environmental contamination risks, thereby promoting the quality of Latvian livestock products and the overall stability of the sector.

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Autoru ieguldījums/ *The contribution of the authors*

Raksts/ *Publication*	Ideja/ Orig*inal idea*	Pētījuma dizains/ *Study design*	Datu ievākšana/ *Data collection*	Datu analīze/ *Data analysis*	Rakstīšana/ *Manuscript preparation*	AZ relatīvais pienesums/ *Relative contribution*
1	DK, AZ, AK	DK, AK, AZ	DK, AK	DK, AZ	AZ	50
2	AZ	AZ	AZ	AZ, ŽŠ	AZ, ŽŠ, MZ	70
3	GD, DK, AZ, MM	GD, AZ, MM	AZ, MM	MM, KT, AZ, SC, GS	GD, KT, GS	30
4	AZ, DK	AZ	AZ	AZ, MZ	AZ, MZ	80
5	AZ, DK	AZ	AZ	AZ, MZ	AZ, MZ	80
6	AZ, DK	AZ	AZ	AZ, MZ	AZ, MZ	80

AZ – Alīna Zolova (ex Derbakova), DK – Dace Keidāne, AK - Anna Krūklīte, MZ - Maksims Zolovs, ŽŠ – Žanete Šteingolde, GD – Gunita Deksne, MM – Maira Mateusa, SC – Svetlana Cvetkova, KT – Karin Troell, GS – Gereon Schares.

SIMBOLI UN SAĪSINĀJUMI

Termini

Izlase – ģenerālkopas daļa, kas ir atlasīta praktiskai novērošanai, lai spriestu par visas ģenerālkopas īpašībām.

Ģenerālkopa – tāda objektu vai organismu kopa, par kuru plāno iegūt informāciju. Tā ir brīvi definējama pētījuma sākumā un ir atkarīga tikai no pētījuma mērķa.

Stratificētā izlase – izlases veids, kurā elementus nejaušināti atlasa no dažādām pētāmās problēmas apakšgrupām.

Invāzijas ekstensitāte – invadēto dzīvnieku procentuālā attiecība pret visu dzīvnieku skaitu izmeklējumu grupā.

Invāzijas intensitāte – vidējais parazītu skaits uz vienu dzīvnieku.

Naivi imūns – termins, kas attiecas uz imūnsistēmas sākotnējo vai nenobriedušo stāvokli, kad imūnsistēma vēl nav saskārusies ar noteiktām infekcijām vai antigēniem un tādējādi nav izveidojusi specifiskus imūnsistēmas atbildes mehānismus pret tiem.

Promocijas darbā lietotie saīsinājumi

Saīsinājums	Latviešu valoda	Angļu valoda
OSG	Oocistu skaits vienā gramā fekāliju	Oocyst per gram feces
Ig	Imūnglobulīns	Immunoglobulin
PCR	Polimerāzes ķēdes reakcija	Polymerase Chain Reaction
PBS	Fosfātu bufera sāļi	Phosphate-Buffered Saline
FITC	Organiskās fluorescējošās krāsvielas veids	Fluorescein Isothiocyanate
ELISA	Enzīmu saistītā imūnsorbentanalīze	Enzyme-Linked Immunosorbent Assay
GLMM	Ģeneralizētā lineārā jauktā modelēšana	Generalized Linear Mixed Model
AIC	Statistisks kritērijs, ko izmanto modelēšanas kontekstā, lai novērtētu modeļa piemērotību un tā atbilstību empīriskajiem datiem	Alaike Information Criterion
TI	Ticamības intervāls	Confidence interval

1. IEVADS

1.1 Jaundzimušo teļu diarejas sindroms

Literatūras dati liecina, ka aptuveni 90 % gadījumu teļu mirstība ir saistīta ar jaundzimušo teļu diarejas sindromu. Slimības klīniskās pazīmes parasti izpaužas teļiem 5–10 dienu vecumā. Klīniski slimība var izpausties gan kā viegla diareja bez sistēmiskām pazīmēm, gan kā akūta, smaga, profūza diareja, kas nepadodas ārstēšanai un izraisa smagu dehidratāciju, elektrolītu līdzsvara traucējumus un nāvi 12 stundu laikā. Sindromu izraisa vairāku bakteriālo un virusālo infekciju kopums (*E. coli, Salmonella*, rotavīruss, koronavīruss). Kopā ar augstākminētajām infekcijām nāves cēloņa noskaidrošanas laikā zarnu traktā tiek diagnosticēti vienšūņi *Cryptosporidium* spp. *C. parvum* ir minēts kā biežākais cēlonis teļu diarejas sindroma attīstībai. Kriptosporīdijas noārda epitēliju, kas pārklāj zarnu bārkstiņas, samazinot barības vielu absorbcijas spējas, un tādā veidā veicina bakteriālo un virusālo slimību ierosinātāju iekļūšanu dzīvnieku organismā (Jasmer, 2007; Cho and Yoon, 2014).

Cryptosporidium spp. ir smagas invāzijas ierosinātājs teļiem, un tas ir viens no svarīgākajiem ekonomisko zaudējumu iemesliem piena lopkopības novietnēs. Lauksaimniekiem var rasties papildu izmaksas, saistītas ar diagnostiku un ārstēšanu, piemēram, papildu izmaksas teļu barībai un audzēšanai, lai tie sasniegtu tirgus svaru. Turklāt invāzija var izraisīt teļu nāvi (Innes et al., 2020).

Kriptosporidioze visā pasaulē ir plaši izplatīta parazitāra vienšūņu ierosināta dzīvnieku un cilvēku invāzija, kura pagājušā gadsimta 80. gados tika atzīta par zoonozi (WHO, 2015). Līdz ar to ir ļoti svarīgi izvērtēt šī parazitārā vienšūņa izplatību mūsu valsts teritorijā, kur lopkopība jau vairākus gadsimtus ir viena no galvenajām lauksaimniecības nozarēm. Tas palīdzēs ne tikai uzlabot ganāmpulku veselību un tādējādi piena govju ražīgumu, bet arī pasargās cilvēkus no saslimšanas ar kriptosporidiozi.

1.2. *Cryptosporidium* spp. uzbūve un attīstības cikls

Kriptosporīdijas ir parazītiski vienšūņi, mikroskopiski mazi organismi, apmēram 4–6 mikronus lieli, kuri lokalizējas govs zarnu epitēlijā.

Vienšūņa iekšējā struktūra ir vienkāršas uzbūves – sastāv no kodola un organellām. Tiem nav ārējo struktūru, piemēram, krāsainu membrānu vai skropstiņu, un tos var identificēt tikai, izmantojot īpašas mikroskopijas tehnikas un krāsojumu. Attīstības periodā kriptosporīdiju uzbūvi veido oocista, kurā ir sporozoīti, bet iztrūkst sporocistu. Ultrastrukturālās un DNS analīzes ir parādījušas augstu morfoloģiskās līdzības pakāpi visām *Cryptosporidium* sugām (Dragomirova, 2022).

Cryptosporidium spp. ir tiešais attīstības cikls – tas nozīmē, ka attīstība notiek bez starpsaimnieku maiņas. Invāzija tiek nodota no viena saimnieka otram ar nobriedušām, invadētspējīgām oocistām. Attīstījušās (sporulējušās) oocistas no saimnieka organisma ar fekālijām izdalās ārējā vidē. Oocistas kļūst invadētspējīgas uzreiz pēc nonākšanas ārējā vidē. Pēc šādu oocistu norīšanas vai ieelpošanas piemērotā saimniekā notiek ekscistācija, kurā tiek atbrīvoti sporozoīti. Šie sporozoīti invadē gremošanas trakta epitēlija šūnas. Šajās šūnās parazīti veic gan aseksuālu (šizogoniju vai merogoniju), gan seksuālu (gametogoniju) vairošanos. Seksuālajā vairošanās procesā tiek radīti mikrogamonti, kas dalās, veidojot mikrogametas jeb vīrišķās gametas, un makrogamonti, kas nobriest par makrogametām jeb sievišķajām gametām. Pēc apaugļošanas oocistas attīstās un sporulē saimnieka zarnu epitēlijšūnās. Kriptosporīdijām izšķir tā saucamās lielās jeb biezsienu oocistas, kas izdalās no gremošanas trakta ārējā vidē, un mazās jeb plānsienu oocistas, kas var radīt autoinvāziju turpat gremošanas traktā (Pinto and Vinayak, 2021).

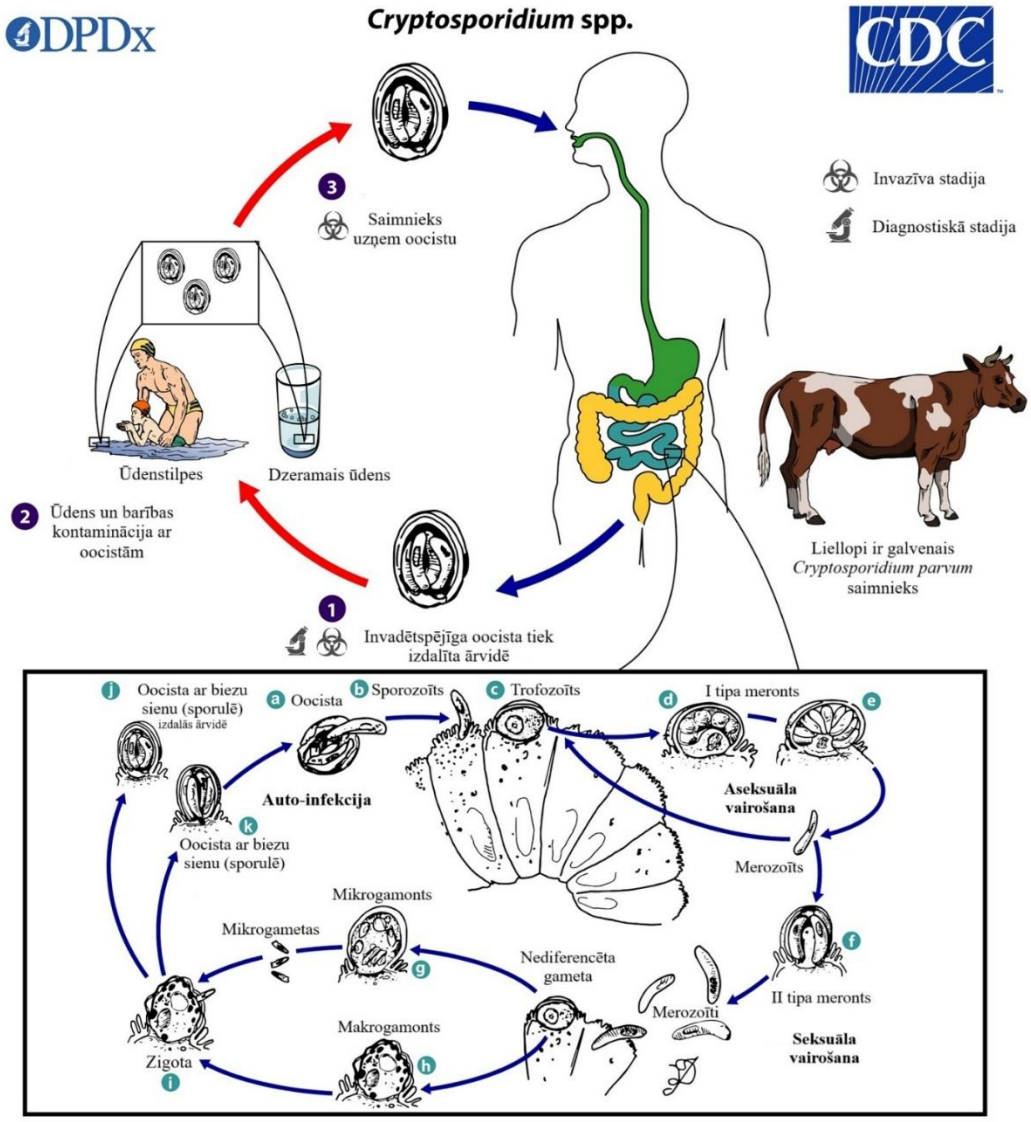
1.3. *Cryptosporidium* spp. sugas

Šobrīd ir atklātas vairāk nekā 44 kriptosporīdiju sugas un virs 120 genotipiem. *C. hominis* (*C. parvum* I tips) ir cilvēkiem specifisks patogēns. *C. parvum* (*C. parvum* II tips) ir zoonoze un invadē gan cilvēkus, gan dzīvniekus. Govīm šobrīd izolētas un zināmas četras kriptosporīdiju sugas: *C. parvum, C. andersoni, C. bovis* un *C. ryanae*. *C. andersoni* invadē pieaugušu govju glumenieku, *C. bovis* un *C. ryanae* izplatās visā gremošanas traktā, kā arī var nokļūt elpošanas sistēmā aerogēnā ceļā vai migrējot pa asinsriti ar fagocītiem. *Cryptosporidium parvum* nav saimnieka sugai specifisks parazīts, tāpēc to var pārnest dažādi dzīvnieki (piemēram, žurkas vai kaķi). Šīs parazīta sugas oocistas tika atrastas 70 % viena līdz trīs nedēļu vecu teļu fekālijās (Ryan et al., 2021; Mahdavi et al., 2024). Daži autori ziņo, ka atsevišķās fermās parazītu invāzijas ekstensitāte var sasniegt 100 % (Santin, Trout and Fayer, 2008). Invāziju var atklāt, sākot ar teļu piekto dzīves dienu, kad oocistas sāk intensīvi izdalīties no gremošanas trakta (Olson, 1999).

Cryptosporidium spp. tiek uzskatītas par vienu no biežākajiem diarejas cēloņiem cilvēkiem, savukārt *C. parvum* un *C. hominis* ir primārie cilvēka kriptosporidiozes izraisītāji. To izplatība valstīs ir ļoti atšķirīga (Vanathy et al., 2017). Pieaugušiem cilvēkiem ar adekvātu imunoloģisko atbildi *C. hominis* un *C. parvum* var izraisīt vieglu, pašlimitējošu diareju. Bērniem, veciem cilvēkiem un imūnkompromitētām personām (piemēram, HIV/AIDS pacientiem) *Cryptosporidium* spp. invāzija izraisa smagu zarnu epitēlija bojājumu, kas savukārt veicina uzturvielu trūkumu, kas var izraisīt dzīvībai bīstamus stāvokļus (Mak, 2004). Paaugstināta riska grupā, kas var saslimt ar kriptosporidiozi, ir iekļauti arī cilvēki, kuri strādā govju novietnēs (Abubakar et al., 2007). Tieši tāpēc ir svarīgi apzināt invāzijas izplatību Latvijā, lai varētu izstrādāt ieteikumus invāzijas izplatības mazināšanai un, saslimšanas gadījumā, patoģenēzes negatīvās ietekmes ierobežošanai.

1.4. Invāzijas avoti

Par invāzijas avotu tiek uzskatītas nobriedušas oocistas, kas izdalās ar fekālijām un izraisa smagu apkārtējās vides kontamināciju. Kriptosporīdijām ir liels izplatīšanās potenciāls, jo tās var izturēt dažādus vides apstākļus un var izdzīvot ūdenī un augsnē vairākus mēnešus (Rahman et al., 2017). Eksperimentāli tika pierādīts, ka lēni sasaldējot kriptosporīdiju oocistas līdz -22 °C temperatūrai un uzturot šo temperatūru 21 stundu, tika iznīcinātas 67 % no kopējā oocistu skaita. Pēc 152 stundām gāja bojā 90 % no kopējā oocistu skaita, bet pat pēc 750 stundām joprojām neliels oocistu daudzums paliek dzīvs. Savukārt *Cryptosporidium* spp. oocistas nav izturīgas pret sausumu: eksperimentāli žāvējot tās ar fēnu istabas temperatūrā, pēc divām stundām visas oocistas zaudēja dzīvotspēju (Robertson, 1992). Invāzija var tikt pārnesta tiešā ceļā no teļa teļam un no govs teļam vai netieši - ar netīriem apaviem, ar kontaminētu apkārtējo vidi, ūdeni vai barību. Ūdens filtrācija un biežāk izmantotās dezinfekcijas metodes nespēj iznīcināt kriptosporīdiju oocistas, tāpēc tieši dzeramais ūdens tiek uzskatīts par galveno invadēšanās avotu (Garber, 1994) (1.1. attēls).



1.1. att. ***Cryptosporidium*** **spp. attīstības cikls** (CDC's Division of Parasitic Diseases and Malaria (DPDM))

Cryptosporidium ģints vienšūņi ir sastopami visā pasaulē, tajā skaitā arī Latvijā. Tie invadē gan cilvēkus, gan daudzas dzīvnieku sugas - lauksaimniecības dzīvniekus, mājdzīvniekus un savvaļas dzīvniekus. Nozīmīgus veselības traucējumus kriptosporīdijas izraisa tieši jaundzimušajiem teļiem. Kriptosporidioze kā invāzija un tās epidemioloģija ir daudzu valstu parazitoloģisko pētījumu uzmanības centrā. Salīdzinoši daudz ir pētījumu par *Cryptosporidium* spp. invāziju ekstensitāti dažādās valstīs, retāk ir pētīti faktori, kuri ietekmē teļu invadēšanās varbūtību ar kriptosporīdijām.

1.5. Pirmpiena nozīme jaundzimušo teļu veselībā

Pirmpiens ir sarežģīts sekrēts, kas satur augstu būtisku ķīmisko savienojumu (barības vielu, augšanas faktoru un imūnfaktoru) daudzumu, kā mērķis ir aktivizēt imūnsistēmu un nodrošināt ar barības vielām jaundzimušo, kā arī stimulēt teļa kuņģa un zarnu trakta attīstību (Elfstrand et al., 2002; McGrath et al., 2016; Puppel et al., 2019). Imūnglobulīna daudzums

pirmpienā ir līdz 100 reizēm lielāks nekā pilnpienā, antimikrobiālo vielu (laktoferīna, laktoperoksidāzes, lizocīma) daudzums ir divas līdz piecas reizes lielāks (Pakkanen and Aalto, 1997; Stelwagen et al., 2009).

Pirmpiens ir pirmais jaundzimušo teļu barības avots, kas ne tikai baro dzīvnieku, bet arī nodrošina nepieciešamos komponentus organisma aizsardzībai. Teļiem ir nepieciešams saņemt atbilstošu kvalitatīva pirmpiena apjomu tūlīt pēc piedzimšanas, lai palīdzētu novērst invāzijas izplatību novietnēs, tālāku slimības klīnisko attīstību un nepieļautu dzīvnieka attīstības aizkavēšanu. Pirmpienā ir nepieciešamie savienojumi: imūnglobulīni (Ig) – glikoproteīni, kas specifiski atpazīst un saistās ar antigēniem uz patogēnu virsmas. Neskatoties uz to, ka ir vairākas Ig klases, tostarp IgA, IgD, IgE, IgG un IgM (Kaskous and Fadlelmoula, 2015), lielākā daļa no tām pirmpienā ir zemā koncentrācijā. IgG ir īpaši nozīmīgs, jo tas ir primārais Ig, kas atrodams govs pirmpienā un pienā, un tam ir galvenā loma humorālās imunitātes veidošanā (McGrath et al., 2016). IgG koncentrācija pirmpienā var sasniegt pat 50–100 mg/ml, un ar pasīvo pārnesi tie nodrošina efektīvu vairāku patogēnu izraisītu cilvēku vai dzīvnieku slimību profilaksi vai ārstēšanu. Tā kā IgG var novērst patogēnu saistīšanās pie zarnu epitēlija šūnām, tie darbojas kā primārā aizsardzība pret lielāko daļu potenciālo kuņģa un zarnu trakta patogēnu. IgG saistīšanās ar *Cryptosporidium* spp. var būtiski ietekmēt parazīta patoģenēzi, kavējot tā izplatību un samazinot invāzijas risku saimniekšūnās (Ulfman et al., 2018).

Kvalitatīva pirmpiena uzņemšana pietiekamā daudzumā ir viens no svarīgākajiem faktoriem, kas ietekmē teļu veselību un izdzīvošanu, jo nodrošina pasīvu imunitātes pārnešanu no govs uz teļu. Tā kā bez IgG pirmpienā ir arī citi faktori, kas potenciāli var ietekmēt invadēšanos ar *Cryptosporidium* spp., šķiet pamatoti novērtēt pirmpiena kvalitāti ietekmējošos faktorus un to saistību ar *Cryptosporidium* spp. invāziju. Turklāt pirmpiena kvalitāte ir atkarīga no daudziem faktoriem, piemēram, govs vecuma (Conneely et al., 2013), šķirnes (Muller and Ellinger, 1981; Morrill et al., 2012), laktācijas reizes (Morrill et al., 2012), atnešanās kalendārās sezonas (Nardone et al., 1997) un cietstāves perioda ilguma (Rastani et al., 2005; Annen et al., 2004).

Pirmpienu govis sāk producēt no nedēļas līdz pāris dienām pirms atnešanās un ražo nedēļu pēc atnešanās. Pēc pirmpiena sekrēcijas govis vienu līdz divas dienas ražo pārejas pienu, kura uzturvērtība un bioloģisko komponentu koncentrācija ir zemākas nekā pirmpienam, bet augstāka nekā nobriedušam pienam jeb pilnpienam (Quinn et al., 2020; O'Callaghan et al., 2020). Savukārt Kargar et al. (2021) norāda, ka ilgstoša teļu ēdināšana ar pārejas pienu (trīs nedēļas) uzlabo teļu augšanu un samazina diarejas varbūtību.

Viena potenciāla kriptosporidiozes kontroles metode ir pirmpiena un pārejas piena izēdināšana. Tā kā teļi piedzimst imunoloģiski naivi (t.i., to imūnsistēma nekad nav bijusi pakļauta antigēnu ietekmei), tiem ir nepieciešama pasīva aizsardzība, ko govs nodrošina ar pirmpienu un pārejas pienu.

Praksē nevar novilkt skaidru līniju, kad pirmpiens pārtop par pārejas pienu un pilnpienu. Ir pieņemts, ka pirmpiens tiek ražots pirmajās trīs dienās pēc govs atnešanās. Tad seko 5–7 dienas, kad tiek ražots pārejas piens (McGrath et al., 2016). Pamatojoties uz jaunākajiem pētījumiem par pirmpiena sastāvu un nozīmi jaundzimušajiem organismiem, tika izstrādāti ieteikumi par pirmpiena daudzumu un savlaicīgu jaundzimušo teļu ēdināšanu. Piemēram, ēdināšana ar pirmpienu atlikšana par sešām stundām (35,6 ± 1,88 %) un 12 stundām (35,1 ± 3,15 %) samazināja IgG maksimālo šķietamo absorbcijas efektivitāti salīdzinājumā ar pirmpiena izēdināšanu tūlīt pēc piedzimšanas (51,8 ± 4,18 %) un pagarināja laiku līdz maksimālajai IgG koncentrācijas sasniegšanai serumā (attiecīgi 24 h pret 15 h). Aizkavēta pirmpiena izēdināšana mēdza samazināt labvēlīgo baktēriju izplatību, kas saistītas ar resnās zarnas gļotādu, īpaši *Bifidobacterium* un *Lactobacillus* sugām, kurām ir svarīga loma zarnu veselībā. Tāpēc ir ļoti svarīgi nodrošināt pirmpiena uzņemšanu pēc iespējas ātrāk pēc teļa piedzimšanas (Fischer et al., 2018; Pyo et al., 2018). Pētījumu par pārejas piena lietderību ir salīdzinoši maz un ieteikumi pārejas piena uzņemšanai nav izstrādāti. Lielajās piena lopkopības

novietnēs teļus atradina no govīm un tur atsevišķi teļu aplokā jau drīz pēc pirmās ēdināšanas, savukārt pārejas pienu ievieto kopējā uzglabāšanas tvertnē, kur to atšķaida ar citu govju pienu.

Kriptosporidiozes ārstēšana ir sarežģīta, jo līdz šim brīdim nebija pieejama efektīva vakcīna slimības profilaksei. Pieejamie medikamenti bieži koncentrējas tikai uz simptomu (piemēram, dehidratācijas) ārstēšanu (Chalmers and Giles, 2010; Meganck, Hoflack and Opsomer, 2014). Nesen (2023. gadā) tirgū ir parādījusies vakcīna *Bovilis Cryptium* ®, kas pasargā teļus no *Cryptosporidium parvum* Gp40 serotipa. Ar to ir jāvakcinē govis divas reizes pirms atnešanās, lai teļš kopā ar pirmpienu saņemtu antivielas pret kriptosporīdijām. Līdz ar to vakcīnas ražotāji pievērš uzmanību tam, ka pirmpiena izēdināšanas režīmam ir vitāli svarīga loma teļa imunitātes izveidošanā (*NOAH Compendium,* 2023). Kopā ar efektīvu novietnes pārvaldību, t.i. ātra pietiekami liela pirmpiena daudzuma izdzirdināšana teļam uzreiz pēc piedzimšanas vai govs atnešanās sezonas izvēle, var kļūt par alternatīvu instrumentu kriptosporidiozes kontrolei vai profilaksei ganampulkā. Bieža fekāliju tīrīšana no govju kūts un teļu aplokiem, kā arī dezinfekcijas līdzekļu, karstā ūdens un mazgāšanas līdzekļu lietošana var palīdzēt ievērojami samazināt oocistu skaitu novietnē (Robertson, Campbell and Smith 1992; Harp and Goff, 1998).

1.6. Promocijas darba hipotēze

Promocijas darbā izvirzīta viena hipotēze: pastāv saistība starp *Cryptosporidium* spp. invāzijas ekstensitāti un intensitāti teļiem un teļu ēdināšanas režīmu, ka arī mātes bioloģisko un vides faktoru kopumu.

1.7. Promocijas darba mērķis

Promocijas darba mērķis ir noteikt piena teļu *Cryptosporidium* spp. invāziju ietekmējošos faktorus un sugu izplatību Latvijā.

1.8. Promocijas darba uzdevumi

Promocijas darbā izvirzīti seši uzdevumi:

1. veikt pilotpētījumu Vidzemes reģionā ar mērķi noskaidrot vispārējo parazitāro invāziju ekstensitāti piena govju ganāmpulkos (**I** publikācija);
2. pārbaudīt, vai pastāv saistība starp IgG līmeni govīm pirmpienā un teļa asins serumā un novērtēt tā saistību ar *Cryptosporidium* spp. invāziju teļiem (**II** publikācija);
3. noteikt *Cryptosporidium* spp. sugas Latvijā (**III** publikācija);
4. pārbaudīt, vai pastāv saistība starp *Cryptosporidium* spp. teļu invadēšanos un tādiem faktoriem kā piena govju novietņu lielumu, govs šķirni, laktācijas reizi, atnešanās kalendāro sezonu un cietstāves perioda ilgumu (**IV** publikācija);
5. izpētīt, vai pastāv saistība starp teļu invāziju ar *Cryptosporidium* spp. un pirmpiena un pārejas piena ēdināšanas režīmu teļiem (**V** publikācija);
6. novērtēt *Cryptosporidium* spp. invāzijas ekstensitāti un intensitāti teļiem un govīm (**I, II, III, IV**, **V** un **VI** publikācijas).

1.9. Promocijas darba novitāte

Promocijas darba tēma ir aktuāla Latvijā un visā pasaulē, jo tiešā veidā skar piensaimniecības nozari, kas Latvijā ir sena un populāra lauksaimniecības nozare. No govīm

iegūtā piena kvalitāti un kvantitāti var ietekmēt tādi faktori kā ēdināšana, turēšana, dažādas bakterioloģiskas vai vīrusu izraisītas infekcijas. Viens no faktoriem, kas būtiski var ietekmēt iegūtās produkcijas kvalitāti un kvantitāti, ir parazītu ierosinātās invāzijas (Knubben-Schweizer, 2010). Teļi, kuri pirmajos dzīves mēnešos tika pakļauti smagām infekcijām, sliktāk pieņemās svarā, ir vājāki, salīdzinot ar vienaudžiem, kuri netika pakļauti infekcijām. Rezultātā šiem dzīvniekiem izaugot, ir zemāka produktivitāte un ekonomiskais labums (Dallago et al., 2024).

Turpretim gaļas govju audzēšana Latvijā ir salīdzinoši jaunāka nozare, bet arī gaļas kvalitāti var būtiski ietekmēt parazītu invāzijas. Pasargāšana no parazitārām invāzijām balstās uz epizootiskās situācijas izvērtēšanu un profilakses pasākumu plānošanu (Forbes et al., 2000; Forbes, 2020). Tomēr, neskatoties uz nozares aktualitāti, pētījuma gaitā tika pieņemts lēmums atteikties no tālākiem praktiskiem gaļas govju izmeklējumiem. Tas skaidrojams ar būtiskām atšķirībām dzīvnieku turēšanas tehnoloģijās un to temperamentā: gaļas šķirņu liellopi pamatā tiek turēti ekstenzīvi (brīvās turēšanas apstākļos aplokos), tie nav pieraduši pie regulāra tieša kontakta ar cilvēku un ir grūtāk fiksējami. Tā kā paraugu ievākšana (īpaši pirmpiena noslaukšana un koproloģiskā testēšana) prasa specifisku dzīvnieka fiksāciju, kas bez speciāla aprīkojuma radītu paaugstinātu traumatisma risku gan personālam, gan pašiem dzīvniekiem, pētījuma turpināšana šajā grupā tika atzīta par nelietderīgu.

Līdz 2015. gadam Latvijā tika veikti fragmentāri pētījumi, kuri nedod pilnīgu *Cryptosporidium* spp. izplatības ainu un nav noteikts, kuras parazītu sugas ir sastopamas valstī, kas bremzē preventīvo pasākumu izstrādi parazītu izplatības mazināšanai un apkarošanai. Jāpiemin, ka *Cryptosporidium parvum* izplatība novietnēs var būt potenciāli bīstama, jo nonākot augsnē un ūdens rezervuāros tā var invadēt cilvēkus, tostarp imūnkompromitētās personās, izraisīt smagu saslimšanu vai pat nāvi.

Promocijas darba ietvaros uz *Cryptosporidium* spp. klātbūtni ir izmeklētas vairāk nekā 2100 govju no vairāk nekā 190 novietnēm dažādos Latvijas reģionos, kā arī izvērtētas dažādu tipu un lieluma novietnes, kas ir pirmais tik apjomīgais pētījums Latvijas teritorijā pēdējos 30 gados.

1.10. Promocijas darba uzbūve

Promocijas darbs sastāv no sešām publikācijām, kurās apkopoti pētījumi par kriptosporīdiju sugu dažādību Latvijas teritorijā un pirmpiena izēdināšanas režīma ietekmi uz kriptosporidiozes invāzijas smaguma pakāpi teļiem pirmajos dzīves mēnešos. Promocijas darba pirmajā publikācijā novērtēta kopējā parazitoloģiskā situācija govju ganāmpulkos Vidzemes reģionā. Otrajā publikācijā novērtēta pirmpiena IgG līmeņa saistība ar *Cryptosporidium* spp. invāzijas intensitāti jaundzimušajiem teļiem. Trešajā publikācijā pētītas *Cryptosporidium* spp. sugas Latvijas teritorijā. Ceturtajā publikācijā pētīta govs laktācijas reizes ietekme uz kriptosporidiozes attīstību teļiem. Piektajā publikācijā novērtēta teļu jutība pret *Cryptosporidium* spp. atkarībā no pirmpiena izdzirdināšanas režīma. Savukārt sestajā publikācijā novērtēta *Cryptosporidium* spp. invāzijas ekstensitāte un intensitāte teļiem un govīm, apkopojot un sintezējot visos iepriekšējos pētījuma posmos iegūtos rezultātus.

2. MATERIĀLI UN METODES

2.1. Izlases datu apraksts (I-VI publikācijas)

Cryptosporidium spp. invāzijas ekstensitātes pētījums piena govju novietnēs tika veikts divos etapos, kas ietver pilotpētījumu (**I**) un pamatpētījumu (**II, III, IV**, **V** un **VI**). Pamatpētījums sastāv no 5 posmiem, kur katrs posms atbilst vienam izvirzītam uzdevumam.

Kopējais izmeklēto dzīvnieku skaits visā pētījuma periodā no 2013. līdz 2022. gadam n = 2655, no tiem:

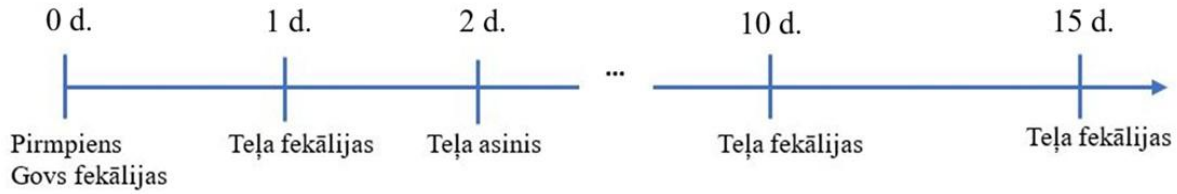
- 975 piena teļi līdz trīs mēnešu vecumam;
- 770 piena teļi vecumā no četriem mēnešiem līdz 24 mēnešiem;
- 910 slaucamās govis, kas vecākas par 24 mēnešiem.

Laika posmā no 2013. līdz 2014. gadam (**I**) tika veikts sākuma pētījuma posms jeb pilotpētījums, kura mērķis bija noskaidrot provizorisko situāciju Latvijā par *Cryptosporidium* spp. invāziju (1. uzdevums). Šī posma ietvaros tika izmeklētas piena (n = 521) un gaļas (n = 119) govis Vidzemes reģionā. Dzīvnieki tika iedalīti četrās grupās: piena govis vecumā no sešiem mēnešiem līdz diviem gadiem (n = 273) un vecākas par diviem gadiem (n = 248); gaļas govis vecumā no sešiem mēnešiem līdz diviem gadiem (n = 90) un vecākas par diviem gadiem (n = 29). Lai noteiktu gremošanas trakta parazītu faunas sastāvu, tai skaitā, lai noteiktu kriptosporīdiju invāzijas ekstensitāti, tika ievākti un izmeklēti koproloģiskie paraugi no 62 govju novietnēm: 50 piena govju novietnēm un 12 gaļas govju novietnēm. Katrs individuālais piena govju koproloģiskais paraugs tika savākts atsevišķā polietilēna maisiņā. Gaļas govju koproloģiskie paraugi tika ņemti no ganībām. Visi paraugi tika marķēti un nogādāti Latvijas Biozinātņu un tehnoloģiju universitātes Veterinārmedicīnas fakultātes Pārtikas un vides higiēnas institūta Parazitoloģijas laboratorijā 24 stundu laikā.

Promocijas darbā izvirzīto 2.-6. uzdevumu sasniegšanai tika veikti pieci neatkarīgi pētījumi, kas veido darba pamatpētījumu un kuru rezultāti tika publicēti zinātniskos rakstos. Pilotpētījumā un pamatpētījuma pirmajā, otrajā, trešajā un ceturtajā posmā izmeklētie dzīvnieki citos pētījumos neatkārtojas. Piektais pamatpētījuma posms apkopo visos iepriekšējos posmos izmeklētos dzīvniekus ar mērķi noskaidrot invāzijas ekstensitāti Latvijas teritorijā. Pirms sākt paraugu vākšanu, tika iegūti dati no Latvijas Lauksaimniecības datu centra par kopējo valstī esošo piena liellopu skaitu 2018. gada 29. jūnijā (pamatpētījuma sākuma brīdis). Balstoties uz šo informāciju, katram pētījumam atsevišķi tika izrēķināts minimālais nepieciešamais (95 % ticamības līmenis un 5 % kļūda) paraugu skaits.

Otrā uzdevuma izpildei, lai pārbaudītu saistību starp IgG līmeni govs pirmpienā un teļa asins serumā un novērtētu tā saistību ar *Cryptosporidium* spp. invāziju teļiem (**II**), no 2018. gada decembra līdz 2019. gada martam tika īstenots pamatpētījuma pirmais posms, kura ietvaros savākti govju pirmpiena un fekāliju paraugi (n = 114), kā arī asins un fekāliju paraugi no jaundzimušajiem teļiem (n = 114) vienā piena govju novietnē. Šajā novietnē teļi tika atšķirti no mātēm uzreiz pēc piedzimšanas un pirmo 24 stundu laikā saņēma divus litrus pirmpiena divās atsevišķās ēdināšanas reizēs. Seši mililitri govs pirmpiena tika noslaukti uzreiz pēc atnešanās, piens tika marķēts, sasaldēts un glabāts -18 ºC temperatūrā līdz nogādāšanai Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta "BIOR" Mikrobioloģijas un patoloģijas laboratorijā. Tāpat pirmajās 24 stundās pēc atnešanās tika paņemts govs fekāliju paraugs (2.1. attēls). Fekāliju paraugi no teļiem tika ņemti pirmajā, desmitajā un piecpadsmitajā dzīves dienā, tie tika marķēti un nogādāti Latvijas Biozinātņu un tehnoloģiju universitātes Veterinārmedicīnas fakultātes Pārtikas un vides higiēnas institūta Parazitoloģijas laboratorijā. Koproloģiskie paraugi tika savākti atsevišķi no katra teļa un govs polietilēna maisiņā. Ja fekāliju daudzums bija pārāk mazs (īpaši pirmajās teļu dzīves dienās), tika veiktas natīvās uztriepes. Asins paraugi ņemti teļiem divu dienu vecumā, kad IgG sasniedz maksimālo līmeni (Fischer et al., 2018). Paraugs tika ņemts no jugulārās vēnas ar 21G izmēra adatu, savākts vakutainerī,

marķēts un glabāts aukstumsomā 4 ºC temperatūrā līdz nogādāšanai Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta "BIOR" Mikrobioloģijas un patoloģijas laboratorijā tālākai izmeklēšanai.



2.1. att. **Paraugu ņemšanas shēma imūnglobulīna G un *Cryptosporidium* spp. invāzijas intensitātes saistības novērtēsanai pamatpētījuma pirmajā posmā**

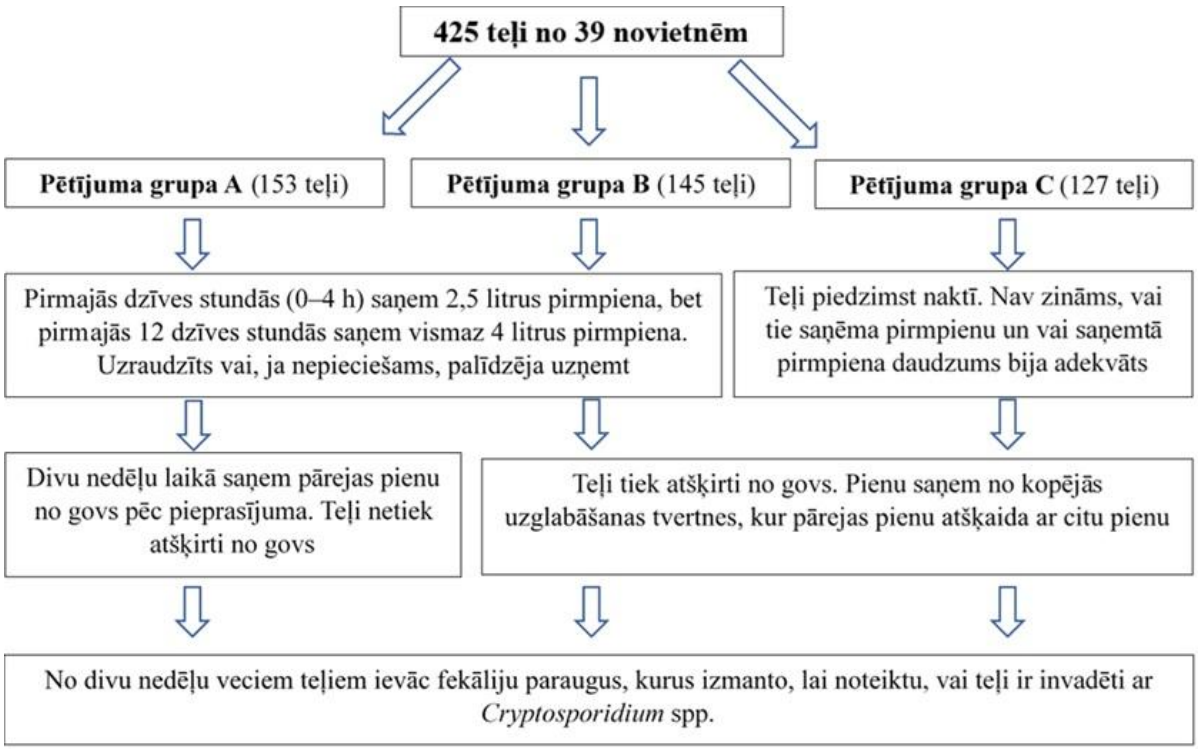
Trešā uzdevuma izpildei tika veikts otrais pamatpētījuma posms, kas iekļauj pētījumu par *Cryptosporidium* spp. invāzijas ekstensitāti un sugu dažādību piena govīm Latvijā. Tas tika veikts laika posmā no 2018. gada jūlija līdz 2019. gada jūnijam (**III**), kura laikā tika savākti 926 individuālie koproloģiskie paraugi (790 no teļiem un govīm novietnēs, 136 no uz kautuvēm atvestajām govīm) no visiem Latvijas novadiem (2.2. attēls). Pētījumā iekļautās novietnes tika sadalītas trīs grupās, atkarībā no novietnes lieluma: mazās novietnes 1–50 govis (n = 113), vidējās novietnes 50–200 govis (n = 39) un lielās novietnes ar vairāk nekā 200 govju novietnē (n = 33). Dzīvnieki tika sadalīti trīs vecuma grupās: teļi vecumā līdz trīs mēnešiem (n = 259), teļi un jaunlopi vecumā no četriem līdz 24 mēnešiem (n = 247) un govis, kas vecākas par 24 mēnešiem (n = 420).

Šis dzīvnieku vecuma grupu sadalījums ir balstīts uz *Cryptosporidium* spp. izraisītas slimības klīniskās ainas un govju reprodukcijas menedžmentu: vissmagāk slimo teļi vecumā līdz trīs mēnešiem; jaundzīvnieki vecumā no trīs līdz 24 mēnešiem reti slimo klīniski un parasti vēl nav neatnesušās; vecumā no 24 mēnešiem notiek pirmā govs atnešanās (Cho and Yoon, 2014; Thompson et al., 2017). Katrā novietnē tika savākts no viena līdz 36 individuālajiem paraugiem, kuri tika marķēti un 24 stundu laikā nogādāti Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta "BIOR" Mikrobioloģijas un patoloģijas laboratorijā. Randomizāciju ierobežoja saimnieku brīvprātīga pieteikšanās izmeklēšanai. Paraugu ņemšana tika veikta arī četrās kautuvēs: tika savākti paraugi no visām nokautajām govīm apmeklējuma dienā (8–62 paraugi katrā kautuvē). Katrs paraugs tika savākts atsevišķā polietilēna maisiņā, marķēts un nogādāts 24 stundu laikā Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta "BIOR" Mikrobioloģijas un patoloģijas laboratorijā.

2.2. att. **Pētījumā iekļauto novietņu atrašanās vietas *Cryptosporidium* spp. sugu dažādības noteikšanai piena govīm Latvijā**

Trešais pamatpētījuma posms tika īstenots visu 2020. gadu ar mērķi izpildīt ceturto uzdevumu un pārbaudīt saistību starp *Cryptosporidium* spp. teļu invadēšanās varbūtību un tādiem faktoriem kā piena govju novietnes lielums, govju šķirne, laktācijas reize, atnešanās sezona un cietstāves perioda ilgums (**IV**). Tika ņemti koproloģiskie paraugi no 153 teļiem 17 novietnēs, kā arī katras novietnes darbinieki atbildēja uz pētījumā izstrādātās anketas jautājumiem (sk. sadaļu "Novietņu aptauja"). Pirms koproloģisko paraugu vākšanas teļi saņēma pirmpienu un pārejas pienu pēc sekojošas shēmas: uzreiz pēc piedzimšanas teļi saņēma ~2,5 litrus pirmpiena un nākamajās 12 stundās vēl vismaz 4 litrus. Teļi netika atšķirti no govs un divu nedēļu laikā saņēma pienu pēc pieprasījuma. Koproloģiskie paraugi no katra teļa tika savākti 14. dienā atsevišķā polietilēna maisiņā, marķēti un nogādāti Latvijas Biozinātņu un tehnoloģiju universitātes Veterinārmedicīnas fakultātes Pārtikas un vides higiēnas institūta Parazitoloģijas laboratorijā 24 stundu laikā.

Laika periodā no 2018. gada decembra līdz 2020. gada decembrim tika īstenots ceturtais pamatpētījuma posms, kura laikā tika izpildīts piektais uzdevums: pārbaudīt saistību starp *Cryptosporidium* spp. invadēšanās ekstensitāti un pirmpiena un pārejas piena ēdināšanas režīmu teļiem (**V**). Šajā posmā tika izmeklēti fekāliju paraugi no 425 teļiem (15 ± 2 dienas veci) 39 novietnēs. Fekāliju paraugus paņēma no teļu taisnās zarnas. Pētījuma dizains ir atspoguļots 2.3. attēlā. Koproloģiskie paraugi no katra teļa tika savākti atsevišķā polietilēna maisiņā, marķēti un nogādāti Latvijas Biozinātņu un tehnoloģiju universitātes Veterinārmedicīnas fakultātes Pārtikas un vides higiēnas institūta Parazitoloģijas laboratorijā 24 stundu laikā.



2.3. att. **Piena izēdināšanas shēma teļiem pirmpiena un pārejas piena ietekmes noteikšanai uz *Cryptosporidium* spp. invāziju**

Lai izpildītu pēdējo, sesto uzdevumu, t.i., noteiktu *Cryptosporidium* spp. invāzijas ekstensitāti Latvijā (VI), pamatpētījuma piektajā posmā tika analizēti visi iepriekš no 2013. gada pavasara līdz 2022. gada rudenim savāktie 2655 individuālie piena govju koproloģiskie paraugi. Tāpāt kā iepriekšējos posmos, pētījumā iekļautie dzīvnieki tika sadalīti trīs vecuma grupās: teļi vecumā līdz trīs mēnešiem (n = 975), teļi un jaunlopi vecumā no četriem līdz 24 mēnešiem (n = 770) un govis, kas vecākas par 24 mēnešiem (n = 910).

2.2. Koproloģisko paraugu laboratoriskā izmeklēšanas metodoloģija (I-V publikācijas)

Koproloģiskie izmeklējumi tika veikti gan pilotpētījumā, gan visos četros pamatpētījuma posmos. Paraugi tika ņemti no teļu un govju taisnās zarnas, ievietoti individuālos polietilēna maisiņā, marķēti un transportēšanas laikā uz laboratoriju uzglabāti aukstuma somā, līdz tika ievietoti laboratorijas ledusskapī 4 °C temperatūrā (Lassen, 2011). Koproloģiskie paraugi tika izmeklēti 24–48 stundu laikā pēc nogādāšanas laboratorijā. Pilotpētījuma ietvaros helmintu (**I**) diagnostikai tika izmantotas standartizētas ovoskopiskās un larvoskopiskās metodes (Roepstorff and Nansen, 1998). Pilotpētījuma un pamatpētījuma pirmā, trešā un ceturtā posma laboratoriskie izmeklējumi veikti Latvijas Biozinātņu un tehnoloģiju universitates Veterinārmedicīnas fakultātes Pārtikas un vides higiēnas institūta Parazitoloģijas laboratorijā (**I, II, IV** un **V**), turpretim pamatpētījuma otrajā posmā iegūtie paraugi tika izmeklēti Pārtikas drošības, dzīvnieku veselības un vides zinātniskajā institūtā "BIOR" (**III**).

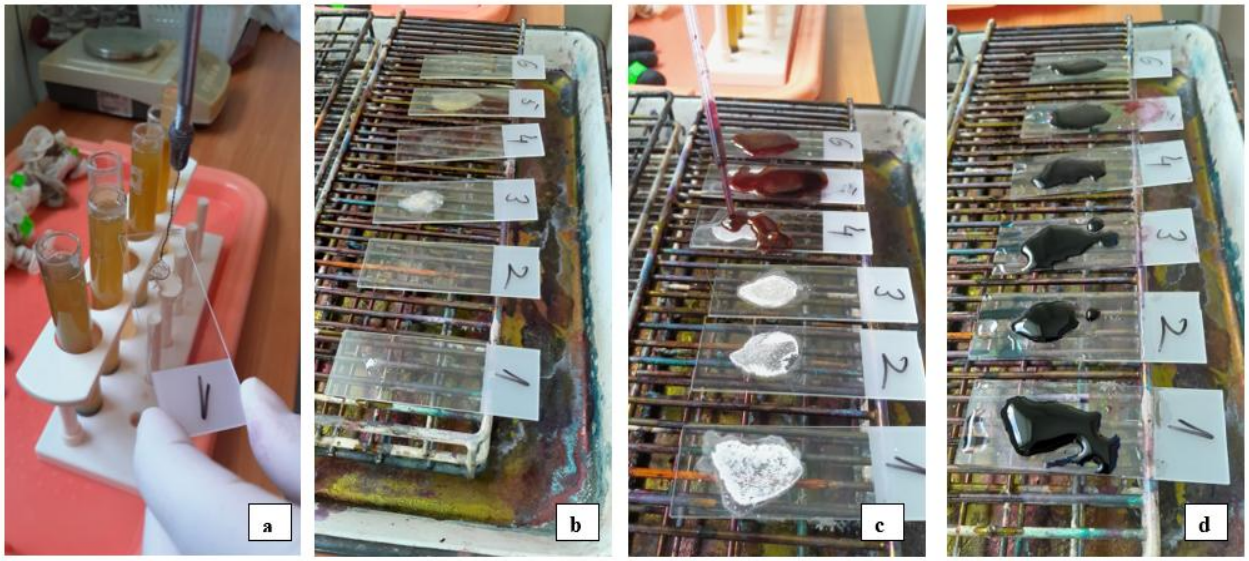
Gadījumos, kad jaundzimušiem teļiem vecumā līdz trim dienām fekāliju daudzums taisnā zarnā nav bijis pietiekams, uz vietas tika ņemtas natīvās uztriepes. Koka irbulītis ar vati tika samitrināts ar fizioloģisko šķidrumu un ievietots teļa taisnajā zarnā 5–6 cm dziļumā. Ar irbulīti tika paslidināts pa zarnu sieniņu, tas apgriezts ap savu asi, izvilkts un ievietots stobriņā ar nelielu fizioloģiskā šķidruma daudzumu, lai neizžūst. Laboratorijā izdara irbulīša nospiedumu uz priekšmetstikliņa un nokrāso paraugu pēc Cīla-Nilsena metodes.

Kā minēts iepriekš, *Cryptosporidium* spp. invāzija bieži izraisa diareju teļiem, retāk govīm. Šī var būt pirmā saslimšanas pazīme, ko saimniekam vai kopējam ir viegli pamanīt. Tāpēc paraugu vākšanas laikā tika reģistrēts dzīvnieku klīniskais stāvoklis (diarejas klātbūtne), lai pēc oocistu saskaitīšanas varētu noteikt, vai invāzijas intensitāte korelē ar gremošanas trakta traucējumu klīnisko smaguma pakapi. Govīm un teļiem fekālijas parasti ir pussķidras līdz cietas, brūnganā krāsā ar raksturīgu smaku, un defekācija ir regulāra - notiek līdz pat vairākām reizēm dienā. Diarejas gadījumā novērojamas izteiktas izmaiņas fekāliju konsistencē – tās kļūst ievērojami šķidrākas vai pat ūdeņainas, biežākas, var saturēt gļotas, asinis, kā arī mainīt krāsu. Šīs izmaiņas bieži pavada dehidratācijas pazīmes, piemēram, iegrimušas acis, sausas gļotādas, letarģija, apetītes zudums un novājēšana (Cho and Yoon, 2014).

Pilotpētījuma ietvaros helmintu (**I**) diagnostikai tika izmantotas standartizētas ovoskopiskās un larvoskopiskās metodes (Roepstorff and Nansen, 1998). Lai noteiktu helmintu invāziju ekstensitāti un intensitāti koproloģiskajos paraugos, tika izmantota McMaster metode. Pēc šīs metodes četri grami fekāliju tika sajaukti ar 56 ml NaCl flotācijas šķīduma, lai iegūtu kopējo tilpumu 60 ml. Fekāliju suspensija tika filtrēta caur sietu glāzē. Izmantojot pipeti, filtrāta maisīšanas laikā tika paņemts paraugs no suspensijas vidusdaļas. Katra McMaster priekšmetstikliņa kamera tika nekavējoties piepildīta ar iegūto filtrātu, izmantojot pipeti. Pēc 5 minūtēm priekšmetstikliņi tika mikroskopēti ar 10x40 objektīvu, koncentrējoties uz augšējo slāni. Olu skaitu uz gramu fekāliju (OSG) tika aprēķināts pēc formulas: saskaitīto olu skaitu abās kamerās reizinot ar 50.

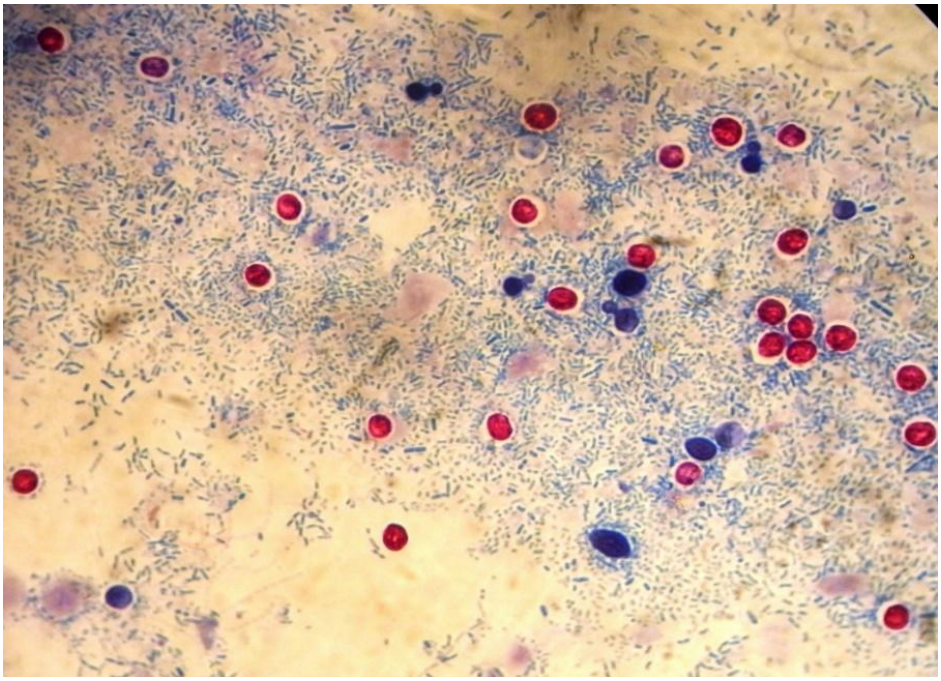
Helmintu kāpuru noteikšanai (**I**) tika izmantota Bērmaņa metode (larvoskopija) (2.4. attēls). Pēc šīs metodes 10 g fekāliju tika ievietoti uz sietiņa, kas novietots konusveida glāzē. Konusveida glāzi piepildīja ar siltu ūdeni, uz glāzes tika uzlikts siets ar koproloģisko materiālu tā, lai aptuveni trešdaļa no sieta ar koproloģisko materiālu atrastos saskarē ar ūdeni. Paraugs tika atstāts uz 30 minūtēm, lai parazītiskie kāpuri nogulsnētos konusveida glāzes apakšā. Pēc 30 minūtēm glāzes augšējā kārta tika nolieta līdz nogulsnēm, bet nogulsnes izlietas uz Petri plates un mikroskopētas 10x40 lielā palielinājumā.

2.4. att. **Koproloģiskā parauga izmeklēšana pēc Bērmaņa metodes: parauga uzlikšanas brīdī un pēc 30 minūtēm**



2.5. att. **Modificētā Cīla-Nilsena (*Ziehl-Neelsen*) krāsošanas metode: a) fekāliju materiāla uzklāšana uz attaukota priekšmetstikliņa, b) priekšmetstikliņa žāvēšana istabas temperatūrā, c) un d) fekāliju materiāla iekrāsošana**

Kriptosporīdiju oocistu noteikšana notika gan pilotpētījumā, gan visos pamatpētījuma posmos (**I, II, III, IV** un **V**). Tika veikta koproloģisko paraugu izmeklēšana pēc flotācijas metodes, un tika izmantota modificēta Cīla-Nilsena krāsošanas metode. Flotācijas metodes veikšanai tika ņemti četri grami fekāliju, ievietoti piestā un sajaukti ar 56 ml piesātināta NaCl šķīdumu un saberzti ar miezeri līdz tika iegūta viendabīga masa. Iegūtais šķīdums caur piltuvi un sietu tika ieliets centrifūgas stobriņā (Kuczynska and Shelton, 1999). Materiāls tika centrifugēts divas minūtes 2000 apgriezienos minūtē. Pēc šķidrās daļas atdalīšanas, tika iegūti divi mililitri koncentrēta materiāla, kas tika izmantoti tālākai krāsošanai.



2.6. att. ***Cryptosporidium* spp. oocistas (palielinājums: x100 eļļa) krāsots ar modificētu Cīla-Nilsena (*Ziehl-Neelsen*) metodi**

Sagatavotā, koncentrētā fekāliju materiāla 10 µl lielu pilienu uzklāj uz attaukota priekšmetstikliņa, nožāvē istabas temperatūrā un iekrāso ar modificētu Cīla-Nilsena tehniku

(Henriksen and Pohlenz, 1981) ar TB Stain komplektu (BD, Īrija) (2.5. attēls). Izžuvusī uztriepe tika fiksēta ar metanolu 10–15 min, nožāvēta un vairākas reizes pārvilkta virs liesmas. Pēc tam uz uztriepes tika uzlieta karbolfuksīna krāsa un izturēta 25–30 minūtes. Preparātu noskaloja ar ūdeni, pārleja ar 8 % sērskābes šķīdumu, paturēja 40–60 sekundes, otro reizi noskaloja ar ūdeni un iekrāsoja ar metilēnzilā šķidrumu. Pēc 3–5 minūtēm preparātu rūpīgi noskaloja ar ūdeni, atstāja izžūt un mikroskopēja imersijas eļļā. Pozitīvas kontroles bija iepriekš noteiktas un tika iekļautas visos krāsojumos. Visos pilienos tika saskaitītas tumši sarkanas līdz rozā oocistas ar tipisku morfoloģiju 100x palielinājumā (2.6. attēls).

Visi negatīvajie paraugi no pamatpētījuma otrā posma (**III**) tika sagatavoti imunofluorescences mikroskopijai, izmantojot AquaGlo komplektu (Waterborne INC, ASV), lai noteiktu antivielām marķētas kriptosporīdiju oocistas. Paraugu sagatavošana fluorescējošajai mikroskopijai tika veikta atbilstoši norādēm: 10 µl fekāliju materiāla pēc desmitkārtīgas rūpīgas atšķaidīšanas tika ievietoti 12 mm teflona drukātā trīskameru priekšmetstikliņā (Immuno-Cell, Mechelen, Vācija), pēc tam paraugi tika žāvēti un fiksēti, iegremdējot priekšmetstikliņu acetonā. Vēlāk materiāls tika iekrāsots ar FITC iezīmētām anti-*Cryptosporidium*/*Giardia* monoklonālajām antivielām (AquaGlo, Waterborne, Inc., ASV) un 30 minūtes inkubēts mitruma kamerā. Pēc tam antivielu šķīdums tika noskalots ar PBS. Visās kamerās tika saskaitītas spilgti iekrāsotās oocistas ar tipisku morfoloģiju 200x palielinājumā. Katra atklātā oocista tika uzskatīta par 200 oocistām vienā gramā (OSG).

2.3. Imūnglobulīna G līmeņa noteikšana jaunpienā un jaundzimušo teļu asinīs (II publikācija)

Lai izpildītu otro uzdevumu un noskaidrotu, vai pastāv saistība starp IgG līmeni govju pirmpienā un teļa asins serumā un novērtētu tā saistību ar *Cryptosporidium* spp. invāziju teļiem, pamatpētījuma pirmā posma ietvaros vienā novietnē četru mēnešu laikā tika vākti govju pirmpiena un teļu asins paraugi. Pirmpiena paraugi tika ņemti klīniski veselām govīm no visām tesmeņa ceturtdaļām pirmajās divās stundās pēc atnešanās 6 ml stobriņos. Asins paraugi tika ņemti no teļu jugulārās vēnas otrajā dzīves dienā, izmantojot vakuuma venipunkciju 6 ml asins seruma stobriņos. Pirmpiens un asins paraugi tika sasaldēti un līdz analīžu veikšanai uzglabāti –80 °C temperatūrā.

Ievāktie teļu seruma un govju pirmpiena paraugi tika izmeklēti, lai noteiktu govs imūnglobulīna G (IgG) koncentrāciju ar ELISA komplektu "Bovine Immunoglobulin" (Bio–X Diagnostics, Beļģija). Izmeklējumi tika veikti Pārtikas drošības, dzīvnieku veselības un vides zinātniskajā institūtā "BIOR" Mikrobioloģijas un patoloģijas laboratorijā.

Paraugu izmeklēšanai tika izveidota kalibrēšanas līkne teļa serumam un govs pirmpienam. Teļu seruma paraugi tika atšķaidīti attiecībā 1:100, bet pirmpiena paraugi – 1:1000. Atšķaidīšanas mikroplates atbilstošajās kamerās tika pārnesti 100 µl kalibrēšanas līknes atšķaidījuma un atšķaidīto paraugu. Pēc tam katrai kamerai tika pievienots konjugāta darba šķīdums, saturs sajaukts un 100 µl no katras kameras tilpuma pārnesti uz komplekta mikroplates atbilstošajām kamerām. Mikroplate tika inkubēta 21 °C ± 3 °C temperatūrā vienu stundu. Pēc tam mikroplati trīs reizes skaloja ar mazgāšanas šķīdumu. Tad katrai kamerai tika pievienoti 100 µl hromatogēna šķīduma, un mikroplate tika inkubēta 21 °C ± 3 °C temperatūrā 10 minūtes tumsā. Reakciju apturēja, katrai kamerai pievienojot 50 µl apturēšanas (stop) šķīduma. Pētīto paraugu optiskais blīvums tika noteikts, izmantojot monohromatisko ELISA lasītāju (Thermo Scientific Multiscan FC) ar 450 nm filtru.

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2.4. DNS izdalīšana, ligzdota PCR reakcija un sekvencēšana (III publikācija)

Pamatpētījuma otrajā posmā *Cryptosporidium* spp. sugu identifikācijai no iegūtā koproloģiskā materiāla tika izdalīta DNS. Izmeklēšana tika veikta Valsts Veterinārajā institūtā Uppsalā, Zviedrijā.

Genomiskā DNS tika izdalīta no granulām, kas iegūtas pēc 2 ml attīrīta fekāliju parauga centrifugēšanas, izmantojot *DNeasy PowerSoil* komplektu (QIAGEN, Hilden, Vācija) saskaņā ar ražotāja norādījumiem. Elūcija tika veikta ar 80 µl šķīduma C6 (t. i., *DNeasy PowerSoil* komplekta elūcijas bufera). Divi mikrolitri no katra DNS parauga tika pakļauti polimerāzes ķēdes reakcijas (PCR) amplifikācijai mērķējot uz 18S rDNS kā aprakstīts iepriekš (Xiao et al., 1999; Åberg et al., 2019). Kā negatīvā un pozitīvā kontrole tika izmantots nukleāzes nesaturošs ūdens un *C. parvum* genomiskā DNS.

Pirmajā amplifikācijas maisījumā bija 1× KAPA2G buferšķīdums (KAPA Biosystems), pa 200 µM no katra deoksinukleozīda trifosfāta (dNTP), pa 0,5 µM no katra ārējā (pirmās kārtas) praimera un 2 µl DNS šķīduma ar kopējo tilpumu 25 µl. Pēc sākotnējās denaturācijas 95 °C temperatūrā trīs minūtes sekoja 40 cikli: 95 °C 30 sekundes, 61 °C 30 sekundes, 72 °C viena minūte un gala pagarinājums 72 °C temperatūrā divas minūtes.

Otrajai amplifikācijai reakcijas maisījumam tika pievienoti 2 µl no pirmās reakcijas, kā norādīts iepriekš, izmantojot iekšējos (otrās kārtas) praimerus. Ligzdotās polimerāzes ķēdes reakcijas (nested-PCR) apstākļi bija 95 °C temperatūrā trīs minūtes, kam sekoja 40 cikli: 95 °C 30 sekundes, 63 °C 30 sekundes, 72 °C temperatūrā viena minūte un pēdējais pagarinājums 72 °C temperatūrā divas minūtes. PCR produkti tika analizēti, izmantojot kapilārās elektroforēzes sistēmu (QIAxcel Advanced, QIAGEN, Vācija). Paredzamā izmēra produkti (apmēram 820 bp) tika pakļauti sekvencēšanai sugu identifikācijai.

PCR produkti tika attīrīti un sekvencēti abos virzienos, izmantojot iekārtu *Applied Biosystems® 3130xl Genetic Analyzer*. Priekšējās un reversās sekvences tika saskaņotas ar programmatūru *BioEdit* v7.2.5 (Hall, 1999), lai izveidotu vienotas konsensa sekvences un koriģētu kļūdas. Iegūtās sekvences tika salīdzinātas ar nukleotīdu sekvencēm, kas deponētas GenBank, izmantojot BLASTn (nukleotīdu pamata vietējās izlīdzināšanas meklēšanas rīks) (Altschul et al., 1990). Lai identificētu jauktas invāzijas, tika izmantots rīks *CryptoGenotyper Galaxy* (Afgan et al., 2016; Yanta et al., 2021). Visas sekvences tika analizētas, izmantojot 18S contig darbplūsmu.

2.5. Aptauja (IV publikācija)

"Lai novērtētu faktorus, kas ietekmē *Cryptosporidium* spp. invāzijas ekstensitāti un ir cieši saistīti ar govju turēšanas apstākļiem, trešajā pamatpētījuma posmā tika izstrādāta aptaujas anketa. Novietņu īpašnieki vai darbinieki uz jautājumiem atbildēja pirms teļu fekāliju paraugu vākšanas.

Aptaujas anketā (1. pielikums) tika iekļauti vairāki jautājumi ar precīzi definētām atbilžu kategorijām, kas ļāva sistematizēt iegūtos datus un veikt to padziļinātu analīzi par:

1) piena govju novietnes lielumu: maza (līdz 10 govis), vidēja (11–50 govis) un liela novietne (virs 50 govis);
2) govju šķirni;
3) laktācijas reizi: 1, 2 un ≥ 3;
4) atnešanās kalendārā sezonu: ziema, pavasaris, vasara un rudens;
5) cietstāves perioda ilgumu: ≤ 45, 46–64 un ≥ 65 dienas.

Iegūtie aptaujas dati tika izmantoti, lai izveidotu matemātisku modeli. Šis modelis ļāva identificēt, kuri no aptaujā iekļautajiem faktoriem ir visciešāk saistīti ar *Cryptosporidium* spp. invāzijas ekstensitāti.

2.6. Statistisko datu analīze (I-VI publikācijas)

Statistikas datu izlasē ņemto govju vecums bija no vienas dienas līdz 24 gadu vecumam. Izmantojot *OpenEpi* sistēmu, tika noteikts, ka 323 govis ir minimālais paraugu skaits, kas nepieciešams šim pētījumam. Aprēķins tika balstīts uz govju populācijas lielumu Latvijā 2018. gada 29. jūnijā (395 320 dzīvnieki; Latvijas Republikas Lauksaimniecības datu centrs, www.ldc.gov.lv), absolūto precizitāti 10 % apmērā un sagaidāmo *Cryptosporidium* spp. proporciju 30 %. Mērķis bija ievākt paraugus vismaz 900 govīm. Izlase tika proporcionāli izkliedēta pa Latvijas reģioniem, sagaidot, ka konkrētajā brīdī vismaz 41 % govju izplatīs *Cryptosporidium* spp. oocistas tajās novietnēs, kurās šis parazīts jau ticis konstatēts (Lassen, 2011).

Lai noteiktu, vai starp divām grupām pastāv statistiski nozīmīgas atšķirības, tika izmantots neatkarīgu izlašu T tests, ja dati bija normāli sadalīti un homogēni. Pretējā gadījumā dati tika analizēti, izmantojot Mann-Whitney U testu. Kruskala–Volisa H tests (*Kruskal–Wallis H test*) tika izmantots, lai pārbaudītu atšķirības starp trīs grupām. Normalitātes pieņēmums tika pārbaudīts ar Šapiro–Vilka testu, bet pieņēmums par dispersiju homogenitāti – ar Levēna testu (**I, II, VI**).

Lai noteiktu lineārās korelācijas stiprumu un virzienu starp diviem nepārtrauktiem mainīgajiem, tika izmantots Pīrsona korelācijas tests. Tika veikta binomiālā loģistiskā regresija, lai noskaidrotu govju pirmpiena un teļu asins seruma IgG līmeņa ietekmi uz *Cryptosporidium* spp. invāzijas varbūtību teļiem (**II**).

Ģeneralizētā lineārā jauktā modelēšana (GLMM) tika veikta, lai noteiktu, vai skaidrojošie mainīgie (piena govju novietnes lielums, šķirne, laktācijas reize, kalendārā sezona un cietstāves perioda ilgums) ir saistīti ar teļu invadēšanās iespējamību ar *Cryptosporidium* spp., par nejaušo efektu (*random effect*) izvēloties novietnes identifikācijas numuru (*FarmID*) (**IV**). GLMM modelis tika izmantots arī, lai noskaidrotu, vai skaidrojošais mainīgais – pirmpiena un pārejas piena ēdināšanas (uzņemšanas) režīms (trīs pētnieciskās grupas) – ir saistīts ar teļu invadēšanās iespējamību, par nejaušo efektu tāpat izvēloties novietnes identifikācijas numuru (*FarmID*) (**V**).

Akaikes informācijas kritērijs (AIC) tika izmantots, lai novērtētu, kurš modelis labāk atbilst datiem. Parazītu ekstensitāte tika aprēķināta kā ar *Cryptosporidium* spp. invadēto govju procentuālā daļa (**I-VI**). Statistiskie testi tika veikti, izmantojot programmatūru *SPSS Statistics* (versija 22; IBM Corporation, Čikāga, Ilinoisa), *Jamovi* (versija 2.0.0; https://www.jamovi.org/) un programmu *R* (versija 3.3.1; http://www.R-project.org), izmantojot pakotni *lme4*. Visas statistiskās analīzes tika veiktas ar nozīmīguma līmeni (α = 0,05). Imūnglobulīna koncentrācijas tika aprēķinātas, izmantojot programmatūru *MyAssays* ar četru parametru loģistiskās līknes (*4PL*) pielāgošanas funkciju (**II**).

Statistisko datu analīzes tika veiktas Vācijā, Frīdriha Lēflera institūtā (Friedrich-Loeffler-Institut) (**III**), Dzīvnieku slimību federālajā pētniecības institūtā, RSU statistikas mācību laboratorijā (**II, IV, V un VI**) un Latvijas Biozinātņu un tehnoloģiju universitātes Veterinārmedicīnas fakultātē (**I**).

2.7. Ētiskie aspekti

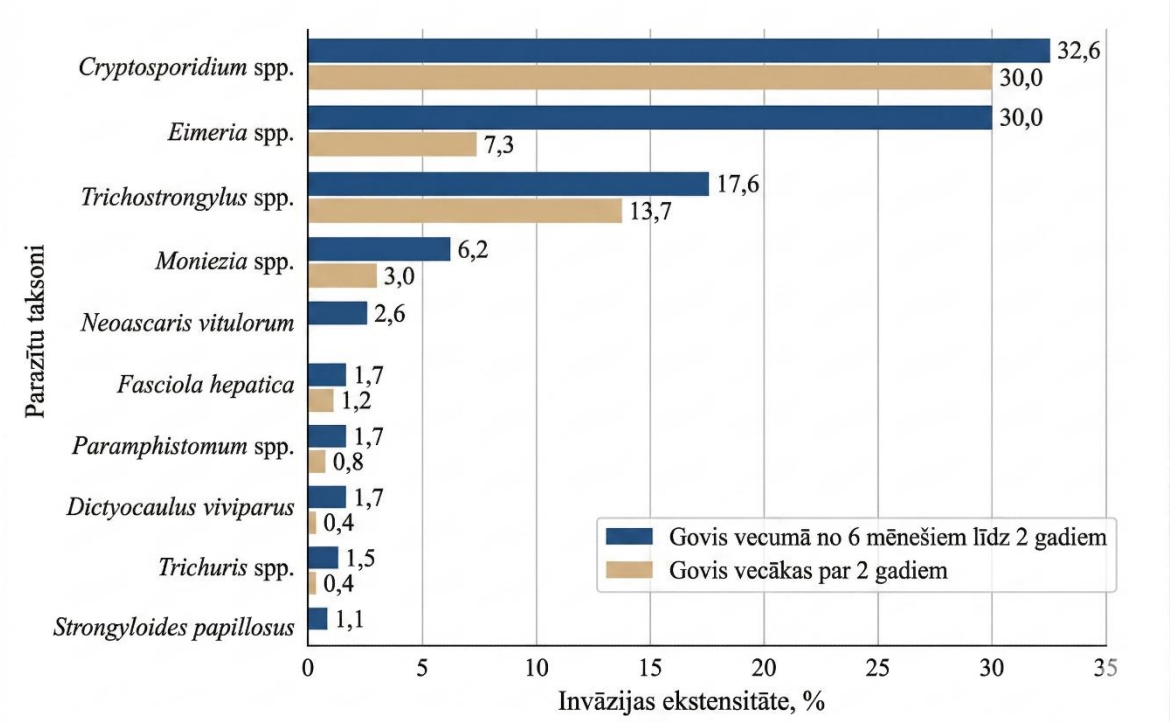
Visas procedūras, kas veiktas pētījumos ar dzīvniekiem, atbilda ētikas standartiem. Visas manipulācijas tika veiktas standarta lauksaimniecības un zootehniskās prakses ietvaros, neradot dzīvniekiem sāpes, ciešanas vai stresu. Asins paraugu vākšanai 2019. gada 7. jūlijā tika saņemta Latvijas Biozinātņu un tehnoloģiju universitātes Dzīvnieku labturības un aizsardzības ētikas padomes atļauja Nr. 19/1.

3. REZULTĀTI UN DISKUSIJA

3.1. Pilotpētījuma galvenie rezultāti (I publikācija)

Cryptosporidium spp. ir globāli izplatīti parazīti, kas rada nopietnus draudus gan dzīvnieku, gan cilvēku veselībai. Līdz sēlim Latvijā dati par šo vienšūņu izplatību ir bijuši salīdzinoši trūcīgi, jo iepriekšējie pētījumi veikti fragmentāri un ir bijuši nepietiekami, lai sniegtu pilnvērtīgu ainu. Tādēļ pētījuma pirmais posms – pilotpētījums, kas veikts no 2013. līdz 2014. gadam Vidzemes reģionā, kļuva par būtisku pirmo soli, lai izpētītu *Cryptosporidium* spp. invāzijas ekstensitāti Latvijas ganāmpulkos. Iegūtie rezultāti pamato nepieciešamību pievērst lielāku uzmanību šo parazītu izpētei Latvijā, jo kriptosporidioze ir potenciāli bīstama zoonoze, kas apdraud gan teļus, gan cilvēkus.

Pilotpētījuma rezultāti (3.1. attēls) liecina, ka starp visiem diagnosticētajiem gremošanas trakta parazītiem tieši vienšūņu izplatība govju novietnēs ir visaugstākā. Starp tiem augstākā invāzijas ekstensitāte tika konstatēta *Cryptosporidium* spp. – robežās no 19,0 % līdz 32,6 %. *Cryptosporidium* spp. invāzijas ekstensitāte būtiski atšķīrās starp govīm un jaunlopiem ($p < 0,05$) — jaunlopiem šis rādītājs bija gandrīz divas reizes augstāks nekā govīm.



3.1. att. **Gremošanas trakta parazītu invāzijas ekstensitāte (%) piena govīm Vidzemes reģionā (2013.–2014. gada pilotpētījuma dati)**

Rēķinot pēc vidējās ekstensitātes rādītāja, aiz vienšūņiem seko cestodes (*Moniezia* spp. – vid. ekstensitāte 6,2 %), nematodes (*Trichostrongylus* spp., *Neoascaris* spp., *Trichuris* spp., *Strongyloides* spp., *Dictyocaulus* spp. – vid. ekstensitāte 4,9 %) un trematodes (*Fasciola* spp., *Paramphistomum* spp. – vid. ekstensitāte 1,7 %).

Lielāku vienšūņu ekstensitāti, salīdzinot ar citiem parazītu tipiem, var izskaidrot ar to augsto reproduktīvo potenciālu, īso attīstības ciklu, autoinvadēšanās iespēju, zemo saimnieka specifiskumu, vieglo izplatīšanos fekāli orālā ceļā un izturību apkārtējā vidē. Vairākos pētījumos ir ziņots, ka *Cryptosporidium* spp., *Eimeria* spp. un *Giardia* spp. parazīti izraisa zarnu slimību uzliesmojumus cilvēkiem un dzīvniekiem (Fu et al., 2023). Turklāt tādas nematodes kā *Strongyloides* spp., *Cooperia* spp., *Chabertia* spp., *Ostertagia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Bunostomum* spp., *Teladorsagia* spp., *Nematodirus* spp. un *Trichuris*

spp. teļiem bieži ierosina kā monoinvāzijas, tā arī jauktas invāzijas kopā ar kriptosporīdijām (Delling and Daugschies, 2022).

Neskatoties uz konstatēto teļu parazitofaunas daudzveidību, tieši kriptosporīdiju invāzija piesaista pastiprinātu kā lauksaimniecības un veterinārmedicīnas, tā arī humanitārās medicīnas speciālistu uzmanību. Katru gadu visā pasaulē tiek ziņots par masveida kriptosporidiozes uzliesmojumiem cilvēkiem, ko izraisa *C. parvum*, turklāt liellopi, mazie atgremotāji un kontaminēts ūdens ir galvenie cilvēka inficēšanās avoti (Caffarena et al., 2020). Parazītu invāzija rada ne tikai veselības riskus cilvēkiem, bet arī ievērojamus ekonomiskus zaudējumus lauksaimniecībā. Piemēram, Meksikā tika aprēķināts, ka govju parazitārās invāzijas izraisa vairāku simtu miljonu eiro zaudējumus, kas saistīti ar piena ražošanas samazināšanos, dzīvmasas pieauguma aizkavēšanos vai lopkopības blakusprodukcijas kontamināciju (Rodríguez-Vivas et al., 2017).

Lai mazinātu parazitāro invāziju negatīvo ietekmi, ir nepieciešams ieviest stingrus kontroles pasākumus. Tomēr tas ir iespējams tikai tad, ja ir zināma precīza parazītu izplatība un invāzijas intensitāte. Līdz šim *Cryptosporidium* spp. invāzija govīm Latvijā nav tikusi padziļināti pētīta, tādēļ turpmākajās darba nodaļās uzmanība ir vērsta uz teļu kriptosporidiozes ekstensitāti un intensitāti ietekmējošiem faktoriem, kā arī invāzijas izplatību un sugu daudzveidību Latvijā.

3.2. Saistība starp *Cryptosporidium* spp. invāziju un IgG koncentrāciju piena govju pirmpienā un teļu asins serumā (II publikācija)

Pirmpiens ir neatņemams jaundzimušo teļu veselības un labturības priekšnoteikums, kas nodrošina ne tikai barības vielas, bet arī pasīvo imunitāti, kas ir kritiski svarīga pirmajās dzīves dienās un nedēļās. Galvenā loma šajā imunitātes pārnesē ir imūnglobulīniem, īpaši imūnglobulīnam G (IgG) (McGrath et al., 2016). Tā kā *Cryptosporidium* spp. invāzija visbiežāk skar tieši jaunākos teļus, likumsakarīgi rodas jautājums: vai pastāv saistība starp IgG līmeni, ko teļš saņem ar pirmpienu un kas cirkulē tā asinīs, un tā uzņēmību pret kriptosporidiozi? Pamatpētījuma pirmais posms tika veltīts atbildes meklēšanai uz šo jautājumu.

Lai izpētītu šo saistību, tika veikts pētījums, kurā tika iekļautas 114 govis un to teļi no piena govju novietnes Latvijā. No katras govs tika ņemts pirmpiena paraugs tūlīt pēc atnešanās un asins paraugs no divu dienu veciem teļiem, kad IgG līmenis sasniedz maksimumu. Kopā ar to tika savākti arī teļu fekāliju paraugi pirmajā, desmitajā un piecpadsmitajā dzīves dienā, lai noteiktu *Cryptosporidium* spp. invāzijas klātbūtni.

IgG koncentrācija pirmpienā un teļu asins serumā tika mērīta, izmantojot imūnfermentatīvo analīzi (ELISA; *Enzyme-Linked Immunosorbent Assay*), kas ir standarta metode antivielu kvantitatīvai noteikšanai. Kriptosporīdiju invāzija tika diagnosticēta, izmantojot Cīla–Nilsena metodi, kas ļauj precīzi identificēt oocistas fekāliju paraugos.

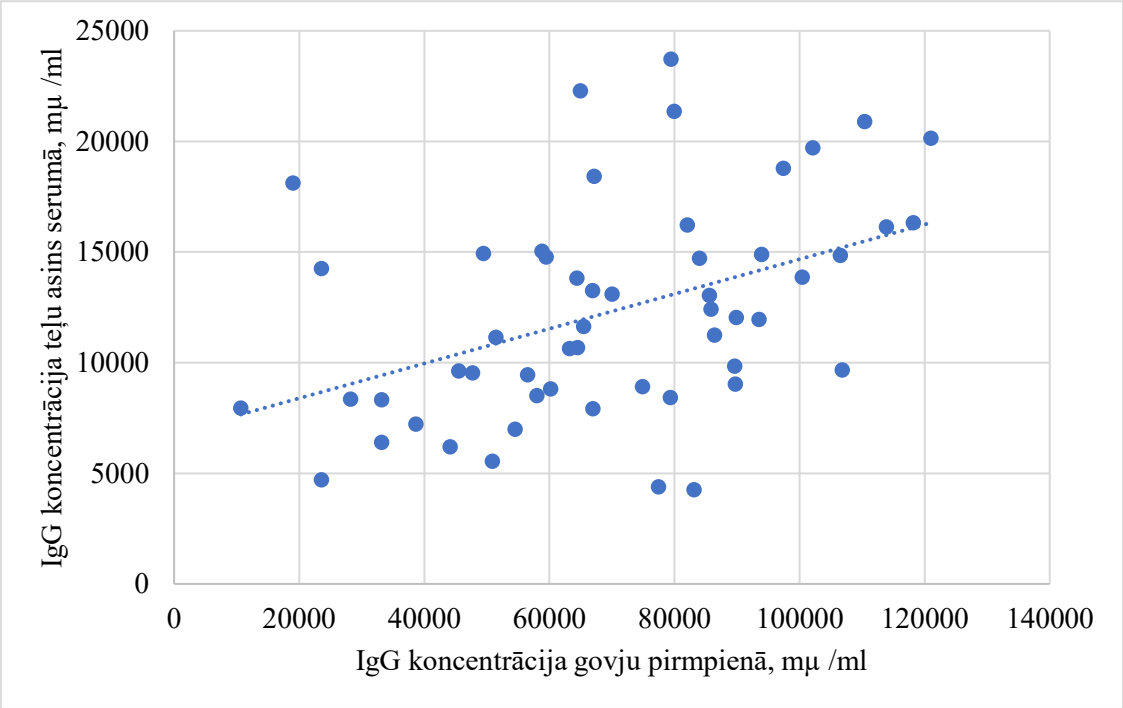
Dati tiek prezentēti kā vidējais aritmētiskais un standartnovirze. IgG koncentrācija govīm pirmpienā bija augstāka (70,7 ± 26,6 g/L) nekā teļu asins serumā (13,2 ± 6,1 g/L), statistiski nozīmīga atšķirība bija 57,4 g/L (95 % TI, 52,4–62,4), $t(124{,}872) = 22{,}536$, $p < 0{,}001$. IgG koncentrācija teļu asinīs ir tieši saistīta ar pirmpiena izēdināšanu (Drikic et al., 2018). Lai gan pastāv imunoloģiska saikne starp mātes imunitāti un pasīvo imunitātes pārnešanu uz pēcnācējiem (Hurley, 2003), un tiek ziņots, ka IgG nodrošina pasīvo imunitāti pret dažādiem patogēniem (*Yersinia enterocolitica*, *Campylobacter jejuni*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella typhimurium*, *Staphylococcus* spp., *Streptococcus* spp.), promocijas darba ietvaros netika konstatēta saistība starp IgG līmeni govju pirmpienā un *Cryptosporidium* spp. invāzijas varbūtību teļiem (Ulfman et al., 2018).

Koproloģisko paraugu analīze uzrādīja skaidru invāzijas dinamiku teļiem pirmajās divās dzīves nedēļās. Proti, pirmajā dienā neviens no teļiem nebija invadēts ar *Cryptosporidium* spp.

(ekstensitāte 0 %). Tomēr vēlāk situācija krasi mainījās: 10. dienā invāzijas ekstensitāte sasniedza 26,3 %, bet 15. dienā tā pieauga līdz 45,6 %.

Šis straujais pieaugums norāda uz intensīvu invadēšanās procesu tieši šajā kritiskajā periodā: pirmajās dzīves dienās, kad specifiska immunitāte vēl nav izveidojusies. Mann-Whitney U tests apstiprināja statistiski nozīmīgu atšķirību oocistu izdalīšanā starp 10. un 15. dienu ($U = 1944$, $z = 2{,}330$, $p = 0{,}020$), ar lielāku oocistu skaita mediānu 15. dienā nekā 10. dienā. Jāatzīmē, ka teļu invāzijas ekstensitāte šajā periodā bija vismaz trīs reizes augstāka nekā to mātēm.

Lai izvērtētu saistību starp IgG koncentrāciju un teļu invāziju ar *Cryptosporidium* spp., tika veikta korelācijas analīze. Tika konstatēta statistiski nozīmīga vidēji cieša pozitīva korelācija starp IgG koncentrāciju govju pirmpienā un teļu asins serumā ($r(114) = 0{,}414$; $p = 0{,}001$) (3.2. attēls). Savukārt statistiski nozīmīga korelācija starp IgG koncentrāciju teļu asins serumā un *Cryptosporidium* spp. invāzijas intensitāti netika konstatēta ($p > 0{,}05$).



3.2. att. **Korelācija starp IgG koncentrāciju govju pirmpienā (0–2 stundas pēc atnešanās) (n = 114) un teļu asins serumā otrajā dzīves dienā (n = 114)**

Lai novērtētu varbūtību, ka *Cryptosporidium* spp. invāzijas iespējamība teļu koproloģiskajos paraugos samazinās, palielinoties IgG līmenim teļu asins serumā, tika veikta loģistiskās regresijas analīze. Rezultāti parādīja, ka izveidotais modelis nav statistiski nozīmīgs ne 10. dienā ($\chi^2(2) = 0{,}013$; $p = 0{,}99$), ne 15. dienā ($\chi^2(2) = 0{,}100$; $p = 0{,}95$). Tas norāda, ka statistiski nozīmīga cēloņsakarība starp IgG koncentrāciju govju pirmpienā vai teļu asins serumā un teļu invadēšanos ar *Cryptosporidium* spp. netika konstatēta.

Iepriekšējie pētījumi par kriptosporīdiju izplatību ASV (Lemeteil et al., 1993) un Ķīnā (Gong et al., 2017) sakrīt ar mūsu iegūtajiem rezultātiem, ka teļiem ir lielāka invāzijas ekstensitāte nekā govīm. Santín et al. (2004) secināja, ka kriptosporīdiju sugu izplatība ir saistīta ar teļu vecumu pirms atšķiršanas un pēcatšķiršanas periodā. Hārps ar līdzautoriem (Harp et al., 1990) pierādīja, ka teļu (līdz 3 mēnešu vecumam) invāzija un atveseļošanās var pasargāt tos no atkārtotas *C. parvum* invāzijas. Visticamāk, šie atklājumi netieši uzsver adaptīvās imunitātes nozīmi kriptosporīdiju invāzijā. Tomēr Gongs ar līdzautoriem (Gong et al., 2017) ir norādījuši, ka *Cryptosporidium* sugas/apakštipi dažādās govju vecuma grupās atšķiras, kas liecina, ka invadēšanās ar vienu kriptosporīdiju sugu un turpmāka atveseļošanās negarantē imunitāti pret citām kriptosporīdiju sugām. Tompsons ar līdzautoriem (Thompson et al., 2017) izteica

pieņēmumu, ka parazīta spēja invadēt dzīvnieku ir saistīta ar izmaiņām zarnu mikroflorā dzīvnieka nobriešanas laikā, tomēr pagaidām trūkst eksperimentālu pētījumu, kas to pierādītu govīm.

Pirmpiens satur ne tikai IgG, bet arī virkni citu imūnglobulīnu, kas arī nodrošina efektīvu imūnreakciju. Piemēram, IgA, kas veido 5-10 % no kopēja imūnglobulīnu daudzuma, ir lielmolekulārs proteīns, kurš netiek absorbēts un paliek uz gļotādas virsmas, nodrošinot lokālo aizsardzību (Pakkanen and Aalto, 1997; Playford, Macdonald and Johnson, 2000; Blum, 2006). Iespējams, tā līmenis precīzāk ļauj prognozēt invadēšanās risku ar kriptosporīdijām, nekā IgG līmenis. Elisons ar līdzautoriem (Ellison et al., 2011) ziņo, ka IgM reakcija notiek tūlīt pēc akūtas invāzijas un tā līmenis samazinās dažu nedēļu laikā, turpretī IgG atbildes reakcija parādās lēnāk, bet saglabājas ilgāku laiku. Līdz ar to kopējo IgA, IgM un IgG līmeņu pētījums pirmajās teļu dzimšanas dienās saistībā ar kriptosporīdiju invāzijas līmeni būtu jauns pētījuma virziens nākotnē. Nedrīkst aizmirst, ka imūnās atbildes reakcijas uz kriptosporīdiju invāziju ietver gan iedzimtu, gan adaptīvu imunitāti. Daudzos pētījumos ir aprakstīta T un B šūnu, dabīgo killeršūnu (NK šūnu), dendrītisko šūnu un makrofāgu, zarnu epitēlija šūnu, gamma interferona, slāpekļa oksīda, pretmikrobu peptīdu, prostaglandīnu, citokīnu un hemokīnu loma imūnās atbildes veidošanā pret kriptosporīdijām (Leitch and He, 2011; Vanathy et al., 2017). Līdz ar to pastāv iespēja, ka nav korekti izvērtēt tikai IgG līmeni pret kriptosporīdijām vērstas imunitātes izveidē. Visdrīzāk tas ir vairāku šūnu un bioloģiski aktīvo vielu mijiedarbība.

Iegūstot rezultātus, tika izvirzīta hipotēze, vai pirmpiena izēdināšana ietekmē kriptosporidiozes invāziju. Tas ļautu pierādīt, ka, lai gan IgG tieši neietekmē invāzijas gaitu, kriptosporidiozes gadījumā pasīvās imunitātes pārnešanai uz pēcnācējiem caur pirmpienu ir lielāka nozīme nekā iedzimtajai un adaptīvajai imunitātei. Šis pieņēmums tika formulēts darba ceturtajā un piektajā uzdevumā un izpētīts pamatpētījuma trešajā un ceturtajā posmā.

3.3. Diagnosticētās *Cryptosporidium* ģints sugas Latvijā (III publikācija)

Lai pilnībā izprastu kriptosporidiozes epidemioloģiju Latvijas govju populācijā, nepietiek tikai ar invāzijas izplatības un intensitātes noteikšanu. Būtiski ir arī noskaidrot, kuras konkrētas *Cryptosporidium* ģints sugas ir sastopamas, jo dažādām sugām var būt atšķirīga patoģenitāte un epidemioloģiskā nozīme, tostarp zoonotiskais potenciāls. Tādēļ trešais promocijas darba uzdevums un pamatpētījuma otrais posms bija veikt molekulāro tipēšanu, lai identificētu Latvijā cirkulējošās kriptosporīdiju sugas.

Lai identificētu *Cryptosporidium* sugas, tika izmantota molekulārā diagnostika, kas šo vienšūņu taksonomiskajā piederībā tiek uzskatītas par diagnostikas zelta standartu. Pētījumā, kas aptver laika posmu no 2018. līdz 2019. gadam, tika savākti 926 koproloģiskie paraugi no liellopiem dažādās Latvijas saimniecībās un kautuvēs. Paraugi tika analizēti, izmantojot polimerāzes ķēdes reakciju (PĶR; *Polymerase Chain Reaction*), amplificējot specifiskus DNS fragmentus, un sekvencēšanas metodi, lai precīzi noteiktu sugas un genotipus. Šī pieeja nodrošināja augstu precizitāti un ļāva atklāt pat reti sastopamas sugas.

Molekulārās analīzes rezultāti apliecināja augstu parazitofaunas daudzveidību: Latvijas govju populācijā tika konstatētas sešas dažādas *Cryptosporidium* sugas. Detalizēti dati par katras sugas sastopamības biežumu, saistību ar dzīvnieka vecumu un diareju ir apkopoti 3.1. tabulā.

3.1. tabula **Kriptosporīdiju sugu epidemioloģiskie rādītāji piena govīm Latvijā**

Suga	Kopējais paraugu skaits	Īpatsvars (95 % TI)	Vecuma mediāna (mēneši)	Vecuma amplitūda (mēneši)	Paraugu skaits govīm ar diareju (95 % TI)
C. parvum	62,0	45,9 (37,8–54,3)	3,0	0,03–111,0	41,9 (30,4–54,3)
C. bovis	29	21,5 (15,4–29,2)	3,5	0,2–172,0	41,4 (25,5–59,3)
C. andersoni	22	16,3 (11,0–23,5)	17,5	0,09–197,0	22,7 (9,7–43,9)
C. ryanae	11	8,1 (4,5–14,1)	6,0	0,2–70,0	18,2 (4,0–48,9)
C. scrofarum	1	0,7 (0,0–4,5)		20,0	0,0
C. ubiquitum	1	0,7 (0,0–4,5)		84,0	100,0
C. parvum / C. bovis	3	2,2 (0,5–6,6)	0,06	0,03–3,0	100,0
C. parvum / C. andersoni	1	0,7 (0,0–4,5)		16,0	0,0
C. parvum / C. ryanae	1	0,7 (0,0–4,5)		1,2	100,0
C. bovis / C. andersoni	1	0,7 (0,0–4,5)		147,0	100,0
C. bovis / C. ryanae	3	2,2 (0,5–6,6)	11,0	1–55,0	33,3 (1,7–86,8)
Visas sugas	135	41,4 (36,5–46,8)	4,5	0,03–197,0	38,5 (30,6–46,9)

Piezīme: Kopējais izolātu īpatsvars (%) aprēķināts pret veiksmīgi genotipēto pozitīvo paraugu skaitu. Diarejas sastopamība izteikta kā procentuālā daļa no attiecīgās sugas vai jaukta tipa invāzijas paraugu skaita. TI – 95% ticamības intervāls.

No 326 govju un teļu koproloģiskajiem paraugiem, kas savākti no 54 novietnēm, un bija mikroskopiski pozitīvi uz *Cryptosporidium* spp., tikai 41,4 % (n = 135) gadījumu tika veiksmīgi amplificēta un sekvencēta *Cryptosporidium* spp. DNS. Kopumā tika konstatētas sešas *Cryptosporidium* sugas: *C. parvum*, *C. bovis*, *C. andersoni*, *C. ryanae*, *C. scrofarum* un *C. ubiquitum*. *Cryptosporidium scrofarum* un *C. ubiquitum* tika konstatēti tikai vienā atsevišķā paraugā, kam epidemioloģiskajā kopainā ir zema nozīmība.

Mūsu pētījumā, izmantojot CryptoGenotyper rīku, 31 no 135 paraugiem tika konstatēta jaukta *Cryptosporidium* spp. invāzija. Trīs paraugos bija *C. parvum* un *C. bovis*, trīs *C. bovis* un *C. ryanae*, vienā *C. parvum* un *C. ryanae*, vienā *C. parvum* un *C. andersoni* un vienā *C. bovis* un *C. andersoni* jaukta tipa invāzijas.

Vismaz viena *Cryptosporidium* spp. suga govīm tika konstatēta 55,6 % (95 % TI 42,2–68,4 %) novietņu, vismaz divas sugas tika konstatētas 31,4 % (95 % TI 20,2–77,7 %) novietņu, bet trīs vai vairāk *Cryptosporidium* sugas bija diagnosticētas 13,0 % (95 % TI 5,9–24,0 %) izmeklēto novietņu.

Cryptosporidium parvum konstatēts govīm no 63,0 % (95 % TI 49,6–75,0 %) novietņu, kam seko *C. bovis* 37,0 % (95 % TI 25,0–50,4 %), *C. andersoni* 37,0 % (95 % TI 25,0–50,4) un *C. ryanae* 26,0 % (95 % TI 15,6-38,8 %). Sugas *C. parvum* un *C. bovis* tika diagnosticētas dažiem teļiem jau otrajā dienā pēc piedzimšanas. Citu autoru publikācijās minēts, ka *C. parvum* biežāk diagnosticē 5 līdz 12 dienu veciem teļiem, bet *C. bovis* – 10 līdz 12 dienu veciem teļiem (Faubert and Litvinsky, 2000; Fayer et al., 2005; Silverlås et al., 2009; Wu et al., 2020). Jāatzīmē, ka invāzija ar kriptosporīdijām notiek jau pirmajās stundās pēc dzimšanas. Tāpēc

gadījumos, kad teļa imunitāte ir pazemināta vai tiem netiek izdzirdināts pietiekams pirmpiena daudzums, kriptosporīdijas var parādīties koproloģiskajos paraugos jau otrajā vai trešajā dzīves dienā (Bjorkman et al., 2015; Garro et al., 2016).

Iepriekšējos pētījumos *C. parvum* bieži tika identificēts teļiem pirmsatšķiršanas periodā, savukārt *C. bovis* un *C. ryanae* jau pēcatšķiršanas periodā un *C. andersoni* pieaugušām govīm (Santín et al., 2008). Tā kā teļu atšķiršanas perioda ilgums dažādās Latvijas novietnēs var atšķirties atkarībā no govju šķirnes un izmitināto dzīvnieku skaita, nevar viennozīmīgi apgalvot, ka pastāv saistība starp konkrētu sugu sastopamību un teļu atšķiršanas vecumu. Vienīgi *C. andersoni* bija vairāk izplatīta vecākiem dzīvniekiem, kuru vidējais vecums bija 17,5 mēneši. Jaunākais ar *C. andersoni* invadētais teļš bija tikai trīs dienas vecs un izdalīja 400 OSG, kas tomēr liecina par izņēmumiem šīs sugas izteiktajā vecumspecifiskumā.

Šajā pētījumā *C. parvum* bija dominējošā suga, kas tika konstatēta jaunajiem teļiem (13 600 OSG), kā arī deviņus gadus vecām govīm (1400 OSG). Vienā bioloģiskajā novietnē septiņus gadus veca govs, kas invadēta ar *C. parvum,* izdalīja lielu oocistu skaitu (128 800 OSG). Informācija par novietnēm ar augstu kriptosporīdiju intensitāti var noderēt arī humānas medicīnas ārstiem, jo mūsu iegūtie skaitļi ļauj apgalvot, ka Latvijā pastāv augsts zoonozes risks. Tādēļ būtu lietderīgi veikt ūdens un augsnes paraugu analīzi novietņu apkārtnē, lai noteiktu vides kontamināciju ar *C. parvum* oocistām.

Mēģinot noskaidrot, kura no sugām visbiežāk izraisa klīniskas saslimšanas pazīmes, t.i., diareju, tika analizēta katras sugas saistība ar diareju dažādās vecuma grupās. Rezultāti skaidri parādīja, ka *C. parvum*, lai gan izplatīta visās vecuma grupās, klīnisku diareju visbiežāk izraisa tieši jaunākajiem teļiem (līdz trīs mēnešu vecumam). 70 % teļu šajā grupā, kuriem tika atrasts *C. parvum*, bija arī diareja (3.2. tabula). Pēc literatūras datiem *C. parvum* var izraisīt spēcīgu saslimšanu teļiem, kas parasti izpaužas kā intensīva diareja (Abeywardena et al., 2015). Uzliesmojumi ar augstu teļu mirstību no *C. parvum* ir aprakstīti arī Igaunijā (Lassen and Talvik, 2009; Niine et al., 2018).

3.2. tabula **Oocistu izolātu ekstensitāte un īpatsvars govīm ar diareju dažādās vecuma grupās atkarībā no *Cryptosporidium* spp. sugas**

Vecuma grupa	*Cryptosporidium* suga	Izolātu skaits / īpatsvars (95 % TI)	Izolātu īpatsvars teļiem ar diareju (95 % TI)
0–3 mēneši	*C. parvums*	30,0 / 52,6 (39,9–65,0)	70,0 (52,1–83,3)
	C. bovis	20,0 / 35,1 (24,0–48,1)	65,0 (43,3–81,9)
	C. andersoni	5,0 / 8,8 (3,8–18,9)	40,0 (11,8–76,9)
	C. ryanae	2,0 / 3,5 (1,0–11,9)	0,0 (0,0–65,8)
	Kopā	57,0 / 45,6 (37,1–54,3)	63,2 (50,2–74,5)
4–24 mēneši	*C. parvums*	14,0 / 38,9 (24,8–55,1)	0,0 (0,0–21,5)
	C. bovis	8,0 / 22,9 (12,1–39,0)	25,0 (7,2–59,1)
	C. andersoni	8,0 / 22,9 (12,1–39,0)	25,0 (7,2–59,1)
	C. ryanae	5,0 / 14,3 (6,3–29,4)	40,0 (11,8–76,9)
	Kopā	35,0 / 28,0 (20,9–36,4)	17,1 (8,1–32,7)
> 24 mēneši	*C. parvums*	13,0 / 41,9 (26,4–59,2)	0,0 (0,0–22,8)
	C. bovis	6,0 / 19,4 (9,2–36,3)	16,7 (3,0–56,4)
	C. andersoni	8,0 / 25,8 (13,7–43,3)	12,5 (2,2–47,1)
	C. ryanae	4,0 / 12,9 (5,1–28,9)	0,0 (0,0–49,0)
	Kopā	31,0 / 24,8 (18,1–33,1)	6,5 (1,8–20,7)

Piezīme: Izolātu īpatsvars (%) aprēķināts kā konkrētās sugas gadījumu skaits pret kopējo genotipēto izolātu skaitu attiecīgajā vecuma grupā. Diarejas īpatsvars (%) norāda to dzīvnieku daļu, kuriem novērotas klīniskas diarejas pazīmes konkrētās sugas invāzijas ietvaros attiecīgajā vecuma grupā. TI – 95 % ticamības intervāls.

Vecākiem teļiem *C. parvum* īpatsvars paraugos joprojām ir visaugstākais, salīdzinot ar citām kriptosporīdiju sugām, bet diarejas klīniskas pazīmes netiek konstatētas. Tas var apgrūtināt invāzijas apkarošanu, jo dzīvnieks bez slimības klīniskām pazīmēm tiek uzskatīts par veselu, netiek norobežots no citiem un netiek ārstēts. *C. parvum* gadījumā šī situacija var būt kritiska un potenciāli bīstama arī cilvēkiem.

Cryptosporidium bovis bija izplatīts visās vecuma grupās, un lielākais invadēto teļu īpatsvars tika konstatēts dzīvniekiem līdz trīs mēnešu vecumam, kā arī lielākais ar *C. bovis* invāziju saistītās diarejas īpatsvars tika novērots šajā pašā vecuma grupā.

Cryptosporidium andersoni invāzijas ekstensitāti teļu vecuma grupā līdz trīs mēnešiem bija ļoti zema, kas ir saistīts ar to, ka *C. andersoni* lokalizējas glumenieka dziedzeršūnās. Attiecīgi, kamēr teļiem glumenieka dziedzeri vēl nav pietiekami attīstīti, šai kriptosporīdiju sugai nevajadzētu izraisīt klīnisko saslimšanu. Interesanti, ka lielāks teļu īpatsvars ar diareju, kur konstatēta arī *C. andersoni* invāzija, tika novērots šajā pašā teļu grupā. Līdzīgi *C. ryanae* izraisa diareju tikai vecuma grupā no 4 līdz 24 mēnešiem. Tas ir skaidrojams ar to, ka šajos gadījumos tās nebija *C. andersoni* vai *C. ryanae* monoinvāzija, bet jaukta invāzija ar *C. parvum* un *C. bovis*. Ir zināmi gadījumi, kad *C. andersoni* klātbūtni konstatēja iepriekš atšķirtiem teļiem, kaut gan šī suga vienmēr ir vairāk saistīta ar pēcatšķiršanas perioda jaulopiem vai pieaugušām govīm (Silverlås et al., 2010; Huetink et al., 2001; Enemark et al., 2002).

Salīdzinot *C. andersoni* un *C. ryanae* invāzijas ekstensitāti, kopējā liellopu populācijā tās tika diagnosticētas reti. Lielākā daļa *C. ryanae* izraisīto invāziju noritēja asimptomātiski, kas atbilst arī Faiera ar līdzautoriem (Fayer et al., 2008) novērojumiem.

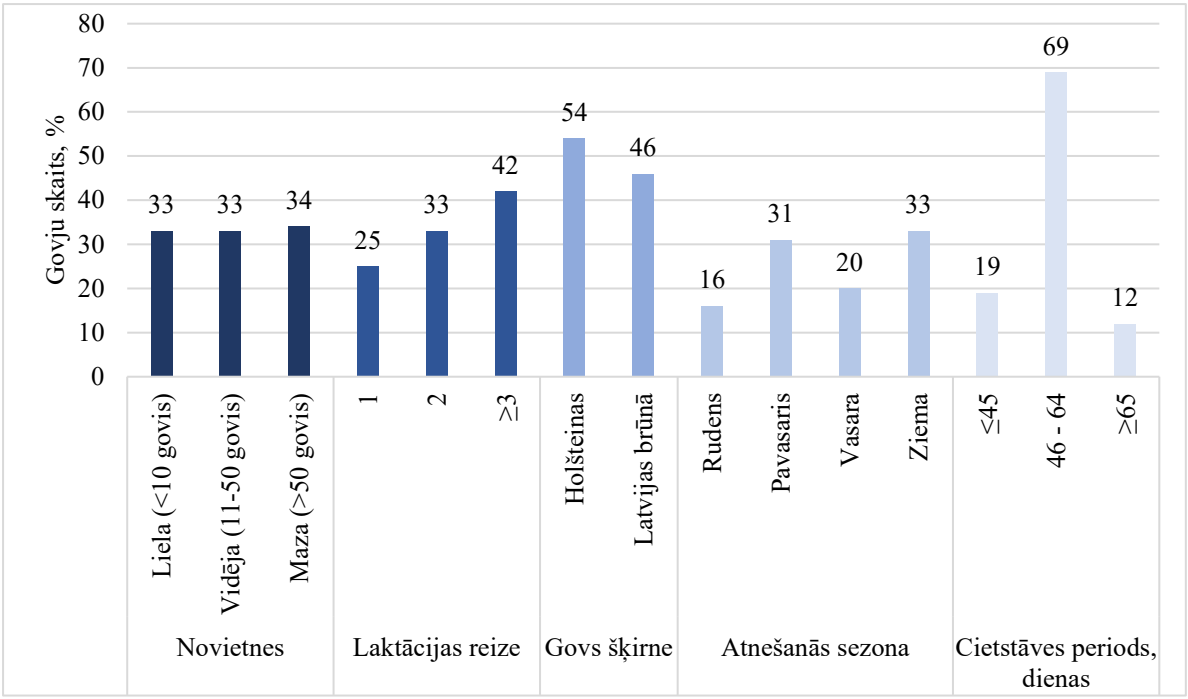
3.4. Govs laktācijas reizes un novietnes lieluma ietekme uz *Cryptosporidium* spp. invāziju teļiem (IV publikācija)

Tā kā tika konstatēti statistiski nesaistoši rezultāti pētījumā par teļu asins seruma IgG līmeņa ietekmi uz kriptosporidiozes izplatību, tika izvirzīts trešais uzdevums: pārbaudīt citus faktorus, kas varētu ietekmēt teļu invadēšanos ar *Cryptosporidium* spp.. Bija būtiski izveidot praktiski pielietojamu zinātnisko pamatojumu kriptosporidiozes riska mazināšanai novietnēs. Tāpēc promocijas darba ietvaros tika vērtēti šādi riska faktori: govs laktācijas reize, novietnes lielums, cietstāvēšanas perioda ilgums, atnešanās sezona un govs šķirne. Šo faktoru ietekme tika pētīta, analizējot kopumā 153 koproloģiskos paraugus, kas vākti no teļiem 17 dažādās piena govju novietnēs Latvijā. Papildus paraugu vākšanai tika veikta arī saimniecību īpašnieku vai darbinieku aptauja. Galvenā datu apstrādes metode bija regresijas analīze, kas ļāva atlasīt būtiskākos faktorus, kuri ietekmē *Cryptosporidium* spp. invāzijas iespējamību teļiem.

Neatkarīgo mainīgo (faktoru) kategoriju proporcija, kas raksturo izlases datus ir atspoguļota 3.3. attēlā.

Izmeklējot koproloģiskos paraugus tika konstatēts, ka 26,1 % teļu tika konstatēta *Cryptosporidium* spp.. Diarejas klīniskās pazīmes tika reģistrētas arī 15 % *Cryptosporidium* spp. pozitīvām govīm. *Cryptosporidium* spp. invāzijas ekstensitāte (26,1 %) bija nedaudz augstāka nekā līdzīgā pētījumā Igaunijā (23 %) (Santoro et al., 2019). Kopumā invāzijas ekstensitāte stipri neatšķiras no tā, kas tika konstatēts citos promocijas darba pētījumos (**I, III**, un **V**).

Lai noskaidrotu, kuri no aptaujā iekļautajiem faktoriem ir visciešāk saistīti ar teļu invadēšanos, tika izmantota ģeneralizētā lineārā jauktā modelēšana (GLMM), kur novietnes identifikācijas numurs tika iekļauts kā nejaušais efekts, lai ņemtu vērā iespējamās atšķirības starp novietnēm. Analīze atklāja statistiski nozīmīgu divu faktoru ietekmi: govs laktācijas reizi ($X^2(2) = 15,83$, $p < 0,001$) un novietnes lielumu ($X^2(2) = 8,68$, $p = 0,013$). Citi faktori, piemēram, govs šķirne, atnešanās sezona un cietstāves perioda ilgums, šajā modelī neuzrādīja statistiski nozīmīgu saistību ar *Cryptosporidium* spp. invāzijas iespējamību teļiem.



3.3. att. **Govju procentuālais sadalījums dažādās izlases grupās mātes bioloģisko un vides faktoru ietekmes novērtēšanai**

Govju skaits procentos ir izrēķināts no kopēja pētījumā izmeklēto dzīvnieku skaita, n=153

Iedziļinoties laktācijas reizes ietekmē, regresijas modelī par references grupu tika izvēlēta pirmā laktācijas reize. Salīdzinājums parādīja, ka teļiem, kas uzņēma otrās laktācijas pirmpienu, bija būtiski mazāka iespēja invadēties ar *Cryptosporidium* spp. (B = –1,723, z = –3,073, p = 0,002, OR = 0,18). Tas nozīmē, ka risks samazinājās vidēji par 82 % (jeb 0,18 reizes; 95 % TI 0,05–0,54), salīdzinot ar teļiem no pirmās laktācijas govīm. Vēl izteiktāks aizsargājošais efekts tika novērots teļiem, kas uzņēma trešās vai vēlākas laktācijas pirmpienu (B = –2,181, z = –3,71, p < 0,001, OR = 0,11). Šajā gadījumā invadēšanās risks samazinājās vidēji par 89 % (jeb 0,11 reizes; 95 % TI 0,03–0,36), salīdzinot ar references grupu. Šī novērotā laktācijas reizes ietekme, visticamāk, ir saistīta ar izmaiņām pirmpiena kvalitātē: govs ar katru gadu vairāk saskaras ar novietnē esošiem patogēniem, tās imūnsistēma izstrādā vairāk antivielu, kas nokļūst pirmpienā. Piemēram, Morila ar līdzautoriem (Morill et al., 2012) atklāja, ka ar katru nākamo laktāciju palielinās IgG koncentrācija un samazinās somatisko šūnu skaits. Kaut gan šajā pētījumā par IgG tika atklāts, ka tā līmenis neietekmē kriptosporīdiju invāziju, šis jautājums netika pētīts no govs laktācijas reizes konteksta. Gulliksena ar līdzautoriem (Gulliksen et al., 2008) norāda, ka vecākas govis ražo pirmpienu ar augstāku antivielu koncentrāciju nekā jaunākas, jo vecākās govis tiek pakļautas antigēnu iedarbībai ilgāk nekā jaunākās (Quigley et al., 1994; Tyler et al., 1999).

Pirmpiens ir būtisks minerālvielu (Ca, P, Mg, Na, Fe, Zn, Cu un Mn) avots jaundzimušajiem teļiem. To koncentrācija pirmpienā ir ievērojami augstāka pirmajās stundās pēc atnešanās, kā arī būtiski atšķiras starp pirmreizēji un vairākkārt atnesušos govju pirmpienu (Kume and Tanabe, 1993). Laktācijas reize ietekmē jaundzimušo teļu minerālvielu stāvokli. Kume un Tanabe (Kume and Tanabe, 1993) pierādīja, ka jaundzimušo teļu hematokrīts un hemoglobīns palielinājās ar katru laktācijas reizi. Laktācijas reize negatīvi korelē ar govs grūsnības periodu (katra nākamā grūsnība ir nedaudz īsāka) un pozitīvi korelē ar piena ražošanas apjomu un teļa dzimšanas svaru (Hoka, Gicheru and Otieno, 2019).

Promocijas darba regresijas modelis parādīja, ka atnešanās mazā novietnē statistiski nozīmīgi samazina teļu iespēju invadēties ar *Cryptosporidium* spp. (B = –1,624, z = –2,843, p = 0,004, OR = 0,20). Govju atnešanās mazās novietnēs samazina teļu iespēju invadēties ar

Cryptosporidium spp. vidēji 0,20 reizes (jeb par 80 %) (95 % TI 0,06–0,60) salīdzinājumā ar lielajām novietnēm. Savukārt **v**idēja lieluma novietņu grupā netika konstatēta statistiski nozīmīga ietekme uz teļu iespēju invadēties ar *Cryptosporidium* spp..

Pirmpiena kvalitāte dažādām govju šķirnēm var atšķirties (Tsuji et al., 1990; Kessler, Bruckmaier and Gross, 2020). Tomēr nav pierādījumu, ka govs šķirne var ietekmēt invāziju ar kriptosporīdijām. Arī šī pētījuma rezultāti neapstiprināja saistību starp kriptosporīdiju invāziju un govs šķirni. Iespējams, ka starp daudzām govju šķirnēm nav acīmredzamas atšķirības aizsardzības spējā pret patogēniem (Murphy et al., 2005).

Apkopojot iegūtos rezultātus, iespējams secināt, ka novietnes lielums un govs laktācijas reize netieši ietekmē piena kvalitāti. Lai gan novietnes lielums nevar tieši ietekmēt pirmpiena vai pārejas piena kvalitāti, pastāv daudzi netieši faktori, kas atšķir lielas un mazas novietnes, piemēram, darba organizācija.

Lielās novietnes, darbojas kā uzņēmumi, kur nodarbināti profesionāļi, lai uzturētu dzīvniekus optimālos apstākļos un lai iegūtu maksimālus produkcijas rezultātus. Lielajās novietnēs retāk tiek nodrošināta individuāla pieeja un govs veselības stāvokļa kontrole. Savukārt mazās novietnes pieder ģimenēm, un tās izmanto dažādu speciālistu pakalpojumus, piemēram, veterinārārstu palīdzību kā ārpakalpojumu. Tādēļ dzīvniekiem no dažāda izmēra novietnēm tiek sniegta atšķirīga aprūpe. Lielajās novietnēs govīm biežāk ir noteikts obligāts cietstāves periods, kamēr mazās novietnēs šo periodu var pielāgot individuāli, ņemot vērā govs veselību, uzvedību un citus faktorus. Arī no vides parazitārā piesārņojuma viedokļa situācija lielajās, vidējās un mazajās novietnēs var atšķirties. Mazajās novietnēs ir vieglāk uzturēt tīru vidi ar zemāku vides parazitāro kontamināciju.

Mazajās novietnēs teļi parasti dzimst ziemas un pavasara sezonā, turpretī lielajās saimniecībās tie dzimst visu gadu. Šī pētījuma rezultāti liecina, ka lielajās novietnēs teļiem ir lielāka iespēja invadēties ar kriptosporīdijām salīdzinājumā ar mazajām novietnēm. Tas skaidrojams ar faktu, ka lielajās novietnēs dzīvnieki tiek turēti augstā blīvumā, kas būtiski palielina patogēnu transmisijas varbūtību un kontaktu ar jauniem saimniekiem. Pētot kriptosporīdiju sugu dažādību tika konstatēts, ka tieši teļi visintensīvāk izdala kriptosporīdiju oocistas apkārtējā vidē. Attiecīgi, ja mazajās saimniecībās teļi dzimst tikai noteiktajā sezonā, tad arī vides piesārņojums būs zemāks salīdzinot ar vidējām un lielajām saimniecībām, kur teļi dzimst visu gadu. Mennerats ar līdzautoriem (Mennerats et al., 2010) ir detalizēti aprakstījuši, kā intensīvā lauksaimniecība var ietekmēt parazītu izplatību.

Sezonālām atšķirībām ir svarīga loma invāziju pārnešanā, piemēram, augsta āra temperatūra un liels nokrišņu daudzums ir saistīts ar kriptosporīdiju invāzijas risku (Jagai et al., 2009). Tomēr šī pētījuma modelī sezonalitātes statistiski nozīmīga ietekme uz teļu invadēšanās iespējamību netika konstatēta. Tas liek domāt, ka galvenais faktors, kas ietekmē kriptosporīdiju invāzijas izplatību teļiem, ir dzīvnieku turēšanas apstākļi novietnēs. Visdrīzāk teļi var invadēties ar parazītiem ne tikai ganībās, kur laika apstākļi sezonāli mainās, bet arī citās vietās, kur teļi tiek turēti ilgākā laika periodā.

Jāatzīmē, ka oocistas ir kriptosporīdiju vides formas ar augstu rezistenci, kas nodrošina to izdzīvošanu nelabvēlīgos eksogēnos apstākļos. Tas norāda, ka parazīti spēj izplatīties un invadēt teļus neatkarīgi no tā, vai ir vasara vai ziema (Robertson, Campbell and Smith, 1992).

Literatūrā ir plaši pētīta cietstāves perioda ilguma ietekme uz piena un pirmpiena ražošanas apjomu, IgG koncentrāciju pirmpienā, mastīta risku, govs pēcdzemdību vielmaiņu un govs enerģijas bilanci (Rastani et al., 2005; Annen et al., 2004; Bertics et al., 1992; Collier, Annen-Dawson and Pezeshki, 2012; Watters et al., 2008). Savukārt, nav gūti viennozīmīgi pierādījum par cietstāves perioda ilguma ietekmi uz teļu veselības stāvokli (Andrée et al., 2018), lai gan govīm ar īsu cietstāves periodu pirmpienam ir zemāka IgG koncentrācija (Rastani et al., 2005). Šajā pētījumā netika atrasta saistība starp cietstāves perioda ilgumu un kriptosporīdiju invāziju. Tomēr nevar izslēgt to, ka pētījuma rezultātu ietekmēja tas, ka lielākajai daļai govju — 69 % gadījumu — bija tieši astoņu nedēļu cietstāves periods. Latvijas piena govju novietnēs praktiski netiek piekāptas variācijas ar cietstāves perioda saīsināšanu vai pagarināšanu.

3.5. *Cryptosporidium* spp. invāzijas varbūtība teļiem ar dažādiem ēdināšanas režīmiem (V publikācija)

Lai gūtu dziļāku izpratni par faktoriem, kas ietekmē *Cryptosporidium* spp. invāziju teļiem, tika izvirzīts ceturtais uzdevums un veikts pētījums, kurā īpaša uzmanība pievērsta dažādu ēdināšanas režīmu lomai. Šī pētījuma mērķis bija noskaidrot, vai un kā konkrētas ēdināšanas stratēģijas, īpaši pirmpiena un pārejas piena izmantošana, korelē ar kriptosporidiozes risku teļiem. Lai to paveiktu, teļi tika iedalīti trīs grupās, katrai piemērojot atšķirīgu ēdināšanas režīmu:

- pētījuma grupa A (reference): teļi saņēma pirmpienu savlaicīgi un pietiekamā daudzumā, un pēc tam turpināja saņemt pārejas pienu vismaz divas nedēļas. Šī grupa kalpoja kā kontroles jeb references grupa regresijas modelī, pret kuru tika salīdzināti pārējie režīmi;
- pētījuma grupa B: teļi saņēma pirmpienu laikus un pietiekamā apjomā, pēc tam pārgāja uz parasto pienu (pilnpienu vai piena aizvietotāju), nevis pārejas pienu;
- pētījuma grupa C: teļi, par kuriem nebija precīzi zināms, vai tie saņēma pirmpienu laikus un pietiekamā daudzumā. Pēc potenciālās pirmpiena saņemšanas šie teļi turpināja saņemt parasto pienu.

Analizējot kopumā 425 teļu koproloģiskos paraugus, tika konstatēts, ka vidēji 35,3 % teļu tika reģistrēta pozitīva *Cryptosporidium* spp. klātbūtne. Diarejas klīniskās pazīmes tika konstatētas 20,6 % invadēto teļu. *Cryptosporidium* spp. pozitīvo un negatīvo teļu procentuālais sadalījums, kā arī teļu ar diarejas (tostarp bez kriptosporīdiju invāzijas) klīniskām pazīmēm procentuālais sadalījums katrā pētījuma grupā apkopots 3.4. attēlā.

Iegūtie rezultāti par kriptosporīdiju oocistu izdalīšanu (vidējā ekstensitāte - 35,3 %; 95 % TI: 26,1–44,1 %) ir tādi paši kā no Igaunijas ziņotie rezultāti – 30 % (Lassen et al., 2009) un 23 % (Santoro et al., 2019), tomēr tie ievērojami atšķiras, salīdzinot ar Lietuvu – 67 % (Lassen and Järvis, 2009). Tas skaidrojams, galvenokārt, ar nelielo izlases apjomu pētījumā (7 novietnes, 15 koproloģiskie paraugi no katras).

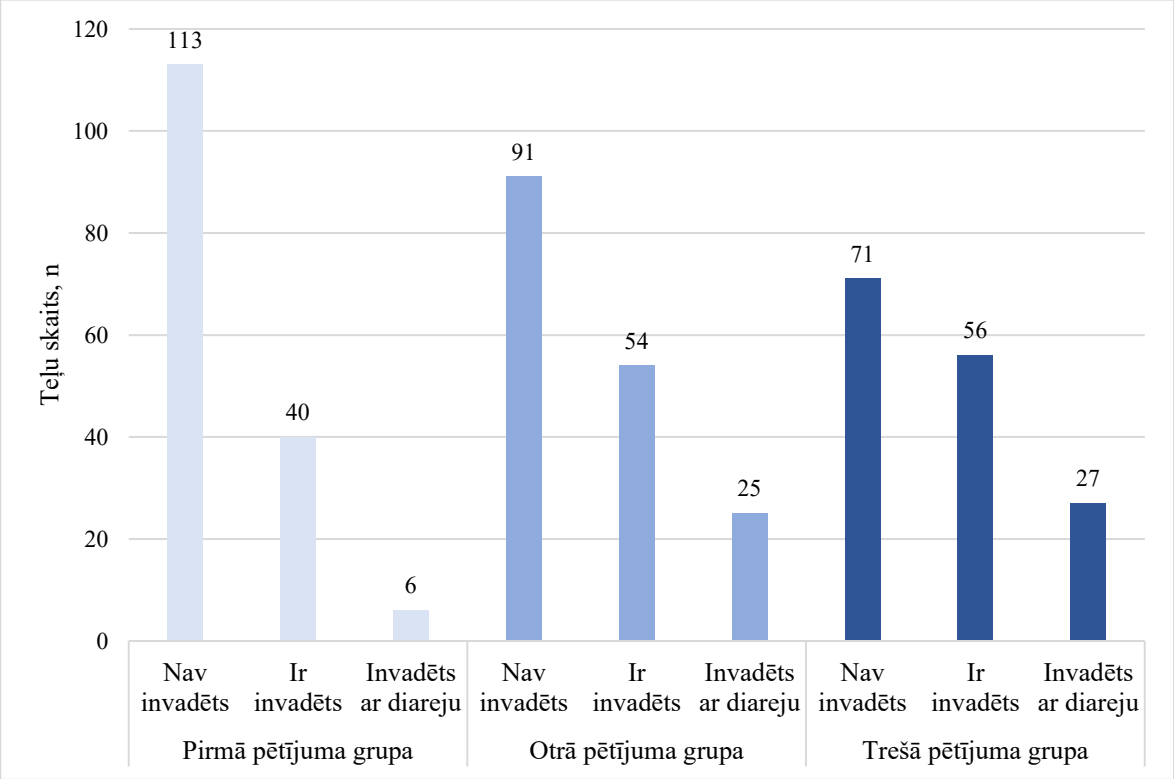
Regresijas modelis bija nozīmīgs ($X^2(2) = 8,62$, $p = 0,0013$), apliecinot, ka dažādi ēdināšanas režīmi ir statistiski nozīmīgi saistīti ar *Cryptosporidium* spp. invāzijas iespējamību teļiem. Pētījuma rezultāti parādīja, ka pirmpiena un pārejas piena izēdināšanas shēmas ir saistīti ar kriptosporīdiju invāziju. Tas norāda, ka gan pirmpienam, gan pārejas pienam ir nozīmīga loma patogēnu invāzijas kontrolē. Visefektīvākā kombinācija pret parazītu invāziju ir savlaicīga atbilstoša pirmpiena daudzuma uzņemšana, kam seko pārejas perioda piena patēriņš vismaz divas nedēļas pirms teļu atšķiršanas.

Regresijas modelis parādīja, ka otrās pētnieciskās grupas ēdināšanas režīms statistiski nozīmīgi prognozēja teļu invadēšanās iespēju ar *Cryptosporidium* spp. ($B = 0,643$, $z = 2,00$, $p = 0,046$, $OR = 1,901$). Tas norāda, ka teļiem, kas pieder pie otrās pētnieciskās grupas, palielinās iespēja invadēties ar *Cryptosporidium* spp. vidēji 1,90 reizes (95 % TI 1,012–3,574), salīdzinājumā ar pirmās pētnieciskās grupas teļiem.

Līdzīgi arī trešās pētnieciskās grupas ēdināšanas režīms statistiski nozīmīgi prognozēja teļu invadēšanās iespējamību ar *Cryptosporidium* spp. ($B = 0,901$, $z = 2,93$, $p = 0,003$, $OR = 2,469$). Tas nozīmē, ka teļiem, kas pieder pie trešās pētnieciskās grupas, palielinās iespēja invadēties ar *Cryptosporidium* spp. vidēji par 2,47 reizēm (95 % TI 1,35–4,52), salīdzinot ar pirmās pētnieciskās grupas teļiem.

Pirmpiena aizsargājošās īpašības tika atkārtoti pētītas un galvenokārt izskaidrotas ar imūnglobulīnu (piemēram, IgG) pārnešanu no govs uz teļu, uzņemot pirmpienu (Robbers et al., 2021). Šī promocijas darba iepriekšējos posmos nav parādījuši saistību starp IgG līmeni govīm pirmpienā un kriptosporīdiju invāziju teļiem; iespējams, iedzimtajai un adaptīvajai imunitātei ir lielāka nozīme imūnreakcijā pret *Cryptosporidium* sugām nekā mātes pasīvās imunitātes pārnešanai uz pēcnācējiem.

Šī pētījuma rezultāti liecina, ka savlaicīgai un atbilstošai pirmpiena daudzuma uzņemšanai tomēr ir nozīmīga loma teļu aizsardzībā pret kriptosporīdiju invāziju, kas norāda, ka nevajadzētu par zemu novērtēt citu pirmpiena bioaktīvo faktoru (augšanas faktoru, hormonu, citokīnu, enzīmu, poliamīnu, nukleotīdu, pretbakteriālo komponentu u.c.) lomu. Imūnglobulīni kopā ar šīm bioaktīvajām vielām veido kompleksu sistēmu, kurā vairāki elementi savstarpēji mijiedarbojas, veidojot vienu universālu barjeru pret patogēniem (Thomson et al., 2017). Turklāt šī pētījuma rezultāti (palielināts diarejas procents) netieši apliecināja nepieciešamību stingri uzraudzīt pirmpiena izēdināšanas laiku, nodrošinot tā uzņemšanu tūlīt pēc piedzimšanas, kas būtiski samazina patogēna kolonizācijas un attīstības varbūtību saimnieka organismā. Teļiem, kas tika ēdināti tūlīt pēc piedzimšana (0 h), bija lielāka IgG koncentrācija serumā, salīdzinot ar teļiem, kas baroti sešas un 12 stundas pēc dzimšanas, kā arī tas ietekmēja teļa zarnu mikrobioma veidošanos (Fischer et al., 2018).



3.4. att. ***Cryptosporidium*** **spp. invāzijas un diarejas sastopamība teļiem atkarībā no pirmpiena un pārejas piena izēdināšanas režīma**

Pirmā pētījuma grupa: pirmajā dzīves stundās teļi saņem adekvātu pirmpiena daudzumu un 2 nedēļas paliek ar govi. Otra pētījumu gruma: pirmajā dzīves stundās teļi saņem adekvātu pirmpiena daudzumu, tiek atdalīti no govs un saņem pienu no kopējas piena uzglabāšanas tvertnes. Trešā pētījumu grupa: teļi piedzima naktī, tiek atdalīti no govs un saņem pienu no kopējas piena uzglabāšanas tvertnes.

Pētījuma rezultāti parādīja, ka teļiem, kas baroti ar pārejas pienu vismaz divas nedēļas pēc pirmpiena izēdināšanas, ir ievērojami mazāka iespēja klīniski saslimt ar kriptosporidiozi un diareju, salīdzinot ar teļiem, kuri pēc pirmpiena saņemšanas uzreiz pārgāja uz parasto pienu (pilnpienu vai piena aizvietotāju). Šos rezultātus apstiprina Konīlijas ar līdzautoriem (Conneeley et al.,2014) un Kargars ar līdzautoriem (Kargar et al., 2021) pētījumi, kuros analizēta pārejas piena izēdināšanas ietekme uz teļu veselību. Kargars ar līdzautoriem (Kargar et al., 2021) pierādīja, ka pārejas piena izēdināšanas ilguma pagarināšana pozitīvi ietekmē teļu dzīvmasas pieaugumu un samazina nespecifiskas diarejas (idiopātiskas šķidras fekālijas) attīstības iespējamību. Tas skaidrojams ar augstāku specifisku bioaktīvo faktoru koncentrāciju salīdzinājumā ar pilnpienu (McGrath et al., 2016; Fischer et al., 2018).

Pārejas piena uzņemšanas perioda saīsināšana vai izlaišana ražošanas praksē bieži sakrīt ar agrīno menedžmenta pāreju, kuras laikā teļš tiek pakļauts kompleksiem stresa faktoriem. Teļš, kas tiek turēts kopā ar māti, saņem pienu *ad libitum* (pēc vajadzības), turpretī ierobežotas ēdināšanas apstākļos dzīvnieks tiek pakļauts stingram ēdināšanas režīmam, kas var pilnībā neatbilst tā individuālajām fizioloģiskajām vajadzībām. Tas var negatīvi ietekmēt kuņģa-zarnu trakta attīstību, kas kalpo par pirmo barjeru infekcijām (Meale et al., 2017). Otrs stresa faktors ir dzīvnieka pārvietošana uz atsevišķu turēšanas vietu, kur tas tiek izmitināts ierobežotā platībā kopā ar citiem dažāda vecuma teļiem (kuriem var būt atšķirīgs veselības stāvoklis un latentas infekcijas), kamēr tā imūnsistēma vēl nav pilnībā nobriedusi. Turklāt atšķiršanas procesa laikā novērotās izmaiņas zarnu mikrobiotā (Li et al., 2012) var palielināt noslieci uz diarejas attīstību.

3.6. *Cryptosporidium* spp. invāzijas ekstensitāte % un intensitāte Latvijā (VI publikācija)

Lai iegūtu vispusīgu raksturojumu par *Cryptosporidium* spp. izplatību Latvijas govju populācijā, promocijas darba ietvaros tika izvirzīts noslēdzošais uzdevums un veikta plaša epidemioloģiskā analīze, īpašu uzmanību pievēršot tam, kā invāzijas rādītājus ietekmē dzīvnieku vecums un novietnes lielums. Šī pieeja ļauj ne tikai konstatēt vispārējo invāzijas līmeni, bet arī identificēt riska grupas un faktorus, kam ir būtiska nozīme parazīta izplatībā.

Lai izpētītu kriptosporīdiju izplatību saistībā ar govju vecumu, tika veikta koproloģiskā analīze dzīvniekiem trīs vecuma grupās: teļiem līdz trīs mēnešu vecumam, teļiem un jaunlopiem no četriem līdz 24 mēnešiem, kā arī pieaugušām govīm, kas vecākas par 24 mēnešiem. Pētījumā, kas aptver laika posmu no 2013. līdz 2022. gadam, tika iekļauti 2655 dzīvnieki, analizējot visus promocijas darba izstrādes gaitā iegūtos koproloģiskos paraugus no dažādām Latvijas saimniecībām, nodrošinot reprezentatīvu izlasi.

Paraugi tika vākti individuāli un analizēti laboratorijā, izmantojot Cīla-Nīlsena (*Zieht-Neelsen*) krāsošanas metodi — standartizētu parazitoloģisko tehniku, kas ļauj precīzi noteikt kriptosporīdiju oocistu klātbūtni un kvantitatīvi novērtēt invāzijas intensitāti. Šāda pieeja garantēja datu ticamību un salīdzināmību ar citiem pētījumiem.

3.3. tabula **Kriptosporīdiju invāzijas ekstensitāte (%) Latvijā un pasaulē**

Valsts	Gads	Dzīvnieks	Ekstensitāte, %	Atsauce
Japāna	2018–2019	Teļi	83,8	Kabir et al., 2020
Lietuva	2009	Govis	67,0	Lassen et al., 2009
Čīle	2007–2008	Piena teļi	56,1	Díaz-Lee et al., 2011
Kalifornija, ASV	2012	Piena teļi	56,0	Li et al., 2019
Taizeme	2016–2017	Piena teļi	51,0	Doungmala et al., 2019
Meksika	2014	Piena teļi	40,0	García-Romo et al., 2014
Itālija	2014	Teļi	38,8	Díaz et al., 2018
Zviedrija	2012–2013	Teļi	38,7	Aberg et al., 2019
Ķīna	2020	Piena teļi	38,4	Wu et al., 2020
Vācija	1993–1997	Piena teļi	36,0	Joachim et al., 2003
Latvija	2013–2020	Govis	27,0	Zolova et al., 2024
Gana	2009	Teļi	29,0	Squire et al., 2013
Kolumbija	2010–2012	Piena teļi	26,6	Avendaño et al., 2018
Brazīlija	2018–2019	Teļi	25,7	Conceição et al., 2021
Beļģija	2019–2020	Piena teļi	25,7	Pinto et al., 2021
Argentīna	2013–2014	Piena teļi	25,5	Lombardelli et al., 2019
Francija	2019–2020	Piena teļi	24,9	Pinto et al., 2021

Valsts	Gads	Dzīvnieks	Ekstensitāte, %	Atsauce
Igaunija	2013–2015	Teļi	23,0	Santoro et al., 2018
Nīderlande	2019–2020	Piena teļi	20,8	Pinto et al., 2021
Koreja	2019–2020	Piena teļi	18,7	Jang et al., 2021
Etiopija	2014–2015	Teļi	18,6	Ayele et al., 2018
Spānija	2016–2018	Govis	16,7	Díaz et al., 2021
Alžīrija	2022	Piena teļi	15,7	Dadda et al., 2022
Irāna	2003–2004	Govis	6,3	Azami, 2007

Apkopojot datus no visiem 2655 paraugiem, tika noteikts, ka kopējā *Cryptosporidium* spp. invāzijas ekstensitāte govīm pētījuma periodā (2013–2020) bija 27 % (95 % TI: 26–29 %). Vidējais izdalīto oocistu skaits uz vienu gramu fekāliju (OSG) pozitīvajos paraugos bija 1000 (starpkvartiļu amplitūda: Q1 = 400, Q3 = 3000). Šie rādītāji iekļaujas kopējā Eiropas un pasaules kontekstā, kur invāzijas līmenis dažādās valstīs ievērojami atšķiras (skat. 3.3. tabulu). Piemēram, šajā pētījumā konstatētā 27 % ekstensitāte ir tuva globālajam vidējam rādītājam (25,5 %, Buchanan et al., 2024) un līdzīgiem rādītājiem kaimiņvalstī Igaunijā (23–30 %, Santoro et al., 2018; Lassen et al., 2009), bet zemāka nekā agrākos pētījumos Lietuvā (67 %, Lassen et al., 2009) vai Dānijā (32 %, Maddox-Hyttel et al., 2006).

Analizējot datus pa vecuma grupām, atklājās ļoti izteikta un statistiski nozīmīga ($p < 0,001$) tendence: gan invadēto dzīvnieku īpatsvars, gan ar invāziju saistītās diarejas biežums samazinājās, pieaugot dzīvnieku vecumam (skat. 3.4. tabulu). Visaugstākā riska grupa ir teļi vecumā līdz trīs mēnešiem. Šajā grupā tika konstatēta visaugstākā invāzijas ekstensitāte (39,4 %) un arī vislielākais vidējais izdalīto oocistu skaits (mediāna 800 OSG). Turklāt tieši šajā vecuma grupā vairāk nekā pusei (56,6 %) invadēto teļu tika novērota diareja. Šie rezultāti saskan ar daudzu citu pētījumu rezultātiem pasaulē, kas norāda uz jaunāko teļu (īpaši pirmajās dzīves nedēļās) augsto jutību (uzņēmību) pret kriptosporīdiju invāziju. Piemēram, Aguilar (2023) ziņoja par 8–14 dienu vecu teļu augsto invāziju ar kriptosporīdijām, bet Jūrie ar līdzautoriem (Urie et al., 2018) konstatēja līdzīgu situāciju divu nedēļu veciem teļiem. Garro ar līdzautoriem (Garro et al., 2016) atklāja, ka teļi līdz 20 dienu vecumam biežāk izdala kriptosporīdiju oocistas, savukārt Dungmala ar līdzautoriem (Doungmala et al., 2019) norādīja, ka augstāka invāzijas ekstensitāte ir 1–3 nedēļu veciem teļiem. Ebijo un Haile (Ebiyo and Haile, 2022) aprēķināja, ka teļiem, kas jaunāki par sešiem mēnešiem, ir 2,7 reizes lielāka iespēja invadēties ar kriptosporīdijām.

3.4. tabula ***Cryptosporidium*** **spp. invāzijas ekstensitāte, intensitāte un ar to saistītās diarejas īpatsvars dažādās govju vecuma grupās**

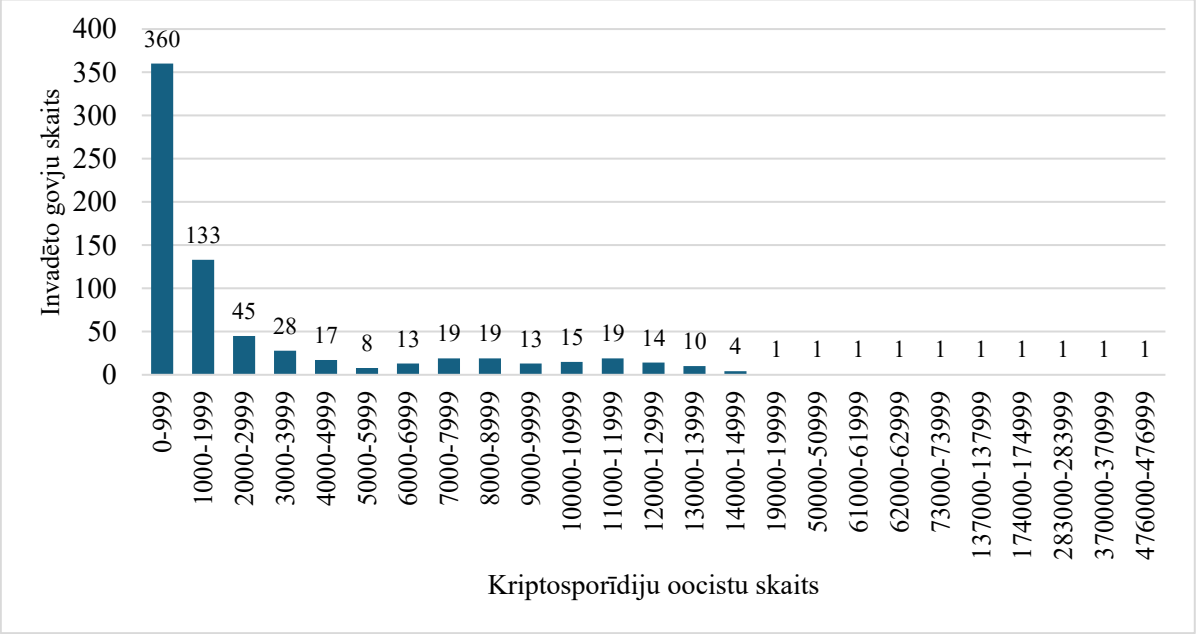
Faktors	Ekstensitāte, % (95 % TI)	Intensitāte (Q1–Q3)	Diareja ar *Cryptosporidium* spp., % (95 % TI)
0–3 mēneši	39,4 (32,6–46,5)	800 (200–2400)	56,6 (44,7–67,9)
4–24 mēneši	20,3 (17,0–23,9)	400 (400–650)	4,2 (0,1–21,1)
> 24 mēneši	19,2 (16,7–21,9)	600 (400–1000)	7,0 (1,9–17,0)
p-vērtība	< 0,001	0,118	< 0,001

Piezīme: Invāzijas rādītāji aprēķināti, pamatojoties uz 2655 dzīvnieku izmeklēšanas rezultātiem. Q1-Q3 – starpkvartiļu amplitūda. TI – 95 % ticamības intervāls.

Šajā pētījumā novērotā saistība starp kriptosporīdiju invāziju un diarejas izpausmēm vēl vairāk uzsver šī parazīta klīnisko nozīmi. Lielāks diarejas biežums invadētiem dzīvniekiem, īpaši jauniem teļiem, atbilst *Cryptosporidium* spp. patogēnajam potenciālam – izraisīt kuņģa-zarnu trakta darbības traucējumus (Fayer et al., 2000). Velss ar līdzautoriem (Wells et al., 2015) ziņoja par augstu kriptosporīdiju izplatību govīm atnešanās sezonā, kas ļauj secināt, ka

atnešanās laiks būtiski ietekmē invāzijas transmisijas dinamiku un līdz ar to arī diarejas sastopamības biežumu: periodā, kad ganāmpulkā palielinās jaundzimušo teļu skaits, palielinās arī izdalīto *Cryptosporidium* spp. oocistu skaits. Tomsons ar līdzautoriem (*Thomson* et al., 2017) ziņoja, ka *Cryptosporidium* spp. bija visbiežāk konstatētais patogēns, kas izraisa diareju teļiem vecumā līdz vienam mēnesim, uzsverot tā ietekmi uz dzīvnieku veselību. Savukārt, Berhanu ar līdzautoriem (Berhanu et al., 2022) atzīmēja, ka pieaugušas govis kalpo kā invāzijas rezervuārs, kas izdala oocistas apkārtējā vidē un pakļauj teļus invāzijas riskam.

Aplūkojot izdalīto oocistu skaitu (invāzijas intensitāti), tika konstatēts ļoti plašs diapazons – no minimālā nosakāmā daudzuma (200 OSG) līdz pat 476 500 OSG. Datu sadalījums atbilda negatīvam binomiālajam sadalījumam (3.5. attēls), kas ir raksturīgs parazītu invāzijām. Tas nozīmē, ka lielākajai daļai invadēto dzīvnieku oocistu skaits bija salīdzinoši neliels (visbiežāk 200–2000 OSG), turpretī neliela daļa dzīvnieku izdalīja ļoti lielu oocistu skaitu (vairāk nekā 15 000 OSG). Šāda pārmērīga dispersija (pārdkliede; *overdispersion*) norāda, ka invāzijas intensitāte nav nejauša, un ka pastāv indivīdi, kas nesamērīgi daudz veicina vides kontamināciju ar oocistām (Ieshko et al., 2024; Gourbière et al., 2015). Šis fakts ir ļoti būtisks kontroles pasākumu plānošanā, jo norāda uz nepieciešamību identificēt un mērķtiecīgi ierobežot invāziju tieši šajos augsta riska dzīvniekos. Teļam pietiek uzņemt tikai 17 *Cryptosporidium* spp. oocistas, lai attīstītos kriptosporidiozes invāzija (Zambrinski et al., 2013).



3.5. att. ***Cryptosporidium* spp. invāzijas intensitāte visos pētījuma posmos izmeklētajām govīm**

Kriptosporīdiju invāzijas ekstensitāte un oocistu skaits vienā gramā fekāliju bija nevienmērīgi sadalīti Latvijas plānošanas reģionos, parādot visaugstāko izplatību Vidzemes reģionā (31 %) un visaugstāko oocistu skaitu Kurzemes reģionā (mediāna = 600, Q1 = 300 un Q3 = 1200). Pašlaik nav iespējams viennozīmīgi interpretēt šāda reģionālā sadalījuma cēloņus, tādēļ turpmāk ir nepieciešami padziļināti ietekmējošo faktoru pētījumi. Kriptosporīdiju invāzijas ekstensitātes un intensitātes sadalījums Latvijā pēc plānošanas reģioniem ir apkopots 3.5. tabulā.

3.5. tabula ***Cryptosporidium*** **spp. vidējā invāzijas ekstensitāte % un vidējā invāzijas intensitāte (OSG) dažādos Latvijas reģionos**

Novads	Ekstensitāte (95 % TI)	Intensitāte (OSG) (Q1–Q3)
Rīgas	28	400 (200-800)
Kurzemes	29	600 (300-1200)
Zemgales	18	600 (400-1000)
Vidzemes	31	600 (400-1000)
Latgales	16	600 (325-900)

Izvērtējot datus, novietnes lieluma griezumā (**III** pētījums, skat. 3.6. tabulu), tika atklāts, ka *Cryptosporidium* spp. bija lielākajā daļā (72,4 %) pētīto ganāmpulku (vismaz viens invadēts dzīvnieks). Invāzijas klātbūtnes varbūtība pieauga līdz ar novietnes lielumu: ja mazajās novietnēs (mazāk nekā 50 govju) vismaz viens invadēts dzīvnieks bija 58,9 % gadījumu, tad vidējās (50–200 govju) šis rādītājs sasniedza 94,4 %, bet lielajās (vairāk nekā 200 govju) – pat 100 %. Savukārt kopējā teļu un govju invāzijas ekstensitāte svārstījās no 43,2 % lielajās novietnēs (n = 13, 8–36 paraugi no vienas novietnes) un 30,9 % vidējās novietnēs (n = 18, 5–30 paraugi no vienas novietnes) līdz 30,8 % mazajās novietnēs (n = 56, 1–14 paraugi no vienas novietnes). Statistiski būtiski augstāks *Cryptosporidium* spp. pozitīvo paraugu īpatsvars tika konstatēts lielajās novietnēs ($p < 0,01$).

3.6. tabula ***Cryptosporidium*** **spp. epidemioloģiskie rādītāji dažāda lieluma govju novietnēs**

Faktors	Faktora kategorijas	Izmeklēti / invadēti	Ekstensitāte, (95 % TI)	Diarejas īpatsvars, (95 % TI)
Novietnes izmērs	1–50 govis	302/93	30,8 (25,8–36,2)	14,2 (10,7–18,7)
	50–200 govis	295/91	30,9 (25,8–36,3)	22,7 (18,3–27,8)
	> 200 govis	329/142	43,2 (37,9–48,6)	28,9 (24,2–34,0)

Augstākais vidējais oocistu skaits vienā gramā fekāliju (725 015) tika novērots teļiem vecuma grupā no nulles līdz pieciem mēnešiem. Savukārt otrs augstākais vidējais oocistu skaits vienā gramā fekāliju (115 620) tika konstatēts govīm, kas vecākas par 24 mēnešiem. Naidams ar līdzautoriem (*Nydam* et al., 2001), pētot kriptosporīdiju oocistu skaitu teļiem vecumā no četrām līdz 12 dienām, konstatēja, ka oocistu skaits pieaug līdz 12. dienai, sasniedzot maksimālo vērtību, bet vēlāk samazinās. Pētījumu gaitā atklājās, ka sešas dienas vecs teļš var producēt apmēram $3,89 \times 10^{10}$ oocistas līdz 12 dienu vecumam. Dipons ar līdzautoriem (*DuPont* et al., 1995) aprēķināja, ka vidējā invāzijas deva, kas izraisa klīnisko saslimšanu veselam cilvēkam, kurš iepriekš nav bijis invadēts ar kriptosporīdijām, ir 132 oocistas, turpretī cilvēkiem ar iepriekšēju imunoloģisko saskarsmi vidējā invāzijas deva ir 1880 oocistu (Chappell et al., 1999). Tas pierāda, ka teļi ir nozīmīgs cilvēka invāzijas avots, jo to izdalīto kriptosporīdiju skaits desmitkārt pārsniedz vidējo cilvēku invāzijas devu. Tas uzsver nepieciešamību uzmanīgi sekot higiēnas prasībām, strādājot ar teļiem, lai samazinātu kriptosporīdiju izplatību un novērstu to potenciālo ietekmi uz cilvēka veselību. Turklāt, ņemot vērā, ka kriptosporidioze ir zoonoze, atbilstoša higiēnas prakse un teļu veselības aizsardzība ir ne tikai dzīvnieku labturības aspekts, bet arī svarīgs pasākums cilvēku veselības aizsardzībai, izvairoties no iespējamās invāzijas.

4. SECINĀJUMI

1. Pilotpētījuma rezultāti parādīja, ka *Cryptosporidium* spp. ir dominējošā invāzija Vidzemes piena govju ganāmpulkos. *Cryptosporidium* spp. invāzijas ekstensitāte (32,6 %) norāda uz būtisku risku dzīvnieku veselībai, ka arī uz potenciālu zoonozes izplatības risku **(I** publikācija).
2. Imūnglobulīna G līmenis govīm pirmpienā nav tieši saistīts ar kriptosporīdiju invāzijas izplatību teļiem, kas liecina, ka IgG līmenim nav būtiskas nozīmes kriptosporīdiju invāzijas attīstībā (**II** publikācija).
3. Identificētas četras tipiskas *Cryptosporidium* sugas: *C. parvum*, *C. bovis*, *C. andersoni* un *C. ryanae*. Teļiem līdz 3 mēnešu vecumam, 52,6 % gadījumu diagnosticēta *C. parvum* suga. 70 % gadījumu tā izraisa diareju. Biežāk diagnosticētas monoinvāzijas. Pieaugušām govīm kriptosporīdiju invāzija klīniskās pazīmes neizraisa, kas norāda uz augstu zoonozes izplatības risku Latvijā. (**III** publikācija).
4. Govs laktācija reize tieši ietekmē teļu iespēju invadēties ar *Cryptosporidium* spp.. Teļiem, kuri saņēma otrās laktācijas pirmpienu un pārejas pienu, iespēja invadēties ar kriposporīdijām samazinās par 82 %, bet saņemot trešās laktācijas pirmpienu un pārejas pienu - par 89 % (**IV** publikācija).
5. Savlaicīga (pirmajās 12 stundās) un atbilstoša apjoma (vismaz 6 litru) pirmpiena daudzuma uzņemšana, kurai seko pārejas perioda piena patēriņš vismaz divas nedēļas pirms atšķiršanas no mātes, būtiski samazina *Cryptosporidium* spp. invāziju (**V** publikācija).
6. Kriptosporīdiju invāzijas ekstensitāte govīm Latvijā ir 27 % (95 % TI 26-29 %). Biežāk invadējas teļi līdz 3 mēnešu vecumam (invāzijas ekstensitāte 39,4 %). Maksimālais vidējais izdalīto oocistu skaits sasniedz 800 oocistu uz gramu fekāliju (200–2400 OSG) (**VI** publikācija).

5. PRIEKŠLIKUMI

1. Tā kā Latvijā dominējošā suga teļiem līdz 3 mēnešu vecumam ir zoonotiskā *C. parvum*, kas rada augstu invadēšanās risku cilvēkiem, nepieciešams ieviest stingrākus individuālās higiēnas protokolus (specifisks darba apģērbs, roku dezinfekcija) personālam, kas strādā ar jaundzimušajiem teļiem, neatkarīgi no klīnisko pazīmju esamības vecākiem dzīvniekiem.
2. Saimniecībās, kurās ir augsta invāzijas ekstensitāte, ieteicams pirmās laktācijas govju teļus izdalīt kā paaugstināta riska grupu. Ja iespējams, šiem teļiem prioritāri nodrošināt augstākas kvalitātes (vairākkārt atnesušos govju) pirmpienu vai papildu preventīvos pasākumus, jo teļiem no pirmās laktācijas govīm ir būtiski augstāka iespēja invadēties.
3. Ņemot vērā, ka novietnes lielums virs 50 govīm ir saistīts ar parazīta klātbūtni visos šāda tipa ganāmpulkos (100 % gadījumu) un augstāku ekstensitāti, lielajās saimniecībās ir kritiski jāsamazina dzīvnieku turēšanas blīvums atnešanās zonās un jāveic regulāra, mērķtiecīga vides dezinfekcija, lai pārtrauktu oocistu uzkrāšanos vidē.
4. Tā kā visaugstākā invāzijas ekstensitāte (39,4 %) un intensitāte (800 OSG) ir tieši teļiem līdz 3 mēnešu vecumam, lielajās novietnēs ir lietderīgi ieviest regulāru koproloģisko skrīningu tieši šajā vecuma grupā. Tas ļautu savlaicīgi identificēt masveida oocistu izdalītājus un ierobežot invāzijas izplatību ganāmpulkā un apkārtējā vidē.

TERMS AND ABBREVIATIONS

Terms

Sample – a part of the population selected for practical observation to draw conclusions about the characteristics of the entire population.

Population (General Population) – a set of objects or organisms about which information is intended to be obtained. It can be freely defined at the start of a study and depends solely on the research objective.

Stratified sample – a sampling method in which elements are randomly selected from various subgroups of the studied problem.

Prevalence (Extensity of infection) – the percentage ratio of infected animals to the total number of animals in the study group.

Intensity of infection – the average number of parasites per animal.

Immune-naive – a term referring to the initial or immature state of the immune system, where the immune system has not yet encountered specific infections or antigens and thus has not developed specific immune response mechanisms against them.

Abbreviations used in the doctoral thesis

Abbreviations	Latvian	English
OSG/ OCG	Oocistu skaits vienā gramā fekāliju	Oocyte count per gram
Ig	Imūnglobulīns	Immunoglobulin
PCR	Polimerāzes ķēdes reakcija	Polymerase Chain Reaction
PBS	Fosfātu bufera sāļi	Phosphate-Buffered Saline
FITC	Organiskās fluorescējošās krāsvielas veids	Fluorescein Isothiocyanate
ELISA	Enzīmu saistītā imūnsorbentanalīze	Enzyme-Linked Immunosorbent Assay
GLMM	Ģeneralizētā lineārā jauktā modelēšana	Generalized Linear Mixed Model
AIC	Statistisks kritērijs, ko izmanto modelēšanas kontekstā, lai novērtētu modeļa piemērotību un tā atbilstību empīriskajiem datiem	Alaike Information Criterion
TI/ CI	Ticamības intervāls	Confidence interval

1. INTRODUCTION

1.1. Neonatal calf diarrhea syndrome

Literature data indicate that in approximately 90 % of cases, calf mortality is associated with neonatal calf diarrhea syndrome. Clinical signs of the disease usually manifest in calves at 5–10 days of age. Clinically, the disease can manifest either as mild diarrhea without systemic signs or as acute, severe, profuse diarrhea that is refractory to treatment and causes severe dehydration, electrolyte imbalances, and death within 12 hours. The syndrome is caused by a complex of multiple bacterial and viral infections (*E. coli*, *Salmonella*, rotavirus, coronavirus). Along with the aforementioned infections, the protozoa *Cryptosporidium* spp. is diagnosed in the intestinal tract during the determination of the cause of death. *C. parvum* is cited as the most frequent cause for the development of calf diarrhea syndrome. Cryptosporidia destroy the epithelium covering the intestinal villi, reducing nutrient absorption capacity, and thereby facilitate the entry of bacterial and viral pathogens into the animal organism (Jasmer, 2007; Cho and Yoon, 2014).

Cryptosporidium spp. is the causative agent of severe infection in calves and is one of the most important causes of economic losses in dairy cattle holdings. Farmers may incur additional expenses related to diagnosis and treatment, such as extra costs for calf feed and rearing to reach market weight. Furthermore, the infection can result in calf mortality (Innes et al., 2020).

Cryptosporidiosis is a globally widespread parasitic protozoan infection of animals and humans, which was recognized as a zoonosis in the 1980s (WHO, 2015). Consequently, it is highly important to evaluate the prevalence of this parasitic protozoan within the territory of our country, where livestock farming has been one of the primary agricultural sectors for several centuries. This will not only help improve herd health and thereby the productivity of dairy cows but will also protect humans from contracting cryptosporidiosis.

1.2. Structure and life cycle of *Cryptosporidium* spp.

Cryptosporidia are parasitic protozoa, microscopically small organisms approximately 4–6 microns in size, which localize in the intestinal epithelium of the cow. The internal structure of the protozoan is simple, consisting of a nucleus and organelles. They lack external structures, such as colored membranes or cilia, and can only be identified using specific microscopy techniques and staining. During its development stage, the structure of the cryptosporidium consists of an oocyst containing sporozoites, but lacking sporocysts. Ultrastructural and DNA analyses have demonstrated a high degree of morphological similarity among all *Cryptosporidium* species (Dragomirova, 2022).

Cryptosporidium spp. has a direct life cycle, meaning that development occurs without a change of intermediate hosts. The infection is transmitted from one host to another via mature, infective oocysts. Developed (sporulated) oocysts are shed from the host's organism into the external environment with feces. Oocysts become infective immediately upon entering the external environment. Following the ingestion or inhalation of such oocysts by a suitable host, excystation occurs, releasing sporozoites. These sporozoites invade the epithelial cells of the gastrointestinal tract. Within these cells, the parasites undergo both asexual (schizogony or merogony) and sexual (gametogony) reproduction. During the sexual reproduction process, microgamonts are produced, which divide to form microgametes (male gametes), as well as macrogamonts, which mature into macrogametes (female gametes). After fertilization, oocysts develop and sporulate within the host's intestinal epithelial cells. Cryptosporidia produce so-called large or thick-walled oocysts, which are shed from the gastrointestinal tract into the

external environment, and small or thin-walled oocysts, which can cause autoinfection directly within the gastrointestinal tract (Pinto and Vinayak, 2021).

1.3. *Cryptosporidium* spp. species

Currently, 44 cryptosporidia species and 120 genotypes have been identified. *C. hominis* (*C. parvum* type I) is a human-specific pathogen. *C. parvum* (*C. parvum* type II) is a zoonosis and infects both humans and animals. In cattle, four cryptosporidia species are currently isolated and known: *C. parvum*, *C. andersoni*, *C. bovis*, and *C. ryanae*. *C. andersoni* infects the abomasum of adult cows, while *C. bovis* and *C. ryanae* spread throughout the entire gastrointestinal tract and can also enter the respiratory system via the aerogenic route or by migrating through the bloodstream with phagocytes. *Cryptosporidium parvum* is not a host-specific parasite, which is why it can be transmitted by various animals (for example, rats or cats). Oocysts of this parasite species were found in the feces of 70 % of one- to three-week-old calves (Ryan et al., 2021; Mahdavi et al., 2024). Some authors report that in certain farms, the infection prevalence can reach 100 % (Santin, Trout and Fayer, 2008). The infection can be detected starting from the fifth day of a calf's life, when oocysts begin to be intensely shed from the gastrointestinal tract (Olson, 1999).

Cryptosporidium spp. is considered one of the most common causes of diarrhea in humans, while *C. parvum* and *C. hominis* are the primary causative agents of human cryptosporidiosis. Their prevalence varies greatly across countries (Vanathy et al., 2017). In adult humans with an adequate immunological response, *C. hominis* and *C. parvum* can cause mild, self-limiting diarrhea. In children, elderly people, and immunocompromised individuals (for example, HIV/AIDS patients), *Cryptosporidium* spp. infection causes severe damage to the intestinal epithelium, which in turn leads to nutrient deficiencies that can result in life-threatening conditions (Mak, 2004). People working in cattle holdings are also included in the high-risk group that can contract cryptosporidiosis (Abubakar et al., 2007). This is precisely why it is important to identify the prevalence of the infection in Latvia, to develop recommendations for reducing its spread and, in case of illness, limiting the negative impact of pathogenesis.

1.4. Sources of infection

Mature oocysts shed with feces are considered the source of infection, causing severe contamination of the environment. Cryptosporidia have a high potential for dissemination because they can withstand various environmental conditions and can survive in water and soil for several months (Rahman et al., 2017). It has been experimentally demonstrated that slowly freezing cryptosporidia oocysts to a temperature of -22 °C and maintaining this temperature for 21 hours destroyed 67 % of the total number of oocysts. After 152 hours, 90 % of the total number of oocysts died, but even after 750 hours, a small number of oocysts still remained alive. Conversely, *Cryptosporidium* spp. oocysts are not resistant to desiccation: in experiments where they were dried with a hair dryer at room temperature, all oocysts lost viability after two hours (Robertson, 1992).

Infection can be transmitted directly from calf to calf and from cow to calf, or indirectly through dirty boots, a contaminated environment, water, or feed. Water filtration and the most used disinfection methods are unable to destroy cryptosporidia oocysts, which is why drinking water is considered the primary source of infection (Garber, 1994) (Figure 1.1).

Protozoans of the genus *Cryptosporidium* are found worldwide, including in Latvia. They infect both humans and many animal species: livestock, domestic pets, or wildlife. Cryptosporidia cause significant health disorders specifically in neonatal calves.

Cryptosporidiosis as an infection and its epidemiology are at the center of attention in parasitological research across many countries. There is a relatively large number of studies on the prevalence of *Cryptosporidium* spp. infection in different countries, whereas the factors influencing the probability of calves becoming infected with cryptosporidia have been studied less frequently.

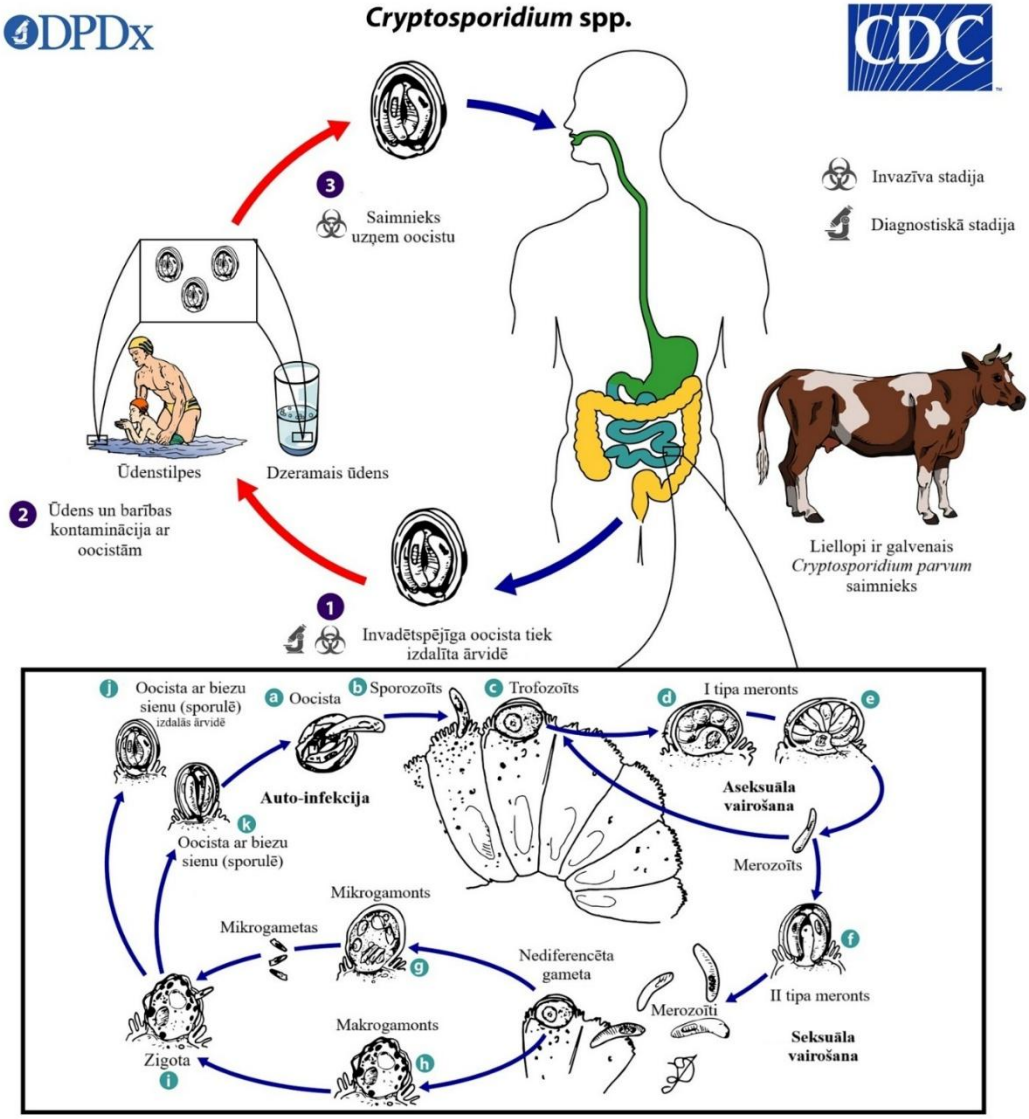


Figure 1.1. **Life cycle of *Cryptosporidium* spp**. (CDC's Division of Parasitic Diseases and Malaria (DPDM))

1.5. Importance of colostrum in neonatal calf health

Colostrum is a complex secretion containing significantly increased amounts of essential chemical compounds (nutrients, growth factors, and immune factors) aimed at activating the immune system, providing nutrients to the newborn, and stimulating the development of the calf's gastrointestinal tract (Elfstrand et al., 2002; McGrath et al., 2016; Puppel et al., 2019). The amount of immunoglobulin in colostrum is up to 100 times higher than in whole milk, while the number of antimicrobial substances (lactoferrin, lactoperoxidase, lysozyme) is two to five times higher (Pakkanen and Aalto, 1997; Stelwagen et al., 2009).

Colostrum is the first source of nutrition for neonatal calves, which not only nourishes the animal but also provides the necessary components for body defence. Calves need to receive

an adequate volume of high-quality colostrum immediately after birth to help prevent the spread of infection within holdings, limit further clinical progression of the disease, and avoid developmental delays in the animal. Colostrum contains essential compounds: immunoglobulins (Ig) – glycoproteins that specifically recognize and bind to antigens on the surface of pathogens. Although there are several classes of Ig, including IgA, IgD, IgE, IgG, and IgM (Kaskous and Fadlelmoula, 2015), most of them are present in low concentrations in colostrum. IgG is particularly significant as it is the primary Ig found in cow colostrum and milk, playing a central role in the development of humoral immunity (McGrath et al., 2016). The concentration of IgG in colostrum can reach up to 50–100 mg/ml, and through passive transfer, they provide effective prevention or treatment for several diseases in humans or animals caused by pathogens. Since IgG can prevent the adhesion of pathogens to intestinal epithelial cells, they act as the primary defence against most potential gastrointestinal pathogens. The adhesion of IgG to *Cryptosporidium* spp. can significantly affect the pathogenesis of the parasite by inhibiting its dissemination and reducing the risk of host cell infection (Ulfman et al., 2018).

Ingestion of high-quality colostrum in sufficient quantities is one of the most critical factors affecting calf health and survival, as it ensures the passive transfer of immunity from the cow to the calf. Since factors other than IgG in colostrum can potentially influence *Cryptosporidium* spp. infection, it seems justified to evaluate the factors affecting colostrum quality and their association with *Cryptosporidium* spp. infection. Furthermore, colostrum quality depends on numerous factors, such as the cow's age (Conneely et al., 2013), breed (Muller and Ellinger, 1981; Morrill et al., 2012), lactation number (Morrill et al., 2012), calendar season of calving (Nardone et al., 1997), and the length of the dry period (Rastani et al., 2005; Annen et al., 2004).

Cows begin to produce colostrum from a week to a few days before calving and continue to produce it for a week after calving. Following colostrum secretion, cows produce transition milk for one to two days, the properties of which are lower than those of colostrum but higher than those of mature milk or whole milk (Quinn et al., 2020; O'Callaghan et al., 2020). Conversely, Kargar et al. (2021) indicate that prolonged feeding of calves with transition milk (for three weeks) improves calf growth and reduces the probability of diarrhea.

One potential control method for cryptosporidiosis is the feeding of colostrum and transition milk. Since calves are born immunologically naive (i.e., their immune system has never been exposed to antigens), they require the support provided by cows through the production of colostrum and transition milk.

In practice, a clear line cannot be drawn as to when colostrum transforms into transition milk and whole milk. It is generally accepted that colostrum is produced during the first three days after calving. This is followed by 5–7 days during which transition milk is produced (McGrath et al., 2016). Based on recent studies regarding the composition and importance of colostrum for neonatal organisms, recommendations were developed concerning the volume of colostrum and the timely feeding of neonatal calves. For example, delaying colostrum feeding by six hours (35.6 ± 1.88 %) and 12 hours (35.1 ± 3.15 %) reduced the maximum apparent efficiency of IgG absorption compared to feeding colostrum immediately after birth (51.8 ± 4.18 %) and delayed the time to peak serum IgG concentration (24 h versus 15 h, respectively). Delayed colostrum feeding tended to reduce the abundance of beneficial bacteria associated with the colonic mucosa, particularly *Bifidobacterium* and *Lactobacillus* species, which play an important role in intestinal health. Therefore, it is highly critical to feed colostrum as soon as possible after the calf's birth (Fischer et al., 2018; Pyo et al., 2018). Studies on the utility of transition milk are relatively scarce, and recommendations for transition milk intake have not been developed. In large dairy holdings, calves are separated from cows and kept individually in calf pens shortly after the first feeding, while the transition milk is placed into a bulk storage tank where it is diluted with milk from other cows.

Treatment of cryptosporidiosis is complex, as no effective vaccine for disease prevention had been available until recently. Available medications frequently focus solely on symptom management, such as treating dehydration (Chalmers and Giles, 2010; Meganck, Hoflack and Opsomer, 2014). Most recently (in 2023), the vaccine Bovilis Cryptium has emerged on the market, which protects calves against the *Cryptosporidium parvum* Gp40 serotype. Cows must be vaccinated with it twice prior to calving, so that the calf receives antibodies against cryptosporidia along with the colostrum. Consequently, the vaccine manufacturers emphasize that the colostrum feeding regimen plays a vitally important role in establishing the calf's immunity (NOAH Compendium, 2023). Combined with effective holding management—namely, the rapid administration of a sufficiently large volume of colostrum to the calf immediately after birth or the selection of the cow calving season can become an alternative tool for the control or prevention of cryptosporidiosis in cattle. Frequent removal of feces from the cowshed and calf pens, as well as the use of disinfectants, hot water, and detergents, can help significantly reduce the number of oocysts within the holding (Robertson, Campbell and Smith, 1992; Harp and Goff, 1998).

1.6. Hypothesis of the Doctoral Thesis

The doctoral thesis puts forward one hypothesis: there is a correlation between the prevalence and intensity of *Cryptosporidium* spp. infection in calves and the calf feeding regimen, as well as a complex of maternal biological and environmental factors.

1.7. Aim of the Doctoral Thesis

The aim of the doctoral thesis is to determine the factors influencing *Cryptosporidium* spp. infection in dairy calves and the species distribution in Latvia.

1.8. Tasks of the Doctoral Thesis

Six tasks have been set for the doctoral thesis:

1. to conduct a pilot study in the Vidzeme region with the aim of determining the general prevalence of parasitic infections in dairy cow herds (Publication **I**);
2. to verify whether a correlation exists between IgG levels in cow colostrum and calf blood serum and to evaluate its association with *Cryptosporidium* spp. infection in calves (Publication **II**);
3. to determine *Cryptosporidium* spp. species in Latvia (Publication **III**);
4. to verify whether a correlation exists between *Cryptosporidium* spp. infection in calves and factors such as dairy cow holding size, cow breed, parity, calving calendar season, and the duration of the dry period (Publication **IV**);
5. to investigate whether a correlation exists between calf infection with *Cryptosporidium* spp. and the colostrum and transition milk feeding regimen in calves (Publication **V**);
6. to evaluate the prevalence and intensity of *Cryptosporidium* spp. infection in calves and cows (Publications **I, II, III, IV, V** and **VI**).

1.9. Novelty of the Doctoral Thesis

The topic of the doctoral thesis is relevant both in Latvia and worldwide, as it directly affects the dairy industry, which is a long-standing and popular agricultural sector in Latvia.

The quality and quantity of milk obtained from cows can be influenced by factors such as feeding, housing, and various infections caused by bacteria or viruses. One factor that can significantly impact the quality and quantity of the produced output is parasitic infection (Knubben-Schweizer, 2010). Calves that were exposed to severe infections in the first months of life gain weight more poorly and are weaker compared to peers who were not exposed to infections. As a result, when these animals grow up, they exhibit lower productivity and economic benefit (Dallago et al., 2024).

Regarding beef cattle farming, it is a relatively newer sector, but meat quality can also be significantly affected by parasitic infections. Protection against parasitic infections is based on the evaluation of the epizootic situation and the planning of preventive measures (Forbes et al., 2000; Forbes, 2020). However, despite the relevance of the sector, a decision was made during the study to withdraw from further practical examinations of beef cattle. This is explained by significant differences in animal housing technologies and their temperament: beef breed cattle are primarily kept extensively (free-range conditions in paddocks), they are not accustomed to regular direct contact with humans and are more difficult to restrain. Since sample collection (especially milking colostrum and coprological testing) requires specific animal restraint, which without specialized equipment would pose an increased risk of trauma to both personnel and the animals themselves, continuing the study in this group was deemed inefficient.

Until 2015, only fragmentary studies were conducted in Latvia, which did not provide a full picture of *Cryptosporidium* spp. distribution, and the parasite species present in the country were not determined, which hindered the development of preventive measures to reduce and combat the spread of parasites. It should be mentioned that the spread of *Cryptosporidium parvum* in holdings can be potentially dangerous because, upon entering soil and water reservoirs, it can infect humans, including immunocompromised individuals, causing severe illness or even death.

Within the framework of the doctoral thesis, more than 2,100 cows were examined for the presence of *Cryptosporidium* spp. from more than 190 holdings across various regions of Latvia and different types and sizes of holdings, which is the first such large-scale study in the territory of Latvia in the last 30 years.

1.10. Structure of the Doctoral Thesis

The doctoral thesis consists of six publications, which combine the study of *Cryptosporidium* species diversity in the territory of Latvia and the impact of the colostrum feeding regimen on the severity of cryptosporidiosis infection in calves in the first months of life. The first publication of the doctoral thesis evaluates the general parasitological situation in cow herds in the Vidzeme region. The second publication evaluates the impact of IgG on the intensity of *Cryptosporidium* spp. infection in newborn calves. The third publication studies *Cryptosporidium* spp. species in the territory of Latvia. The fourth publication studies the impact of cow lactation (parity) on the development of cryptosporidiosis in calves. The fifth publication evaluates calf susceptibility to *Cryptosporidium* spp. depending on the colostrum feeding regimen.

2. MATERIALS AND METHODS

2.1. Description of sample data (Publications I–VI)

A study on the prevalence of *Cryptosporidium* spp. infection in dairy cow holdings was conducted in two stages, including a pilot study (**I**) and a main study (**II, III, IV, V** and **VI**). The main study consists of 5 stages, where each stage corresponds to one defined task. The total number of animals examined throughout the study period from 2013 to 2022 was n = 2655, including:

- 975 dairy calves up to three months of age;
- 770 dairy calves aged four to 24 months;
- 910 milking cows older than 24 months.

During the period from 2013 to 2014 (**I**), the initial study phase or pilot study was conducted, the aim of which was to determine the provisional situation in Latvia regarding *Cryptosporidium* spp. infection (first task). Within this phase, dairy (n = 521) and beef (n = 119) cows were examined in the Vidzeme region. Animals were divided into four groups: dairy cows aged six months to two years (n = 273) and older than two years (n = 248); beef cows aged six months to two years (n = 90) and older than two years (n = 29). To determine the composition of the gastrointestinal parasite fauna, including the prevalence of *Cryptosporidium* infection, coprological samples were collected and examined from 62 cattle holdings: 50 dairy cow holdings and 12 beef cattle holdings. Each individual dairy cow coprological sample was collected in a separate polyethylene bag. Beef cattle coprological samples were taken from pastures. All samples were labelled and delivered to the Parasitology Laboratory of the Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, within 24 hours.

To achieve the tasks set in the doctoral thesis, five independent studies were conducted, forming the main study of the work, and their results were published in scientific articles. The animals examined in the first, second, third, and fourth stages of the main study do not overlap in other studies. The fifth stage of the main study summarizes the animals examined in all previous stages with the aim of determining the prevalence of infection within the territory of Latvia. Before starting sample collection, data were obtained from the Agricultural Data Centre of Latvia regarding the total number of dairy cattle in the country as of June 29, 2018 (the start of the main study). Based on this information, the minimum required sample size (95 % confidence level and 5 % margin of error) was calculated separately for each study.

To achieve the second task—to verify the correlation between IgG levels in bovine colostrum and calf blood serum and evaluate its association with *Cryptosporidium* spp. infection in calves (**II**) — the first stage of the main study was implemented from December 2018 to March 2019, during which bovine colostrum and fecal samples (n = 114), as well as blood and fecal samples from newborn calves (n = 114), were collected in one dairy cow holding. In these holdings, calves were separated from their mothers immediately after birth and received two litres of colostrum in two separate feedings within the first 24 hours. Six millilitres of bovine colostrum were milked immediately after calving; the milk was labelled, frozen, and stored at –18 °C until delivery to the Microbiology and Pathology Laboratory of the Institute of Food Safety, Animal Health and Environment "BIOR". A bovine fecal sample was also taken within the first 24 hours after calving (Fig. 2.1). Fecal samples from calves were taken on the first, tenth, and fifteenth days of life; they were labelled and delivered to the Parasitology Laboratory of the Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies. Coprological samples were collected separately from each calf and cow in a polyethylene bag. If the amount of feces was too small (especially during the first days of the calves' lives), native smears were performed. Blood samples were taken from calves at two days of age, when IgG reaches its maximum level (Fischer et al., 2018). The sample was taken from the jugular vein using a 21G

size needle, collected in a vacutainer, labelled, and stored in a cooler bag at 4 °C until delivery to the Microbiology and Pathology Laboratory of the Institute of Food Safety, Animal Health and Environment "BIOR" for further investigation.

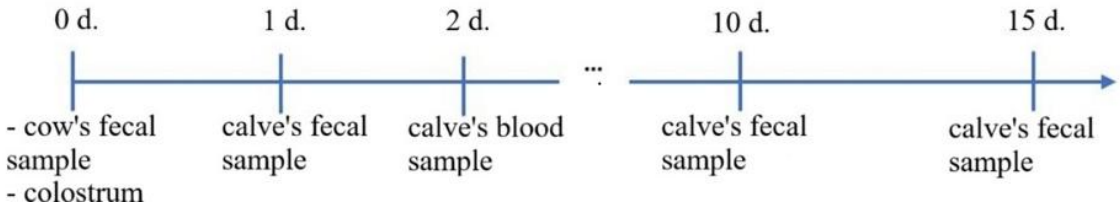


Figure 2.1. **Sampling scheme for the evaluation of the association between immunoglobulin G and *Cryptosporidium* spp. infection intensity in the first stage of the main study**

To fulfil the third task, the second stage of the main study was conducted, which includes a study on the prevalence and species diversity of *Cryptosporidium* spp. in dairy cows in Latvia. This was carried out from July 2018 to June 2019 (**III**), during which 926 individual coprological samples were collected (790 from calves and cows in holdings, 136 from cows brought to slaughterhouses) from all regions of Latvia (Figure 2.2). Holdings included in the study were divided into three groups depending on holding size: small holdings (1–50 cows, n = 113), medium holdings (50–200 cows, n = 39), and large holdings with more than 200 cows in the holding (n = 33). Animals were divided into three age groups: calves up to three months of age (n = 259), calves and youngstock aged four to 24 months (n = 247), and cows older than 24 months (n = 420).

Figure 2.2. **Locations of the holdings included in the study for the determination of *Cryptosporidium* spp. species diversity in dairy cows in Latvia**

This animal age group distribution is based on the clinical picture of the disease caused by *Cryptosporidium* spp. and bovine reproduction management: calves up to three months old are most severely affected; young animals aged three to 24 months rarely show clinical signs and usually have not yet calved; at 24 months of age, the first calving occurs (Cho and Yoon, 2014; Thompson et al., 2017). In each holding, from one to 36 individual samples were collected, which were labelled and delivered to the Microbiology and Pathology Laboratory of the Institute of Food Safety, Animal Health and Environment "BIOR" within 24 hours.

Randomization was limited by the voluntary application of owners for investigation. Sampling was also conducted in four slaughterhouses: samples were collected from all slaughtered cows on the day of the visit (8–62 samples per slaughterhouse). Each sample was collected in a separate polyethylene bag, labelled, and delivered to the Microbiology and Pathology Laboratory of the Institute of Food Safety, Animal Health and Environment "BIOR" within 24 hours.

The third stage of the main study was implemented throughout 2020 to fulfil the fourth task and verify the association between the probability of *Cryptosporidium* spp. infection in calves and factors such as dairy cow holding size, cow breeds, parity, calving season, and the duration of the dry period (**IV**). Coprological samples were taken from 153 calves in 17 holdings, and employees of each holding answered the questions of our developed

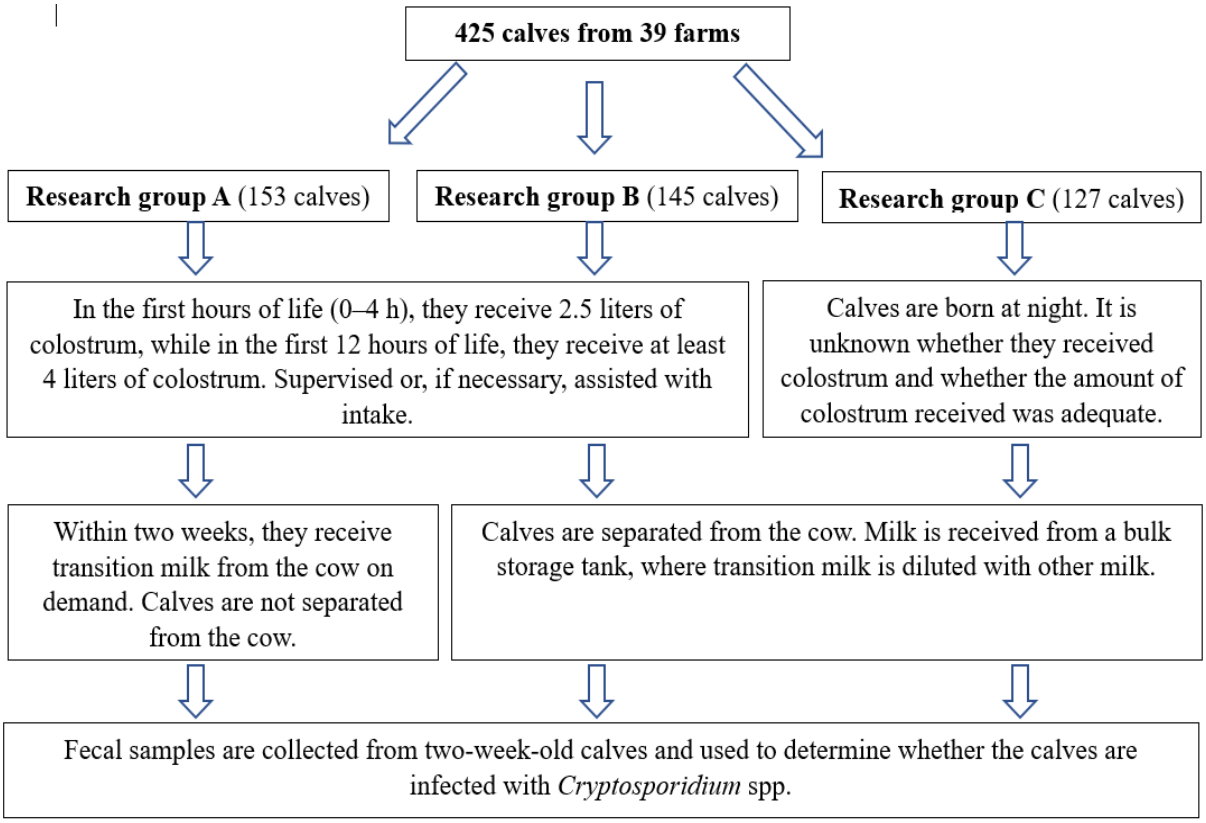


Figure 2.3. **Calf milk feeding regimen for determining the impact of colostrum and transition milk on *Cryptosporidium* spp. infection**

questionnaire (see apex "Survey"). Before collecting coprological samples from the calves, they were fed colostrum and transition milk according to the following scheme: immediately after birth, calves received ~2.5 litres of colostrum, and at least another 4 litres in the next 12 hours. Calves were not separated from the cow and received milk on demand for two weeks. Coprological samples from each calf were collected on day 14 in a separate polyethylene bag, labelled, and delivered to the Parasitology Laboratory of the Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies within 24 hours.

From December 2018 to December 2020, the fourth stage of the main study was implemented, during which the fifth task was fulfilled: to verify the association between the prevalence of *Cryptosporidium* spp. infection and the colostrum and transition milk feeding regimen in calves (**V**). Fecal samples from 425 calves (15 ± 2 days old) in 39 holdings were examined in this stage. Fecal samples were taken from the rectum of the calves. The study design is reflected in Figure 2.3. Coprological samples from each calf were collected in a separate polyethylene bag, labelled, and delivered to the Parasitology Laboratory of the Institute

of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies within 24 hours.

To fulfil the last, sixth task, i.e., to determine the prevalence of *Cryptosporidium* spp. infection in Latvia (**VI**), all 2655 individual dairy cow coprological samples collected previously from the spring of 2013 to the autumn of 2022 were analysed in the fifth stage of the main study. As in previous stages, the animals included in the study were divided into three age groups: calves up to three months (n = 975), calves and youngstock aged four to 24 months (n = 770), and cows older than 24 months (n = 910).

2.2. Laboratory methodology for coprological examination (Publications I–V)

Coprological examinations were performed in the pilot study and in all four stages of the main study. Samples were taken from the rectum of calves and cows, placed in an individual polyethylene bag, labelled, and stored in a cooler bag during transport to the laboratory until they were placed in a laboratory refrigerator at 4 °C (Lassen, 2011). Coprological samples were examined within 24–48 hours after delivery to the laboratory. Within the pilot study, standardized ovoscopic and larvoscopic methods were used for the diagnosis of helminths (**I**) (Roepstorff and Nansen, 1998). Laboratory examinations for the pilot study and the first, third, and fourth stages of the main study were performed at the Parasitology Laboratory of the Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture (**I, II, IV,** and **V**), but samples obtained in the second stage of the main study were examined at the Institute of Food Safety, Animal Health and Environment "BIOR" (**III**).

In cases where the amount of feces in the rectum of newborn calves up to three days old was insufficient, native smears were taken on-site. A wooden cotton swab was moistened with saline and inserted 5–6 cm deep into the calf's rectum. The swab was glided along the intestinal wall, rotated around its axis, withdrawn, and placed in a tube with a small amount of saline to prevent drying. In the laboratory, an impression of the swab was made on a slide, and the sample was stained using the Ziehl-Neelsen method.

As mentioned previously, *Cryptosporidium* spp. infection often causes diarrhea in calves and less frequently in cows. This can be the first sign of illness that is easy for the owner or keeper to notice. Therefore, samples with clinical signs of diarrhea were noted during sample collection so that, after counting oocysts, it could be determined whether infection intensity correlates with clinical manifestations of gastrointestinal tract disorders. In cows and calves, feces are usually semi-liquid to solid, brownish in colour with a characteristic odor, and defecation is regular, occurring up to several times a day. In the case of diarrhea, marked changes in fecal consistency are observed — they become significantly more liquid or even watery, more frequent, and may contain mucus, blood, and change colour. These changes are often accompanied by signs of dehydration, such as sunken eyes, dry mucous membranes, lethargy, loss of appetite, and emaciation (Cho and Yoon, 2014).

Within the pilot study, standardized ovoscopic and larvoscopic methods were used for the diagnosis of helminths (**I**) (Roepstorff and Nansen, 1998). To determine the prevalence and intensity of helminth infection in coprological samples, the McMaster method was used. According to this method, four grams of feces were mixed with 56 ml of NaCl flotation solution to obtain a total volume of 60 ml. The fecal suspension was filtered through a sieve into a beaker. Using a pipette, a sample was taken from the middle of the filtrate while stirring. Each chamber of the McMaster slide was immediately filled with the mixture using a pipette. After 5 minutes, the slides were examined with a 10x40 objective, focusing on the upper layer. The number of eggs per gram (EPG) of feces can be calculated using the formula: count the eggs in both chambers and multiply by 50.

The Baermann method (larvoscopy) was used for the detection of helminth larvae (Publication **I**, Figure 2.4.). According to this method, 10 g of feces were placed in a conical glass with a sieve placed on it. The conical glass was filled with warm water, and the sieve with coprological material was placed on the glass so that approximately one-third of the sieve with the material was in contact with the water. The sample was left for 30 minutes to allow the parasitic larvae to settle at the bottom of the conical glass. After 30 minutes, the upper layer of the glass was poured off down to the sediment, and the sediment was poured into a Petri dish and microscoped at 10x40 magnification.

Figure 2.4. **Coprological sample examination by the Baermann method: at the time of sample setup and after 30 minutes**

The detection of *Cryptosporidium* oocysts took place in both the pilot study and all stages of the main study (**I, II, III, IV, and V**). Examination of coprological samples was performed using the flotation method and a modified Ziehl-Neelsen staining method. To perform the flotation method, four grams of feces were taken, placed in a mortar, mixed with 56 ml of saturated NaCl solution, and stirred with a pestle until a homogeneous mass was obtained. The resulting solution was poured through a funnel and sieve into a centrifuge tube (Kuczynska and Shelton, 1999). The material was centrifuged for two minutes at 2000 rpm. As a result, after separating the liquid part, two millilitres of concentrated material were obtained and used for further staining.

A 10 µl drop of the prepared concentrated fecal material was applied to a degreased microscope slide, dried at room temperature, and stained using the modified Ziehl-Neelsen technique (Henriksen and Pohlenz, 1981) with a TB Stain kit (BD, Ireland) (Figure 2.5.). The dried smear was fixed with methanol for 10–15 min, dried, and passed over a flame several times. Then, carbol fuchsin stain was poured onto the smear, waited for 25–30 min, rinsed with water, then 8 % sulfuric acid solution was poured on, held for 40–60 sec, rinsed a second time with water, and methylene blue stain solution was poured on. After waiting for 3–5 min, the preparation was thoroughly rinsed with water, allowed to dry, and microscoped under oil immersion. Positive controls were predetermined and included in all stainings. For enumeration, dark red to pink oocysts with typical morphology were counted in all drops at 100x magnification (Figure 2.6.).

All negative samples from the second stage of the main study (**III**) were prepared for fluorescent microscopy using the AquaGlo kit (Waterborne INC, USA) to detect antibody-labelled *Cryptosporidium* oocysts. Preparation of samples for fluorescent microscopy was

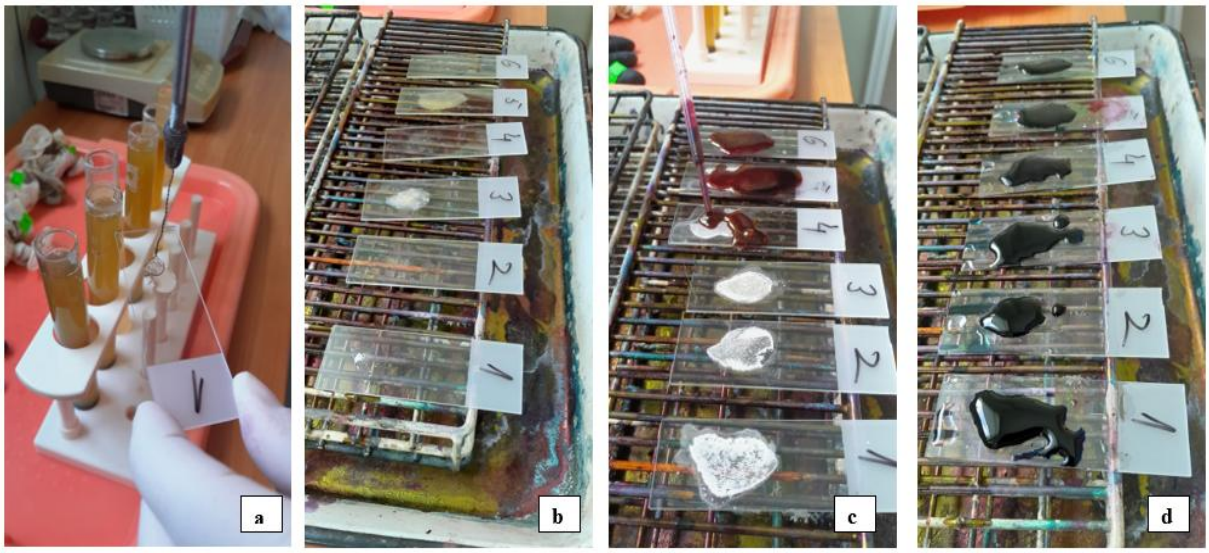


Figure 2.5. **Modified Ziehl-Neelsen technique: a) application of fecal material onto a degreased slide, b) drying of the slide at room temperature, c) and d) staining of the fecal material**

performed according to the instructions: 10 µl of fecal material, after a ten-fold thorough dilution, was placed on a 12 mm Teflon-printed three-chamber slide (Immuno-Cell, Mechelen, Belgium); then the samples were dried and fixed by immersing the slide in acetone. Later, the material was stained with FITC-labeled anti-*Cryptosporidium/Giardia* mAb (AquaGlo, Waterborne, Inc., USA) for 30 minutes in a humidity chamber, after which the antibody solution was rinsed with PBS. Brightly stained oocysts with typical morphology were counted in all chambers at 200x magnification. Each detected oocyst was considered as 200 oocysts per gram (OPG).

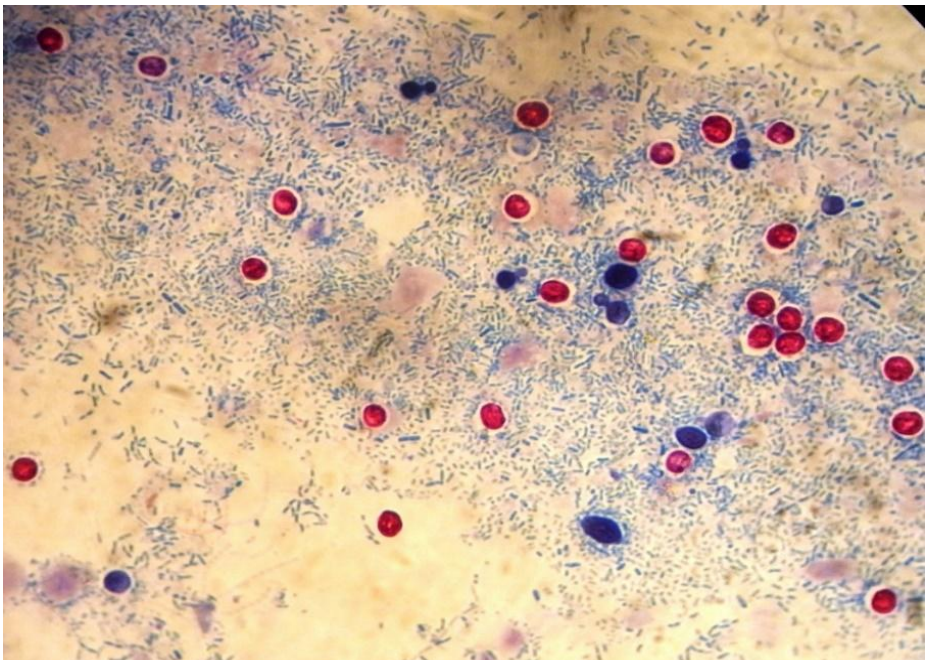


Figure 2.6. ***Cryptosporidium*** **spp. oocysts (magnification: x100 oil) stained with the modified Ziehl-Neelsen technique**

2.3. Determination of Immunoglobulin G levels in colostrum and neonatal calf blood (Publication II)

To answer the second task and determine whether there is a correlation between IgG levels in bovine colostrum and calf blood serum and evaluate its association with *Cryptosporidium* spp. infection in calves, bovine colostrum and calf blood samples were collected in one holding over four months within the first stage of the main study. Colostrum samples were taken from clinically healthy cows from udder quarters within the first two hours after calving in 6 ml tubes. Blood samples were taken from the jugular vein of calves on the second day of life using vacuum venipuncture in 6 ml blood serum tubes. Colostrum and blood samples were frozen and stored at –80 ºC until testing.

The collected calf serum samples and bovine colostrum samples were examined for bovine immunoglobulin IgG with the ELISA kit "Bovine Immunoglobulin" (Bio-X Diagnostics, Belgium). Investigations were performed at the Microbiology and Pathology Laboratory of the Institute of Food Safety, Animal Health and Environment "BIOR". A calibration curve was created for calf serum and bovine colostrum for sample examination. Calf serum samples were diluted 1/100, and colostrum samples were diluted 1/1000. In the appropriate chambers of the dilution microplate, 100 µl of the calibration curve dilutions and diluted samples were transferred; then diluted conjugate was added to each chamber, mixed, and then 100 µl of the contents were transferred to the appropriate chambers of the kit microplate.

The microplate was incubated at 21 °C ± 3 °C for one hour. Then the microplate was rinsed three times with washing solution. Then 100 µl of chromogen solution was added to each chamber, and the microplate was incubated at 21 °C ± 3 °C for 10 minutes, protected from light. The reaction was stopped by adding 50 µl of stop solution to each chamber. The optical density of the studied samples was determined using a monochromatic ELISA reader (Thermo Scientific Multiscan FC) with a 450 nm filter.

2.4. DNA extraction, nested PCR, and sequencing (Publication III)

In the second stage of the main study, DNA was extracted from the obtained coprological material to determine *Cryptosporidium* spp. species. The investigation was performed at the National Veterinary Institute in Uppsala, Sweden. Genomic DNA was extracted from the pellets obtained after centrifuging 2 ml of purified fecal sample using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Elution was performed with 80 µl of solution C6 (i.e., the DNeasy PowerSoil kit elution buffer). Two microliters of each DNA sample were subjected to polymerase chain reaction (PCR) amplification targeting 18S rDNA, as described previously (Xiao et al., 1999; Åberg et al., 2019). Nuclease-free water and *C. parvum* genomic DNA were used as negative and positive controls.

The first amplification mixture contained 1 × KAPA2G buffer solution (KAPA Biosystems), 200 µM of each deoxynucleoside triphosphate, 0.5 µM of each primary primer, and 2 µl of DNA solution with a total volume of 25 µl. Initial denaturation at 95 °C for three minutes was followed by 40 cycles consisting of 95 °C for 30 seconds, 61 °C for 30 seconds, 72 °C for one minute, and a final extension at 72 °C for two minutes. For the second amplification, 2 µl from the first reaction were added to the reaction mixture as specified previously, except for the secondary primers. The conditions for the nested PCR reaction were 95 °C for three minutes, followed by 40 cycles at 95 °C for 30 seconds, 63 °C for 30 seconds, 72 °C for one minute, and a final extension at 72 °C for two minutes. PCR products were run by capillary electrophoresis (QIAxel Advances, QIAGEN, Germany). Products of the expected size (approximately 820 bp) were subjected to sequencing for species identification.

PCR products were cleaned and sequenced in both directions with the Applied Biosystems® 3130xl Genetic Analyzer. Forward and reverse sequences were aligned with BioEdit v7.2.5 software (Hall, 1999) to generate individual consensus sequences and correct discrepancies. The obtained sequences were compared with nucleotide sequences deposited in GenBank using BLASTn (Basic Local Alignment Search Tool) (Altschul et al., 1990). To identify mixed infections, the CryptoGenotyper Galaxy tool was used (Afgan et al., 2016; Yanta et al., 2021). All sequences were analyzed using the 18S contig workflow.

2.5. Survey (Publication IV)

To evaluate the factors affecting the prevalence of *Cryptosporidium* spp. infection, which are closely related to cow housing conditions, a survey questionnaire was developed in the third stage of the main study. Questions were answered by the holding owners or employees before fecal sampling of calves. The survey questionnaire included several questions with precisely defined answer categories, which allowed for systematizing the obtained data and performing deeper analysis:

1) dairy cow holding size: small (≤ 10 cows), medium (11–50 cows), and large holding (> 50 cows);
2) cow breed;
3) parity: 1, 2, and ≥ 3;
4) calving calendar season: winter, spring, summer, and autumn;
5) duration of the dry period: ≤ 45, 46–64, and ≥ 65 days.

The obtained survey data were used to create a mathematical model. This model allowed for identifying which of the factors included in the survey are most closely related to the prevalence of *Cryptosporidium* spp. infection in cows.

2.6. Statistical data analysis (Publications I–VI)

The age of the cows taken in the statistical data sample ranged from one day to 24 years. Sample size calculation was performed using the OpenEpi system (Dean et al., 2015). According to the OpenEpi system, 323 cows were the minimum number required for this study. The calculation was based on the cow population size in Latvia as of June 29, 2018 — 395320 cows (Agricultural Data Centre of the Republic of Latvia, www.ldc.gov.lv), an absolute precision of 10 %, and an expected proportion of *Cryptosporidium* spp. of 30 %. The aim was to take samples from at least 900 cows. The sample was proportionally dispersed across the regions of Latvia, expecting that at a given moment at least 41 % of cows would shed *Cryptosporidium* spp. oocysts in the holding where *Cryptosporidium* spp. was detected (Lassen, 2011).

To determine whether statistically significant differences exist between two groups, an independent samples T-test was used if the data were normally distributed and homogeneous. Otherwise, data were analysed using the Mann-Whitney U test. The Kruskal–Wallis H test was used to verify the difference between three groups. The normality assumption was verified with the Shapiro–Wilk test and the assumption of homogeneity of variances with Levene's test (**I, II, VI**).

To determine the strength and direction of linear correlation between two continuous variables, the Pearson correlation test was used. Binomial logistic regression was performed to determine the influence of IgG in bovine colostrum and calf blood serum on the probability of *Cryptosporidium* spp. infection in calves (**II**). Generalized Linear Mixed Modeling (GLMM) was performed to determine whether explanatory variables (dairy cow holding size, breed, parity, calendar season, and duration of the dry period) are associated with the probability of

calf infection with *Cryptosporidium* spp., where the holding identification number ("FarmID") was set as a random effect variable (**IV**). GLMM was also performed to determine whether the explanatory variable —colostrum and transition milk feeding regimens with three categories (three study groups) — is associated with the probability of calf infection with *Cryptosporidium* spp., where the holding identification number ("FarmID") was set as a random effect variable (**V**).

Akaike Information Criteria (AIC) were used to evaluate which model better fits the data. Parasite prevalence was calculated as the percentage of cows infected with *Cryptosporidium* spp. (**I-VI**). Statistical tests were performed using SPSS Statistics version 22 (IBM Corporation, Chicago, Illinois), Jamovi version 2.0.0 (https://www.jamovi.org/), or R (http://www.R-project.org) version 3.3.1, using the lme4 package. All statistical analyses were performed with a significance level of $\alpha = 0.05$. Immunoglobulin concentrations were calculated using My Assays software with four-parameter curve-fitting options (**II**). Statistical data analyses were performed in Germany at the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (**III**), at the RSU Statistical Education Laboratory (**II, IV, V, and VI**), and at the Faculty of Veterinary Medicine of the Latvia University of Life Sciences and Technologies (**I**)

2.7. Ethical aspects

All procedures performed in studies involving animals were in accordance with ethical standards. The activities were carried out as part of standard agricultural and zootechnical practice, without causing pain, suffering, or stress to the animals. For the collection of blood samples, permission No. 19/1 was obtained on July 7, 2019, from the Animal Welfare and Protection Ethics Council of the Latvia University of Agriculture.

3. RESULTS AND DISCUSSION

3.1. Main results of the pilot study (Publication I)

Cryptosporidium spp. are parasites known worldwide, causing serious problems for both animal and human health. To date, relatively little has been known about these protozoa in Latvia, as studies have been fragmentary and insufficient to provide a comprehensive picture of their distribution. This is precisely why the initial research phase — the pilot study conducted from 2013 to 2014 in the Vidzeme region — became a crucial first step in investigating the prevalence of *Cryptosporidium* spp. infection in Latvian herds. This work emphasized the need to pay greater attention to this problem in our country, as cryptosporidiosis is a potentially dangerous disease not only for calves but also for humans due to the risk of zoonosis.

The pilot study (Figure 3.1.) showed that the prevalence of protozoa in cow holdings is the highest. Among them, *Cryptosporidium* spp. ranked first with a prevalence ranging from 19 % to 32.6 %. The prevalence of *Cryptosporidium* spp. significantly differed between cows and youngstock ($p < 0.05$), with youngstock being exposed to infection nearly twice as often as cows.

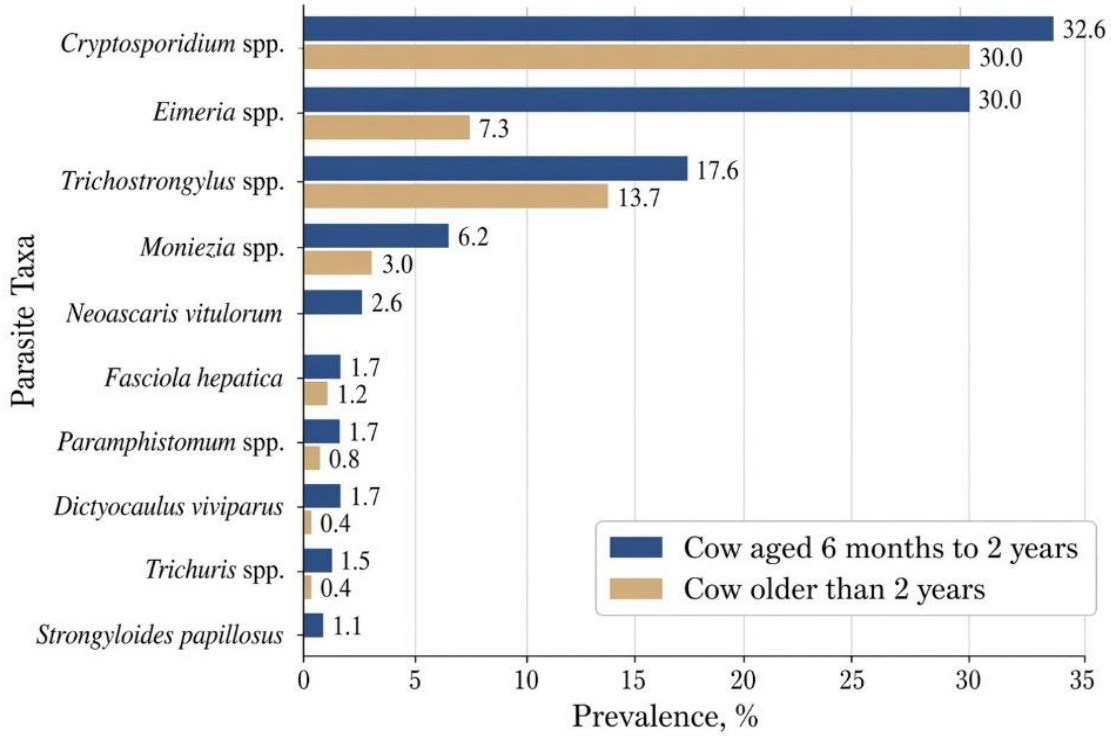


Figure 3.1. **Prevalence (%) of gastrointestinal parasite infections in dairy cows in the Vidzeme region (2013–2014 pilot study data)**

Based on the average prevalence rate, protozoa were followed by cestodes (*Moniezia* spp.—average prevalence 6.2 %), nematodes (*Strongylus* spp., *Neoscaris* spp., *Trichuris* spp., *Strongyloides* spp., *Dictyocaulus* spp. — average prevalence 4.9 %), and trematodes (*Fasciola* spp., *Paramphistomum* spp. —average prevalence 1.7 %).

The higher prevalence of protozoa compared to other types of parasites can be explained by their high reproductive potential, speed, and possibility of autoinfection, low definitive host specificity, easy fecal-oral transmission, and resistance in the environment. Several studies have reported that *Cryptosporidium* spp., *Eimeria* spp., and *Giardia* spp. parasites cause intestinal disease outbreaks in humans and animals (Fu et al., 2023). Furthermore, parasites such as *Strongyloides* spp., *Cooperia* spp., *Chabertia* spp., *Ostertagia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Buenostomum* spp., *Teladorsagia* spp., *Nematodirus* spp., and *Trichuris*

spp. are frequent infections in calves, both individually and as co-infections with cryptosporidia (Delling and Daugschies, 2022).

Despite the identified diversity of calf parasitofauna, it is specifically *Cryptosporidium* infection that causes concern among farmers, veterinarians, and physicians. Every year, mass outbreaks of human cryptosporidiosis caused by *C. parvum* are reported worldwide, where cows, small ruminants, and water serve as sources of human infection (Caffarena et al., 2020). Parasite infection poses not only health risks to humans but also significant economic losses in agriculture. For example, in Mexico, it was estimated that bovine parasitic infections cause losses of several hundred million euros related to reduced milk production, delayed weight gain, or contamination of livestock by-products (Rodríguez-Vivas et al., 2017).

To effectively avoid the negative impact of parasitic infection, it is necessary to implement strict parasite control. However, this is only possible if the exact distribution and intensity of the infection are known. Until now, *Cryptosporidium* spp. infection in cows in Latvia has not been studied; therefore, further studies within the doctoral thesis will focus on factors influencing the intensity of calf cryptosporidiosis, as well as the distribution and species diversity of the infection in Latvia.

3.2. Correlation between *Cryptosporidium* spp. infection and IgG concentration in dairy cow colostrum and calf blood serum (Publication II)

Colostrum is an integral component of newborn calf health, providing not only nutrients but also passive immunity, which is critical during the first days and weeks of life. The main role in this transfer of immunity is played by immunoglobulins, especially immunoglobulin G (IgG) (McGrath et al., 2016). Since *Cryptosporidium* spp. infection most often affects the youngest calves, a logical question arises: is there a correlation between the IgG level a calf receives with colostrum and circulates in its blood, and its susceptibility to cryptosporidiosis? The first stage of the main study was dedicated to seeking an answer to this question.

To investigate this correlation, a study was conducted including 114 cows and their calves from dairy cow holdings in Latvia. A colostrum sample was taken from each cow immediately after calving, and a blood sample was taken from two-day-old calves, when IgG levels reach their peak. Along with this, calf fecal samples were collected on days 1, 10, and 15 after birth to determine the presence of *Cryptosporidium* spp. infection.

IgG concentration in colostrum and calf blood serum was measured using the ELISA (Enzyme-Linked Immunosorbent Assay) method, which is a standard technique for quantitative antibody determination. *Cryptosporidium* infection was diagnosed using the Ziehl-Neelsen method, which allows for the accurate identification of oocysts in fecal samples.

Data are presented as the arithmetic mean and standard deviation. IgG concentration in cow colostrum was higher (70.7 ± 26.6 g/L) than in calf blood serum (13.2 ± 6.1 g/L); the statistically significant difference was 57.4 g/L (95 % CI, 52.4–62.4), $t\ (124.872) = 22.536$, $p < 0.001$. IgG concentration in calf blood is directly related to colostrum feeding (Drikic et al., 2018). Although an immunological link exists between maternal immunity and passive transfer of immunity to offspring (Hurley, 2003), and it is reported that IgG is a protective substance against various pathogens (*Yersinia enterocolitica*, *Campylobacter jejuni*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella typhimurium*, *Staphylococcus*, *Streptococcus*), no correlation was found within the doctoral thesis between the IgG level in cow colostrum and the probability of *Cryptosporidium* spp. infection in calves (Ulfman et al., 2018).

Analysis of coprological samples showed clear infection dynamics in calves during the first two weeks of life. Specifically, on day 1, none of the calves were infected with *Cryptosporidium* spp. (prevalence 0 %). However, the situation changed drastically later: on day 10, the prevalence of infection reached 26.3 %, and on day 15, it increased to 45.6 %.

This rapid increase indicates an intensive infection process specifically during this critical period: the first days of life, when specific immunity has not yet developed. The Mann-Whitney U test confirmed a statistically significant difference in oocyst shedding between day 10 and day 15 ($U = 1944$, $z = 2.330$, $p = 0.020$), with a higher median oocyst count on day 15 than on day 10. It should be noted that the level of infection in calves during this period was at least three times higher than that of their mothers (prevalence in cows was lower).

To evaluate the correlation between IgG concentration and *Cryptosporidium* spp. infection in calves, a correlation analysis was performed. A statistically significant moderately strong positive correlation was found between IgG concentration in cow colostrum and calf blood serum ($r(114) = 0.414$; $p = 0.001$) (Figure 3.2.). In contrast, no statistically significant correlation was found between IgG concentration in calf blood serum and the intensity of *Cryptosporidium* spp. infection ($p > 0.05$).

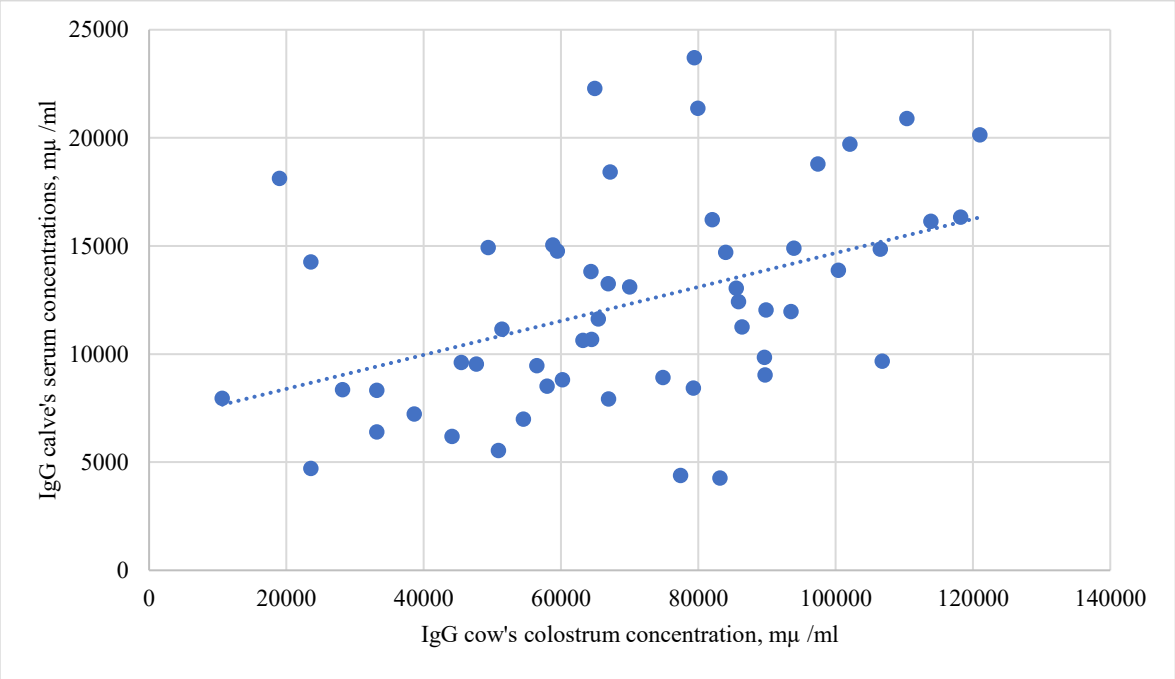


Figure 3.2. **Correlation between IgG concentration in cow colostrum (0–2 hours post-calving) (n = 114) and in calf blood serum on the second day of life (n = 114)**

To evaluate the probability that the amount of *Cryptosporidium* spp. oocysts in calf coprological samples decreases as the IgG level in calf blood serum increases, a logistic regression analysis was performed. The results showed that the developed model was not statistically significant either on day 10 ($X^2(2) = 0.013$; $p = 0.99$) or on day 15 ($X^2(2) = 0.100$; $p = 0.95$). This means that no causal relationship was found between the IgG concentration in either cow colostrum or calf blood serum and *Cryptosporidium* spp. infection in calves.

Previous studies on the distribution of *Cryptosporidium* in the USA by Lemeteil et al. (1993) and in China by Gong et al. (2017) coincide with our findings that calves have a higher prevalence of infection than cows. Santín et al. (2004) concluded that the distribution of *Cryptosporidium* species is related to the age of calves during the pre-weaning and post-weaning periods. Harp et al. (1990) proved that infection and recovery in calves (up to 3 months of age) can protect them from repeat *C. parvum* infection. Most likely, these findings indirectly emphasize the importance of adaptive immunity in *Cryptosporidium* infection. However, Gong et al. (2017) indicated that *Cryptosporidium* species/subtypes differ across various cow age groups, suggesting that infection with one *Cryptosporidium* species and subsequent recovery does not guarantee immunity against other *Cryptosporidium* species. Thompson et al. (2017) assumed that the parasite's ability to infect an animal is related to changes in the intestinal

microflora during animal maturation, but unfortunately, there are no experimental studies proving this in cows.

Colostrum contains not only IgG but also a range of other immunoglobulins that also ensure an effective immune response. For example, IgA, which accounts for 5-10 % of the total amount of immunoglobulins, is a high-molecular-weight protein that is not absorbed but remains on the surface of the mucosa, providing local protection (Pakkanen and Aalto, 1997; Playford, Macdonald and Johnson, 2000; Blum, 2006). It is possible that its level is a better predictor of the possibility of *Cryptosporidium* infection than the IgG level. Elison et al. (2011) report that the IgM response occurs immediately after acute infection and its level decreases within a few weeks, whereas the IgG response appears more slowly but persists for a longer period. Thus, a study of total IgA, IgM, and IgG levels in the first days of calf life in relation to the level of *Cryptosporidium* infection would be a new research direction for the future. It must not be forgotten that the immune response to *Cryptosporidium* infection includes both innate and adaptive immunity. Numerous studies have described the roles of T and B cells, natural killer cells, dendritic cells and macrophages, intestinal epithelial cells, gamma interferon, nitric oxide, antimicrobial peptides, prostaglandins, cytokines, and chemokines in building the immune response against *Cryptosporidium* (Leitch and He, 2011; Vanathy et al., 2017). Consequently, it may not be correct to evaluate only the IgG level in the development of immunity directed against *Cryptosporidium*. Most likely, it is the result of the action of multiple cells and chemical compounds.

Upon obtaining these results, the idea arose to test whether colostrum feeding affects the *Cryptosporidium* infection. This would prove that even though IgG does not directly affect the infection, in the case of cryptosporidiosis, the passive transfer of immunity to offspring through colostrum is of greater importance than innate and adaptive immunity. This assumption was formulated in the fourth and fifth tasks of the work and investigated in the third and fourth stages of the main study.

3.3. Diagnosed species of the genus *Cryptosporidium* in Latvia (Publication III)

To fully understand the epidemiology of cryptosporidiosis in the Latvian cow population, it is not sufficient only to determine the prevalence and intensity of infection. It is also essential to find out which specific species of the genus *Cryptosporidium* are present, as different species may have varying pathogenicity and epidemiological significance, including zoonotic potential. Therefore, the third task of the doctoral thesis and the second stage of the main study was to perform molecular typing to identify the *Cryptosporidium* species circulating in Latvia.

To identify *Cryptosporidium* species, molecular diagnostics was used, which is the gold standard for detecting such protozoa. In the study covering the period from 2018 to 2019, 926 coprological samples were collected from cattle in various Latvian farms and slaughterhouses. The samples were analyzed using polymerase chain reaction (PCR), which amplified specific DNA fragments, and a sequencing method to accurately determine species and genotypes. This approach ensured high precision and allowed for the detection of even rare species.

Molecular analysis revealed a diverse picture: six different *Cryptosporidium* species were identified in Latvian cows. Detailed data on the frequency of occurrence of each species, their association with animal age, and diarrhea are summarized in Table 3.1.

Table 3.1. **Epidemiological parameters of *Cryptosporidium* species in dairy cows in Latvia**

Species	Total samples	Proportion (95 % TI)	Median age (months)	Age range (months)	Samples from cows with diarrhea (95 % CI)
C. parvum	62	45.9 (37.8–54.3)	3.0	0.03–111.0	41.9 (30.4–54.3)
C. bovis	29	21.5 (15.4–29.2)	3.5	0.2–172.0	41.4 (25.5–59.3)
C. andersoni	22	16.3 (11.0–23.5)	17.5	0.09–197.0	22.7 (9.7–43.9)
C. ryanae	11	8.1 (4.5–14.1)	6.0	0.2–70.0	18.2 (4.0–48.9)
C. scrofarum	1	0.7 (0.0–4.5)		20.0	0.0
C. ubiquitum	1	0.7 (0.0–4.5)		84.0	100.0
C. parvum / *C. bovis*	3	2.2 (0.5–6.6)	0.06	0.03–3.0	100.0
C. parvum / *C. andersoni*	1	0.7 (0.0–4.5)		16.0	0.0
C. parvum / *C. ryanae*	1	0.7 (0.0–4.5)		1.2	100.0
C. bovis / *C. andersoni*	1	0,7 (0.0–4.5)		147.0	100.0
C. bovis / *C. ryanae*	3	2.2 (0.5–6.6)	11.0	1.0–55.0	33.3 (1.7–86.8)
All species	135	41.4 (36.5–46.8)	4.5	0.03–197.0	38.5 (30.6–46.9)

Note: The total proportion of isolates (%) is calculated relative to the number of successfully genotyped positive samples. The occurrence of diarrhea is expressed as a percentage of the total number of samples for the respective species or mixed-type infection. CI – 95 % confidence interval.

Out of 326 cow and calf coprological samples collected from 54 holdings and microscopically positive for *Cryptosporidium* spp., *Cryptosporidium* spp. DNA was successfully amplified and sequenced in only 41.4 % (n = 135) of the samples. In total, six *Cryptosporidium* species were identified: *C. parvum*, *C. bovis*, *C. andersoni*, *C. ryanae*, *C. scrofarum*, and *C. ubiquitum*. *Cryptosporidium scrofarum* and *C. ubiquitum* were identified in only one separate sample each, which is not diagnostically significant.

In our study, using the CryptoGenotyper tool, 31 out of 135 samples were found to have *Cryptosporidium* spp. infection with mixed pathogens. Three samples had *C. parvum* / *C. bovis*, three had *C. bovis* / *C. ryanae*, one had *C. parvum* / *C. ryanae*, one had *C. parvum* / *C. andersoni*, and one had *C. bovis* / *C. andersoni* mixed infections.

At least one *Cryptosporidium* spp. species was identified in cows in 55.6 % (95 % CI 42.2–68.4 %) of holdings, at least two species were identified in 31.4 % (95 % CI 20.2–77.7 %) of holdings, but three or more *Cryptosporidium* species were diagnosed in 13.0 % (95 % CI 5.9–24.0 %) of the examined holdings.

Cryptosporidium parvum was found in cows from 63.0 % (95 % CI 49.6–75.0 %) of holdings, followed by *C. bovis* in 37.0 % (95 % CI 25.0–50.4 %), *C. andersoni* in 37.0 % (95 % CI 25.0–50.4 %), and *C. ryanae* in 26.0 % (95 % CI 15.6–38.8 %).

The species *C. parvum* and *C. bovis* were diagnosed in some calves as early as the second day after birth. Other authors' publications mention that *C. parvum* is more often diagnosed in calves aged 5 to 12 days, but *C. bovis* in calves aged 10 to 12 days (Faubert and Litvinsky, 2000; Fayer et al., 2005; Silverlås et al., 2009; Wu et al., 2020). It should be noted that infection

with cryptosporidia occurs as early as the first hours after birth. Therefore, in cases where a calf's immunity is lowered or they are not fed a sufficient amount of colostrum, cryptosporidia can appear in coprological samples on the second or third day of life (Bjorkman et al., 2015; Garro et al., 2016).

In previous studies, *C. parvum* was often identified in calves during the pre-weaning period, whereas *C. bovis* and *C. ryanae* were found in weaned calves, and *C. andersoni* in adult cows (Santín et al., 2008). Since the duration of the calf weaning period in various Latvian holdings may differ depending on the cow breed and the number of housed animals, it cannot be claimed that there is a link between species occurrence and the weaning period. Only *C. andersoni* was more prevalent in older animals, whose median age was 17.5 months. The youngest calf infected with *C. andersoni* was only three days old and shed 400 OPG, which once again proves that this species is age specific.

In this study, *C. parvum* was the dominant species found in young calves (13600 OPG), as well as in nine-year-old cows (1400 OPG). In one organic holding, a seven-year-old cow infected with *C. parvum* shed many oocysts (128800 OPG). Information about holdings with high *Cryptosporidium* intensity can also be useful for human physicians, as the figures we obtained allow us to state that there is a very high zoonotic risk in Latvia. It is worthwhile to check the water and soil around holdings to determine their contamination with *C. parvum* oocysts.

To find out which species most often causes clinical signs of illness, i.e., diarrhea, the association of each species with diarrhea in different age groups was analysed. The results clearly showed that although *C. parvum* is prevalent in all age groups, it most often causes clinical diarrhea specifically in the youngest calves (up to three months of age). 70% of calves in this group who were found to have *C. parvum* also had diarrhea (Table 3.2). According to literature data, *C. parvum* can cause severe illness in calves, which usually manifests as intense diarrhea (Abeywardena et al., 2015). Outbreaks with high calf mortality from *C. parvum* have also been described in Estonia (Lassen and Talvik, 2009; Niine et al., 2018).

Table 3.2. **Prevalence and proportion of oocyst isolates in cows with diarrhea in different age groups depending on the *Cryptosporidium* species**

Age group	*Cryptosporidium* species	Number / proportion of isolates (95 % CI)	Proportion of isolates in calves with diarrhea (95 % TI)
0–3 months	*C. parvums*	30.0 / 52.6 (39.9–65.0)	70.0 (52.1–83.3)
	C. bovis	20.0 / 35.1 (24.0–48.1)	65.0 (43.3–81.9)
	C. andersoni	5.0 / 8.8 (3.8–18.9)	40.0 (11.8–76.9)
	C. ryanae	2.0 / 3.5 (1.0–11.9)	0.0 (0.0–65.8)
	Total	57.0 / 45.6 (37.1–54.3)	63.2 (50.2–74.5)
4-24 months	*C. bovis*	8.0 / 22.9 (12.1–39.0)	25,0 (7,2–59,1)
	C. andersoni	8.0 / 22.9 (12.1–39.0)	25,0 (7,2–59,1)
	C. ryanae	5.0 / 14.3 (6.3–29.4)	40,0 (11,8–76,9)
	Total	35.0 / 28.0 (20.9–36.4)	17,1 (8,1–32,7)

Age group	*Cryptosporidium* species	Number / proportion of isolates (95 % CI)	Proportion of isolates in calves with diarrhea (95 % TI)
> 24 months	*C. parvums*	13.0 / 41.9 (26.4–59.2)	0.0 (0.0–22.8)
	C. bovis	6.0 / 19.4 (9.2–36.3)	16.7 (3.0–56.4)
	C. andersoni	8.0 / 25.8 (13.7–43.3)	12.5 (2.2–47.1)
	C. ryanae	4.0 / 12.9 (5.1–28.9)	0.0 (0.0–49.0)
	Total	31.0 / 24.8 (18.1–33.1)	6.5 (1.8–20.7)

Note: The total proportion of isolates (%) is calculated relative to the number of successfully genotyped positive samples. The occurrence of diarrhea is expressed as a percentage of the total number of samples for the respective species or mixed-type infection. CI – 95 % confidence interval.

In older calves, the proportion of *C. parvum* in samples is still the highest compared to other *Cryptosporidium* species, but clinical signs of diarrhea are not identified. This can complicate infection control, as an animal without clinical signs of disease is considered healthy, is not isolated from others, and is not treated. In the case of *C. parvum*, this situation can be critical and potentially dangerous for humans as well.

Cryptosporidium bovis was prevalent in all age groups, and the highest proportion of infected calves was in animals up to three months old, as well as the highest proportion of diarrhea associated with *C. bovis* infection was observed in this same age group.

The prevalence of *Cryptosporidium andersoni* infection in the calf age group up to three months is very low, which is related to the fact that *C. andersoni* localizes in the glandular cells of the abomasum. Accordingly, until these are sufficiently developed in calves, this *Cryptosporidium* species should not cause clinical disease. Interestingly, a higher proportion of calves with diarrhea where *C. andersoni* infection was also identified was observed in this same calf group. Similarly, *C. ryanae* causes diarrhea only in the age group from 4 to 24 months. This is explained by the fact that in these cases, they were not *C. andersoni* or *C. ryanae* mono-infections, but mixed infections with *C. parvum* and *C. bovis*. There are known cases where the presence of *C. andersoni* was identified in previously weaned calves, although this species is always more associated with post-weaning calves or mature cows (Silverlås et al., 2010; Huetink et al., 2001; Enemark et al., 2002).

If we compare the prevalence of *C. andersoni* and *C. ryanae* infection in the context of the general situation in calves, it was rarely diagnosed. Most of the infections caused by *C. ryanae* were asymptomatic, which also corresponds to the observations of Fayer et al. (2008).

3.4. Impact of cow lactation number and holding size on *Cryptosporidium* spp. infection in calves (Publication IV)

Having obtained negative results in the study on the effect of IgG levels in calf blood serum on the possibility of developing cryptosporidiosis, a fourth task was set: to check other factors that could influence calf infection with *Cryptosporidium* spp. It was important to create a knowledge base that could be easily applied in holdings to reduce the risks of cryptosporidiosis. Therefore, the following factors were considered within the doctoral thesis: cow lactation number (parity), holding size, dry period, calving season, and cow breed. The impact of these factors was studied by analyzing a total of 153 coprological samples collected from calves in 17 different dairy cow holdings in Latvia. In addition to sampling, a survey of farm owners or employees was also conducted. The main data processing method was

regression analysis, with the help of which the most significant factors affecting the probability of *Cryptosporidium* spp. infection in calves were selected.

The proportion of independent variable (factor) categories characterizing the sample data is reflected in Figure 3.3.

When examining coprological samples, it was found that 26.1 % of calves were positive for *Cryptosporidium* spp. The presence of diarrhea signs was also recorded in 15 % of *Cryptosporidium* spp. positive cows. The prevalence of *Cryptosporidium* spp. infection (26.1 %) was slightly higher than in a similar study in Estonia (23 %) (Santoro et al., 2019). Overall, the prevalence of infection does not differ significantly from what was identified in other studies of the doctoral thesis (**I**, **III**, and **V**).

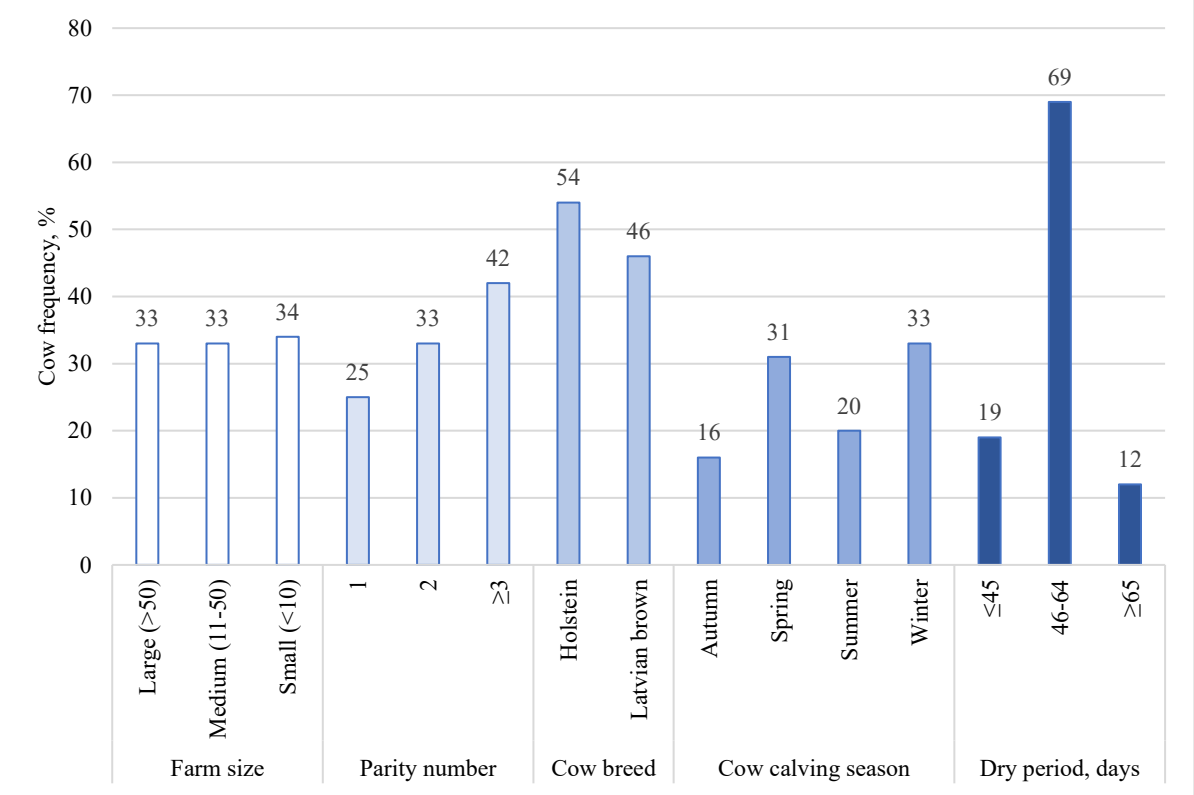


Figure 3.3. **Percentage distribution of cows across different sample groups for the assessment of maternal biological and environmental factors.**
The percentage of cows is calculated from the total number of animals examined in the study (n=153).

To find out which of the factors included in the survey are most closely related to calf infection, generalized linear mixed modelling (GLMM) was used, where the holding identification number was included as a random effect to take into account possible differences between holdings. The analysis revealed a statistically significant impact of two factors: cow lactation number ($X^2(2) = 15.83$, $p < 0.001$) and holding size ($X^2(2) = 8.68$, $p = 0.013$). Other factors, such as cow breed, calving season, and duration of the dry period, did not show a statistically significant association with the probability of *Cryptosporidium* spp. infection in calves in this model.

Delving into the impact of lactation number, the first lactation was chosen as the reference group in the regression model. Comparison showed that calves that consumed colostrum from the second lactation had a significantly lower chance of being infected with *Cryptosporidium* spp. ($B = –1.723$, $z = –3.073$, $p = 0.002$, $OR = 0.18$). This means the risk decreased on average by 82 % (or 0.18 times; 95 % CI 0.05–0.54) compared to calves from first-lactation cows. An even more pronounced protective effect was observed in calves that consumed colostrum from the third or later lactation ($B = –2.181$, $z = –3.71$, $p < 0.001$, $OR = 0.11$). In this case, the risk of infection decreased on average by 89% (or 0.11 times; 95 % CI 0.03–0.36) compared to the

reference group. This observed effect of the lactation number is most likely related to changes in colostrum quality: with each year, a cow is exposed to more pathogens present in the holding, and her immune system produces more antibodies, which enter the colostrum. For example, Morrill et al. (2012) discovered that with each subsequent lactation, IgG concentration increases and the somatic cell count decreases. Although it was identified in this study regarding IgG that its level does not affect *Cryptosporidium* infection, this question was not studied from the perspective of the cow's lactation number. Gulliksen et al. (2008) point out that older cows produce colostrum with a higher antibody concentration than younger ones, as older cows are exposed to antigens for a longer period than younger ones (Quigley et al., 1994; Tyler et al., 1999).

Colostrum is an essential source of minerals (Ca, P, Mg, Na, Fe, Zn, Cu, and Mn) for newborn calves. Their concentration in colostrum is significantly higher in the first hours after calving and also differs significantly between cows calving for the first time and multiparous cows (Kume and Tanabe, 1993). The lactation number affects the mineral status of newborn calves. Kume and Tanabe (1993) proved that the hematocrit and hemoglobin of newborn calves increased with each lactation number. The lactation number negatively correlates with the cow's gestation period (each subsequent pregnancy is slightly shorter) and positively correlates with milk production volume and calf birth weight (Hoka, Gicheru, and Otieno, 2019).

The regression model of the doctoral thesis showed that small holdings were a statistically significant predictor of the chance of calves being infected with *Cryptosporidium* spp. (B = –1.624, z = –2.843, p = 0.004, OR = 0.20). Cow calving in small holdings reduces the chance of calves being infected with *Cryptosporidium* spp. on average by 0.20 times (or by 80 %; 95 % CI 0.06–0.60) compared to large holdings. Factors such as medium and large holding size did not show a significant impact on the chance of calves being infected with *Cryptosporidium* spp.

Colostrum quality may differ across various cow breeds (Tsuji et al., 1990; Kessler, Bruckmaier, and Gross, 2020). However, there is no evidence that cow breed can influence *Cryptosporidium* infection. Our study also did not show a correlation between *Cryptosporidium* infection and cow breed. It is possible that there is no obvious difference in the protective ability against pathogens among many cow breeds (Murphy et al., 2005).

From everything mentioned above, it can be concluded that holding size and cow lactation number indirectly affect milk quality. Although holding size cannot directly influence the quality of colostrum or transition milk, there are many indirect factors that distinguish large and small holdings, such as labor organization.

Large holdings operate as enterprises where professionals are employed to maintain animals in optimal conditions and to obtain maximum production results. In large holdings, an individual approach and tracking of a cow's health status are provided less frequently. In contrast, small holdings are family-owned and use services from various specialists, such as veterinary assistance, as an external service. Therefore, animals from holdings of different sizes are provided with different care. In large holdings, an obligatory dry period is more often set for cows, while in small holdings, this period can be adjusted individually, considering the cow's health, behaviour, and other factors. Also, from the perspective of environmental parasitic contamination, the situation in large, medium, and small holdings may differ. In small holdings, it is easier to maintain a clean environment with the lowest parasitic contamination.

In small holdings, calves are usually born in the winter and spring seasons, but in large ones, calves are born all year round. The results of this study suggest that in large holdings, calves have a higher chance of being infected with cryptosporidia compared to small holdings. This can be explained by the fact that in large holdings, animals are kept in high density in one place, and this density affects the possibility that parasites will encounter new hosts. When studying the species diversity of *Cryptosporidium*, we identify that it is specifically the calves that shed more *Cryptosporidium* oocysts into the environment. Accordingly, if in small farms calves are born only in a specific season, then the environmental contamination will also be lower compared to medium and large farms where calves are born throughout the year.

Mennerat et al. (2010) have described in detail how intensive agriculture can influence the distribution of parasites.

Seasonal differences play an important role in the transmission of infections; for example, high outdoor temperatures and high rainfall are associated with the risk of *Cryptosporidium* infection (Jagai et al., 2009). However, no effect of seasonality was found on the probability of parasitic infection in calves. This suggests that the main factor influencing the distribution of *Cryptosporidium* infection in calves is the animal housing conditions in the holdings. Most likely, calves can be infected with parasites not only in pastures, where weather conditions change seasonally, but also in other places where calves are kept for a longer period.

It should be noted that oocysts are the resistant forms of cryptosporidia, which allows them to survive in unfavourable environmental conditions. This indicates that parasites can spread and infect calves regardless of whether it is summer or winter (Robertson, Campbell, and Smith, 1992).

The duration of the dry period was carefully studied and proved to affect milk and colostrum production volume, IgG concentration in colostrum, mastitis risk, cow postpartum metabolism, and cow energy balance (Rastani et al., 2005; Annen et al., 2004; Bertics et al., 1992; Collier, Annen-Dawson, and Pezeshki, 2012; Watters et al., 2008). In contrast, there is no evidence regarding the impact of the dry period duration on calf health status (Andrée et al., 2018), although for cows with a short dry period, colostrum has a lower IgG concentration (Rastani et al., 2005). No correlation was found in this study between the duration of the dry period and *Cryptosporidium* infection. However, it cannot be excluded that the results of the study were influenced by the fact that too large a percentage of cows, i.e., 69 %, had an eight-week dry period. In Latvia, farmers rarely shorten or lengthen a cow's dry period.

3.5. Probability of *Cryptosporidium* spp. infection in calves with different feeding regimens (Publication V)

To gain a deeper understanding of the factors influencing *Cryptosporidium* spp. infection in calves, a fifth task was set and a study was conducted with a particular focus on the role of different feeding regimens. The aim of this study was to find out if and how specific feeding strategies, especially the use of colostrum and transition milk, correlate with the risk of cryptosporidiosis in calves. To do this, calves were divided into three groups, each with a different feeding regimen:

- Study group A (reference): calves received colostrum timely and in sufficient quantity and then continued to receive transition milk for at least two weeks. This group served as the control or reference group in the regression model, against which the other regimens were compared;
- study group B: calves received colostrum timely and in sufficient quantity, but then switched to regular milk (whole milk or milk replacer) instead of transition milk;
- study group C: calves for whom it was not exactly known if they received colostrum timely and in sufficient quantity. After potential colostrum intake, these calves continued to receive regular milk.

Analysing a total of 425 calf coprological samples, it was found that on average 35.3 % of calves had a positive *Cryptosporidium* spp. finding. Clinical signs of diarrhea were recorded in 20.6 % of infected calves. The percentage distribution of *Cryptosporidium* spp. positive and

negative calves, as well as the percentage distribution of calves with clinical signs of diarrhea (including those without cryptosporidia) in each study group, is summarized in Figure 3.4.

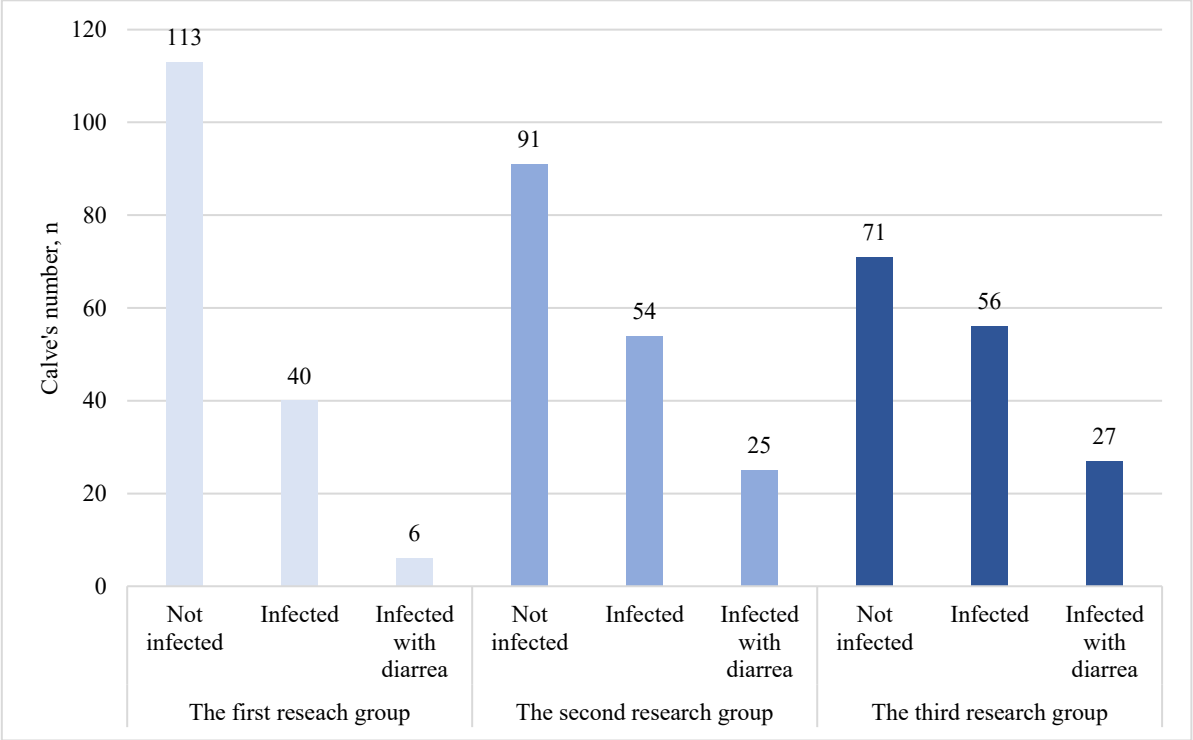


Figure 3.4. **Occurrence of *Cryptosporidium* spp. infection and diarrhea in calves according to colostrum and transition milk feeding regimens**

First study group: calves receive an adequate amount of colostrum during the first hours of life and remain with the cow for 2 weeks. Second study group: calves receive an adequate amount of colostrum during the first hours of life, are separated from the cow, and receive milk from a bulk milk storage tank. Third study group: calves are born at night, are separated from the cow, and receive milk from a bulk milk storage tank.

The obtained results regarding *Cryptosporidium* oocyst shedding (average prevalence = 35.3 %, from 26.1 % to 44.1 %) are the same as those reported from Estonia — 30 % (Lassen et al., 2009) and 23% (Santoro et al., 2019); however, they significantly differ compared to Lithuania — 67 % (Lassen and Järvis, 2009), which is primarily explained by the small sample size in that study (7 holdings, 15 coprological samples from each).

The regression model was significant (X^2 (2) = 8.62, p = 0.0013), showing that different feeding regimens are statistically significantly associated with the probability of *Cryptosporidium* spp. infection in calves. The study results showed that colostrum and transition milk feeding regimens are associated with *Cryptosporidium* infection. This means that both colostrum and transition milk play an important role in pathogen infection control. The most effective combination against parasitic infection is the timely intake of an appropriate amount of colostrum, followed by transition milk consumption for at least two weeks before weaning from the mother.

The regression model showed that the feeding regimen of the second research group statistically significantly predicted the chance of calves being infected with *Cryptosporidium* spp. (B = 0.643, z = 2.00, p = 0.046, OR = 1.901). This indicates that for calves belonging to the second research group, the chance of being infected with *Cryptosporidium* spp. increases on average by 1.90 times (95 % CI 1.012–3.574) compared to calves from the first research group.

Similarly, the feeding regimen of the third research group statistically significantly predicted the chance of calves being infected with *Cryptosporidium* spp. (B = 0.901, z = 2.93, p = 0.003, OR = 2.469). This means that for calves belonging to the third research group, the chance of being infected with *Cryptosporidium* spp. increases on average by 2.47 times (95 % CI 1.35–4.52) compared to calves from the first research group.

The protective properties of colostrum were repeatedly studied and mainly explained by the transfer of immunoglobulin (e.g., IgG) from cow to calf upon colostrum intake (Robbers et al., 2021). Our previous studies have not shown a correlation between IgG levels in cow colostrum and *Cryptosporidium* infection in calves; possibly innate and adaptive immunity play a greater role in the immune response against *Cryptosporidium* species than the transfer of maternal passive immunity to offspring.

The results of this study suggest that timely and appropriate colostrum intake still plays an important role in *Cryptosporidium* infection in calves, suggesting that the role of other colostrum bioactive compounds (growth factors, hormones, cytokines, enzymes, polyamines, nucleotides, antibacterial components, etc.) should not be underestimated. Immunoglobulins together with bioactive compound components form a complex system in which several elements interact with each other, forming a single universal barrier against pathogens (Thomson et al., 2017). Furthermore, the results of this study (increased diarrhea percentage) indirectly showed that colostrum intake should be controlled so that it is taken immediately after birth, which significantly reduces the chance that a pathogen will enter and develop in the organism. Calves fed immediately after birth (0 h) had a higher serum IgG concentration compared to calves fed six and 12 hours after birth, and this also influenced the formation of the calf's intestinal microbiome (Fischer et al., 2018).

The results of this study showed that calves fed with transition milk for at least two weeks after colostrum feeding have a significantly lower chance of clinically falling ill with cryptosporidiosis and diarrhea compared to calves receiving colostrum and then whole milk. These results are confirmed by the study of Conneeley et al. (2014) and Kargar et al. (2021) on the health status of calves who were also fed transition milk. Kargar et al. (2021) proved that extending the duration of transition milk feeding positively affects calf weight gain and reduces the probability of diarrhea (watery feces — the cause of diarrhea is unknown). This is explained by the higher concentration of some bioactive compounds compared to whole milk (McGrath et al., 2016; Fischer et al., 2018).

Skipping transition milk intake is always related to the weaning process, when a calf is exposed to several stressful situations. A calf that is not weaned from the mother receives milk on demand in an unlimited quantity, whereas a weaned calf is subjected to a specific feeding program that may not meet the calf's individual physiological needs. This affects the development of the gastrointestinal system, which serves as the first barrier to infections (Meale et al., 2017). The second stress factor is the movement of the animal to a separate housing area, where it lives in a restricted territory together with other calves of different ages (with different health status and infections) while its immune system is still immature. Furthermore, changes in the intestinal microbiota observed during the weaning process (Li et al., 2012) can influence the predisposition to develop diarrhea.

3.6. Prevalence (%) and intensity of *Cryptosporidium* spp. infection in Latvia (Publication VI)

To gain a more detailed picture of the distribution of *Cryptosporidium* spp. in the Latvian cow population, a final sisth task was set within the doctoral thesis, and a broad epidemiological analysis was performed, paying special attention to how infection indicators are influenced by animal age and holding size. This approach allows not only for determining the overall infection level but also for identifying risk groups and factors that are essential in the parasite's distribution.

To investigate the distribution of cryptosporidia in relation to cow age, coprological analysis was performed in three age groups: calves up to three months of age, calves and youngstock from four to 24 months, as well as adult cows older than 24 months. The study, covering the period from 2013 to 2022, included 2,655 animals (coprological samples from all

animals collected during the doctoral thesis) from various Latvian farms, ensuring a representative sample.

Samples were collected individually and analysed in the laboratory using the Ziehl-Neelsen method — a standardized parasitological technique that allows for the accurate detection of *Cryptosporidium* oocysts and quantitative evaluation of the infection intensity. This approach guaranteed data reliability and comparability with other studies.

Table 3.3. **Prevalence of *Cryptosporidium* spp. infection (%) in Latvia and the world**

Country	Year	Animal	Prevalence, %	Reference
Japan	2018–2019	Calves	83.8	Kabir et al., 2020
Lithuania	2009	Cows	67.0	Lassen et al., 2009
Chile	2007–2008	Dairy calves	56.1	Díaz-Lee et al., 2011
California, USA	2012	Dairy calves	56.0	Li et al., 2019
Thailand	2016–2017	Dairy calves	51.0	Doungmala et al., 2019
Mexico	2014	Dairy calves	40.0	García-Romo et al., 2014
Italy	2014	Calves	38.8	Díaz et al., 2018
Sweden	2012–2013	Calves	38.7	Aberg et al., 2019
Chine	2020	Dairy calves	38.4	Wu et al., 2020
Germany	1993–1997	Dairy calves	36.0	Joachim et al., 2003
Latvia	2013–2020	Cows	27.0	Zolova et al., 2024
Ghana	2009	Calves	29.0	Squire et al., 2013
Columbia	2010–2012	Dairy calves	26.6	Avendaño et al., 2018
Brazil	2018–2019	Calves	25.7	Conceição et al., 2021
Belgium	2019–2020	Dairy calves	25.7	Pinto et al., 2021
Argentina	2013–2014	Dairy calves	25.5	Lombardelli et al., 2019
France	2019–2020	Dairy calves	24.9	Pinto et al., 2021
Nigeria	2010	Calves	23.4	Ayinmode and Fagbemi, 2010
Estonia	2013–2015	Calves	23.0	Santoro et al., 2018
Netherlands	2019–2020	Dairy calves	20.8	Pinto et al., 2021
Korea	2019–2020	Dairy calves	18.7	Jang et al., 2021
Ethiopia	2014–2015	Calves	18.6	Ayele et al., 2018
Spain	2016–2018	Cows	16.7	Díaz et al., 2021
Algeria	2022	Dairy calves	15.7	Dadda et al., 2022
Iran	2003–2004	Cows	6.3	Azami, 2007

Summarizing data from all 2,655 samples, it was determined that the total prevalence of *Cryptosporidium* spp. infection in cows during the study period (2013–2020) was 27 % (95 % confidence interval [CI] 26–29 %). The average number of oocysts shed per gram of feces (OPG) in positive samples was 1,000 (interquartile range: Q1 = 400, Q3 = 3,000). These indicators fit into the general European and global context, where infection levels significantly differ across countries (see Table 3.3). For example, the 27 % prevalence identified in this study is close to the global average (25.5 %, Buchanan et al., 2024) and similar indicators in neighbouring Estonia (23–30 %, Santoro et al., 2018; Lassen et al., 2009), but lower than in earlier studies in Lithuania (67 %, Lassen et al., 2009) or Denmark (32 %, Maddox-Hyttel et al., 2006).

Analysing data by age groups revealed a very distinct and statistically significant ($p < 0.001$) trend: both the proportion of infected animals and the frequency of infection-related diarrhea decreased as the animal's age increased (see Table 3.4). The highest risk group is calves up to three months of age. In this group, the highest prevalence of infection (39.4 %) and also

the highest average number of shed oocysts (median 800 OPG) were identified. Furthermore, specifically in this age group, more than half (56.6 %) of the infected calves were observed to have diarrhea. These results align with the results of many other studies worldwide, pointing to the high susceptibility of the youngest calves (especially in the first weeks of life) to cryptosporidiosis. For example, Aguilar (2023) reported a high infection rate of *Cryptosporidium* in 8–14-day-old calves, while Urie et al. (2018) identified a similar situation in two-week-old calves. Garro et al. (2016) discovered that calves up to 20 days of age shed *Cryptosporidium* oocysts more frequently, whereas Doungmala et al. (2019) indicated that the highest prevalence of infection is in 1–3-week-old calves. Ebiyo and Haile (2022) calculated that calves younger than six months have a 2.7 times greater chance of being infected with cryptosporidia.

Table 3.4. **Prevalence and intensity of *Cryptosporidium* spp. infection and the proportion of associated diarrhea in different cattle age groups**

Factor	Prevalence, % (95 % CI)	Median (Q1–Q3)	Diarrhea with *Cryptosporidium* spp., % (95 % CI)
0–3 months	39.4 (32.6–46.5)	800 (200–2400)	56.6 (44.7–67.9)
4–24 months	20.3 (17.0–23.9)	400 (400–650)	4.2 (0.1–21.1)
> 24 months	19.2 (16.7–21.9)	600 (400–1000)	7.0 (1.9–17.0)
p-value	< 0,001	0.118	< 0,001

Note: Infection parameters were calculated based on the examination results of 2655 animals. Q1–Q3 – interquartile range.

The link observed in this study between *Cryptosporidium* infection and diarrhea in dairy cows further emphasizes the clinical significance of this parasite. The higher frequency of diarrhea in infected animals, especially young calves, corresponds to the pathogenic potential of *Cryptosporidium* spp. to cause gastrointestinal tract disorders (Fayer et al., 2000). Wells et al. (2015) reported a high distribution of cryptosporidia in cows during the calving season, suggesting that calving time significantly influences the infection rate and thus the frequency of diarrhea incidence: in the period when the number of newborn calves in the herd increases, the number of shed *Cryptosporidium* spp. oocysts also increases. Thompson et al. (2017) reported that *Cryptosporidium* spp. was the most frequently identified pathogen causing diarrhea in calves up to one month old, emphasizing its impact on animal health. In contrast, Berhanu et al. (2022) noted that adult cows serve as an infection reservoir, shedding oocysts into the environment and exposing calves to the risk of infection.

Looking at the number of shed oocysts (intensity of infection), a very wide range was identified—from the minimum detectable number (200 OPG) to as many as 476500 OPG. The data distribution corresponded to a negative binomial distribution (Figure 3.5), which is characteristic of parasitic infections. This means that for most infected animals, the number of oocysts was relatively small (most often 200–2000 OPG), but a small proportion of animals shed a very large number of oocysts (more than 15000 OPG). Such overdispersion indicates that the infection intensity is not random and that individuals exist who contribute disproportionately to environmental contamination with oocysts (Ieshko, Gorbach & Parshukov 2024; Gourbière, Morand & Waxman 2015). This fact is very crucial in planning control measures, as it points to the need to identify and purposefully limit infection specifically in

these high-risk animals. It is enough for a calf to ingest only 17 *Cryptosporidium* spp. oocysts for a cryptosporidiosis infection to develop (Zambrinski et al., 2013).

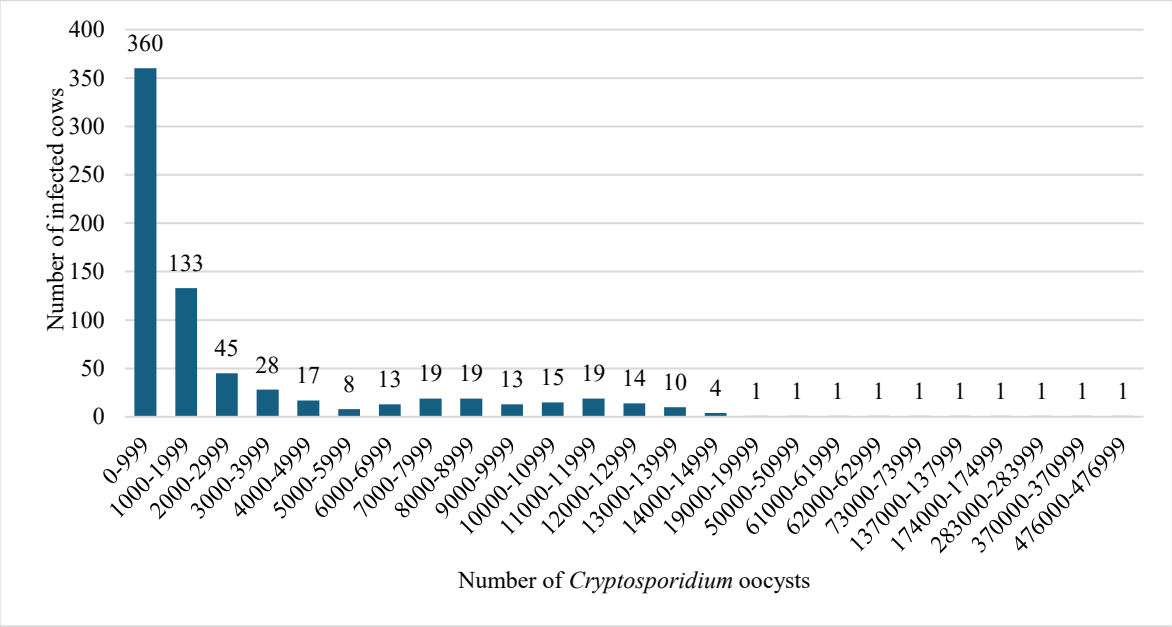


Figure 3.5 ***Cryptosporidium*** **spp. infection intensity in cows examined across all stages of the study**

Cryptosporidium prevalence and the number of oocysts per gram were unevenly distributed across Latvia's planning regions, showing the highest distribution in the Vidzeme region (31 %) and the highest number of oocysts in the Kurzeme region (median = 600, Q1 = 300, and Q3 = 1,200). Currently, it cannot be said what such a distribution of results is related to; in-depth studies of influencing factors are necessary. The distribution of the prevalence and intensity of *Cryptosporidium* infection in Latvia by planning regions is summarized in Table 3.5.

Table 3.5. **Mean prevalence (%) and mean intensity (OPG) of *Cryptosporidium* spp. infection in various regions of Latvia**

Region	Prevalence (95 % CI)	Intensity (OSG) (Q1–Q3)
Rīga	28	400 (200-800)
Kurzeme	29	600 (300-1200)
Zemgale	18	600 (400-1000)
Vidzeme	31	600 (400-1000)
Latgale	16	600 (325-900)

Evaluating the data by holding size (publication **III**, Table 3.6), it was found that *Cryptosporidium* spp. was present in the majority (72.4 %) of the studied herds (at least one infected animal). The probability of the presence of infection increased with the size of the holding: while in small holdings (< 50 cows), at least one infected animal was found in 58.9 % of cases, in medium holdings (50–200 cows) this indicator reached 94.4 %, and in large holdings (> 200 cows)—even 100 %. In turn, the total prevalence of infection in calves and cows ranged from 43.2 % in large farms (n = 13, 8–36 samples from one holding) and 30.9 % in medium farms (n = 18, 5–30 samples from one holding), to 30.8 % in small farms (n = 56, 1–14 samples from one holding). A significantly higher proportion of *Cryptosporidium* spp. was observed in large farms ($p < 0.01$).

Table 3.6. ***Cryptosporidium*** **spp. epidemiological indicators in holdings of various sizes**

Factor	Factor category	Examinated / infected	Prevalence (95 % TI)	Diarrhea proportion (95 % CI)
Farm size	1–50 cows	302/93	30.8 (25.8–36.2)	14.2 (10.7–18.7)
	50–200 cows	295/91	30.9 (25.8–36.3)	22.7 (18,3–27.8)
	> 200 cows	329/142	43.2 (37.9–48.6)	28.9 (24.2–34.0)

The highest average number of oocysts per gram of feces (725015) was observed in calves in the age group from zero to five months. In contrast, the second-highest average number of oocysts per gram of feces (115 620) was identified in cows older than 24 months. Nydam et al. (2001), who studied the number of *Cryptosporidium* oocysts in calves aged four to 12 days, found that the number of oocysts increases until day 12, reaching the maximum value, but later decreases. During the multiple studies, it was found that a six-day-old calf can produce approximately 3.89 x 10^{10} oocysts up to 12 days of age. DuPont et al. (1995) calculated that the average infectious dose that causes clinical illness in a healthy person who has not previously been infected with *Cryptosporidium* is 132 oocysts, but for a person who has previously encountered *Cryptosporidium*, the average infectious dose is 1880 oocysts (Chappell et al., 1999). This proves that calves are a significant source of human infection, as the number of *Cryptosporidium* they shed ten times exceeds the average human infectious dose. This emphasizes the need to carefully follow hygiene requirements when working with calves to reduce the distribution of *Cryptosporidium* and prevent its potential impact on human health. Furthermore, considering that cryptosporidiosis is a zoonosis, appropriate hygiene practice and care for calf health is not only an aspect of animal welfare but also an important measure for human health protection, avoiding possible infection.

4. CONCLUSIONS

1. Pilot study results showed that *Cryptosporidium* spp. is the dominant infection in dairy cow herds in the Vidzeme region. The prevalence of *Cryptosporidium* spp. infection (32.6 %) indicates a significant risk to animal health, as well as a potential risk for the spread of zoonosis (Publication **I**).
2. Immunoglobulin G levels in cow colostrum are not directly related to the distribution of *Cryptosporidium* infection in calves, indicating that IgG levels do not play a significant role in the development of *Cryptosporidium* infection (Publication **II**).
3. Four typical *Cryptosporidium* species were identified: *C. parvum*, *C. bovis*, *C. andersoni*, and *C. ryanae*. In calves up to 3 months of age, the *C. parvum* species was diagnosed in 52.6 % of cases. In 70 % of cases, it causes diarrhea. Monoinfections were diagnosed more frequently. In adult cows, *Cryptosporidium* infection does not cause clinical signs, which indicates a high potential zoonotic risk in Latvia (Publication **III**).
4. The cow's lactation number (parity) directly affects the probability of calves becoming infected with *Cryptosporidium* spp. For calves that received colostrum and transition milk from the second lactation, the probability of infection decreases by 82 %, while receiving colostrum and transition milk from the third lactation reduces it by 89 % (Publication **IV**).
5. Timely (within the first 12 hours) and appropriate (at least 6 litres) colostrum intake, followed by the consumption of transition milk for at least two weeks before weaning from the mother, significantly reduces *Cryptosporidium* spp. infection (Publication **V**).
6. The prevalence of *Cryptosporidium* infection in cows in Latvia is 27 % (95 % CI 26–29 %). Calves up to 3 months of age are infected more frequently (prevalence 39.4 %). The highest median number of oocysts shed per gram is 800 OPG (200–2400) (Publication **VI**).

5. RECOMMENDATIONS

1. Since the dominant species in calves up to 3 months of age in Latvia is the zoonotic *C. parvum*, which poses a high risk of infection to humans, it is necessary to implement stricter individual hygiene protocols (specific work clothing, hand disinfection) for personnel working with newborn calves, regardless of the presence of clinical signs in older animals.
2. In holdings with a high prevalence of infection, it is recommended to identify calves from first-lactation cows as a high-risk group. If possible, these calves should be prioritized to receive higher quality colostrum (from multiparous cows) or additional preventive measures, as calves from first-lactation cows have a significantly higher probability of infection.
3. Due to the fact that a holding size of over 50 cows is associated with a 100 % presence of infection in the herd and a higher prevalence, it is critical in large farms to reduce housing density in calving areas and perform regular, targeted environmental disinfection to interrupt the accumulation of oocysts in the environment.
4. As the highest infection prevalence (39.4 %) and intensity (800 OPG) occur specifically in calves up to 3 months of age, it would be appropriate for large holdings to implement regular coprological screening specifically for this age group. This would allow for the timely identification of mass oocyst shedders and limit the spread of infection within the herd and the surrounding environment.

IZMANTOTIE LITERATŪRAS AVOTI/ *REFERENCES*

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PIELIKUMI/ APEX

Aptaujas anketa par piena govju novietnes apsaimniekošanas un bioloģiskajiem faktoriem

Lūdzu, atbildiet uz šādiem jautājumiem par novietni un govīm pirms teļu koproloģisko paraugu ņemšanas.

1. Cik slaucamas govis ir Jūsu ganāmpulkā?

[ ] Maza novietne (≤ 10 govis)

[ ] Vidēja novietne (11–50 govis)

[ ] Liela novietne (> 50 govis)

2. Kāda ir govs (teļa mātes) šķirne?

[ ] Latvijas brūnā (LB)

[ ] Holšteinas melnraibā (HM)

[ ] Cita (krustojumi u.c.)

3. Kura laktācija ir govij pēc pēdējās atnešanās?

[ ] 1. laktācija

[ ] 2. laktācija

[ ] ≥ 3. laktācija (vairākkārt dzemdējusi govs)

4. Kurā gadalaikā teļš ir dzimis?

[ ] Ziema

[ ] Pavasaris

[ ] Vasara

[ ] Rudens

5. Cik dienas govs atradās cietstāvē pirms atnešanās?

[ ] ≤ 45 dienas

[ ] 46–64 dienas

[ ] ≥ 65 dienas

Scientific articles

1. Dace Keidāne, Anna Krūklīte, Alīna Derbakova, 2015. Prevalent parasitosis in beef and dairy cattle farms in Vidzeme region. Rural Sustainability Research. Vol. 34(329), p.21–25. DOI:10.1515/plua-2015-0009. (uz 2023. gadu – SCOPUS).
2. Alīna Derbakova, Maksims Zolovs, Dace Keidāne, Žanete Šteingolde, 2020. Effect of immunoglobulin G concentration in dairy cow colostrum and calf blood serum on Cryptosporidium spp. invasion in calves. Veterinary World. Vol. 13(1) p.165–169. DOI:10.14202/vetworld.2020.165-169. (uz 2023. gadu – SCOPUS: IF = 1.6, Q1).
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Prevalent Parasitosis in Beef and Dairy Cattle Farms in Vidzeme Region

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Abstract. The aim of the study was to investigate the beef and dairy cow parasitosis epizootic situation in Vidzeme region. Research was done throughout Vidzeme territory during the period of the years 2013-2014. The total number of animals examined was: 273 dairy and 90 young beef cattle aged from 6 months to two years and 248 dairy and 29 beef cows older than two years. For the diagnosis of helminthes standardized ovoscopic and larvoscopic methods were used. For the diagnosis of protozoa flotation and modified Ziehl-Neelsen methods were used. The main species in the samples were *Cryptosporidium* spp., *Eimeria* spp. and *Strongylus* spp. In the young dairy and beef cattle aged from 6 months to two years and cattle older than two years *Cryptosporidium* spp. invasion accordingly was 32.6% and 19% (dairy cattle) and 62.2% and 65.5% (beef cattle); the invasion of *Eimeria* spp. 30% and 7.3% (dairy cattle) and 55.6% and 10.3% (beef cattle); and the invasion of *Strongylus* spp. was 17.6% and 13.7% (dairy cattle) and 43.3% and 27.6% (beef cattle). Both dairy and beef cattle were infected with *Moniezia* spp., *Paramphistomum* spp., *Strongyloides* spp. Dairy cows aged from 6 months to two years had *Trichuris* spp., *Dictyocaulus* spp. and *Neoscaris* spp. invasion.
Key words: cryptosporidium, eimeria, strongylus, parasitosis, cattle, Latvia.

Introduction

In Latvia, there have been no requirements for farmers to carry out any coprological examination of cattle in the last 20 years. Only few researches have been carried out in the last 5-10 years (Keidāne, Krūklīte, & Medne, 2012; Lassen, 2011). Thereby, the situation regarding the most common cattle parasitosis in this country has still not been fully described.

In addition to climate change and the import of cattle, there is a great opportunity to introduce the parasite species which are not registered in the republic (Demiaszkiewicz, 2014).

Overall, worldwide cattle show a very high prevalence (95.5%) of parasite infections. Out of this percentage, 75.1% had multiple parasites while 20.4% had a single parasite infection. Prevalence of *Strongyles* spp. (63.1%) was the highest, followed by *Fasciola* spp. (51.1%), *Eimeria* spp. (29.4%), *Paramphistomum* spp. (25.9%), *Schistosoma* spp. (21.7%), *Ascaris* spp. (6.1%) and then *Moniezia* spp. (2.3%) (Squire, Amafu-Dey & Beyuo,2013; Уркхарт , 2000). The prevention of parasitic diseases is based on the study of the epizootic situation and planning of preventive measures (Jasmer, Lahmers, & Brown,2007; Heinrichs *et al.*, 2003; Forbes *et al*., 2000; Уркхарт *et.al*., 2000).

The aim of this study was to find out prevalent parasitosis in dairy and beef cattle herds in Vidzeme region.

Materials and Methods

The research was done in Vidzeme region in the period from 2013-2014. The dairy and beef cattle were investigated. Animals were divided into the following four groups: dairy cattle aged from six months to two years (n = 273) and older than two years (n = 248); beef cattle aged from six months to two years (n = 90) and older than 2 years (n = 29). Samples were obtained from 62 cattle farms: 50 dairy cattle farms and 12 beef cattle farms. Laboratory tests were made in the laboratory of the department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture.

Rectal fecal samples collected into plastic bags were kept in a refrigerator at 4˚C prior to examination. For the diagnosis of helminthes standardized ovoscopic and larvoscopic methods were used (Roepstorff & Nansen, 1998). For the diagnosis of protozoa flotation and modified Ziehl-Neelsen methods (Henriksen & Pohlenz, 1981) were used.

Parasitosis invasion extensive margin or prevalence (IE) was computed by dividing the number of infected animals by the total number of animals in a group. The correlation between the age of the animals and the validity of invasion was calculated using the t-test function in Microsoft Excel 2013 programme.

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Results and Discussion

The present study showed that in Vidzeme parasitosis were found in both age groups of dairy (Figure 1) and beef cattle (Figure 2).

In both age groups of dairy cattle, the highest infestation extensive margin (IE)was showed by *Cryptosporidium* spp. IE 32.6% and 19% ($p < 0.05$), *Eimeria* spp. IE 30% and 7.3% ($p < 0.01$) and *Strongylus* spp. IE 17.6% and 13.7% ($p < 0.05$) correspondingly. The prevalence of *Eimeria* spp. and *Strongylus* spp. in both groups is quite similar to studies in other countries (Kounty *et al.*, 2012; Knubben-Schweizer *et al*., 2010; Lassen & Talvik, 2009; Forbes *et al*., 2000).

In group of young cattle from six months to two years in addition to the above mentioned invasions, we diagnosed *Moniezia* spp. IE 6.2%, *Neoscaris* spp. (ascarids) IE 2.6%, *Trichuris* spp. IE 1.5%, *Fasciola* spp. IE 1.7%, *Paramphistomum* spp. IE 1.7%, *Strongyloides* spp. IE 1.1% and *Dictyocaulus* spp. IE 1.7%.

In cattle older than two years, we diagnosed *Moniezia* spp. IE 3%, *Trichuris* spp. IE 0.4%, *Fasciola* spp. IE 1.2%, *Paramphistomum* spp. IE 0.8% and *Dictyocaulus* spp. IE 0.4%.

Neoscaris vitulorum or calf ascarids was diagnosed in young cattle aged six months to two years IE 2.6%. As we know, adult cattle do not suffer from ascarids (Davila, Irsic, & Greiner, 2010; Zajac & Conboy, 2006).

Dictyocaulus spp. invasion in cattle from six months to two years was IE 1.7%, in cows older than two years IE 0.4% ($p > 0.05$). It is noted that the invasion of *Dictyocaulus*is spp. is more often diagnosed in young animals. (Elsheikla, 2011; Holzhauer *et al*., 2011; Hoglund, Ganheim, & Alenius, 2003). The diagnosed invasion of *Dictyocaulus* spp. in the cattle older than two years could be explained by the import of cattle from different regions of the world.

Describing the invasion of *Fasciola* spp. IE 1.7% and 1.2%, *Paramphistomum* spp. IE 1.7% and 0.8%, it can be concluded that the situation in Vidzeme region is similar to that of the previous studies about Latvia (Keidāne, Krūklīte, & Medne, 2012), but comparing with the research conducted in other countries, the prevalence of parasites in Latvia is lower. Similarly, a study in Austria found that cattle *Fasciola* spp. IE was 16% (Knubben-Schweizer *et al*., 2010). Regarding *Paramphistomum* spp., in Turkey the average IE throughout the year was 10.4% (Ozdal *et al*., 2010).

In beef cattle, like in dairy cattle, the highest IE both for young cattle from six months to two years and cattle older than two years has been observed in *Cryptosporidium* spp. IE 62.2% and 65.5% ($p < 0.01$), *Eimeria* spp. 55.6% and 10.3% ($p < 0.01$) and *Strongylus* spp. IE 43.3% and 27.6% ($p < 0.01$) invasions (Figure 2). Completely different results were obtained from researchers in Alberta, Canada – the prevalence of *Cryptosporidium* spp. in beef cattle calves is 5% (Ralston, McAllister, & Olson, 2003; Gow & Waldner, 2006). Compared with other countries, *Eimeria* spp. IE in Latvia is slightly higher: studies in Turkey found *Eimeria* spp. IE for calves in beef cattle herds 27.2% (Cieek *et al*., 2007), 33.3% in Hungary (Farkas, Szeidemann & Majors,2007), 22.6% in Brazil (Almeda *et al*., 2011). *Strongylus* spp. IE in Latvia is lower than in other countries, for example, in Thailand IE is 100% (Lwin, 2011).

Along with the invasions mentioned above, young cattle were infected with *Moniezia* spp. IE 8.9% and *Strongyloides* spp. IE 1.1%, whereas in cattle older than two years *Paramphistomum* spp. IE 3.4% was diagnosed. In comparison with other countries, the invasion of these parasitosis in our country is

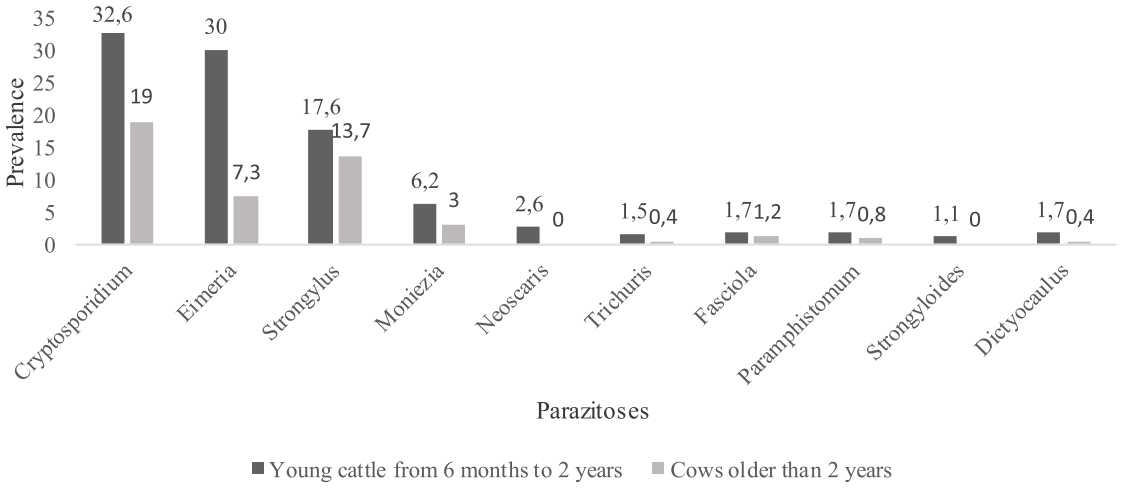

Figure 1. Prevalence (%) of parasite invasion in dairy cattle.

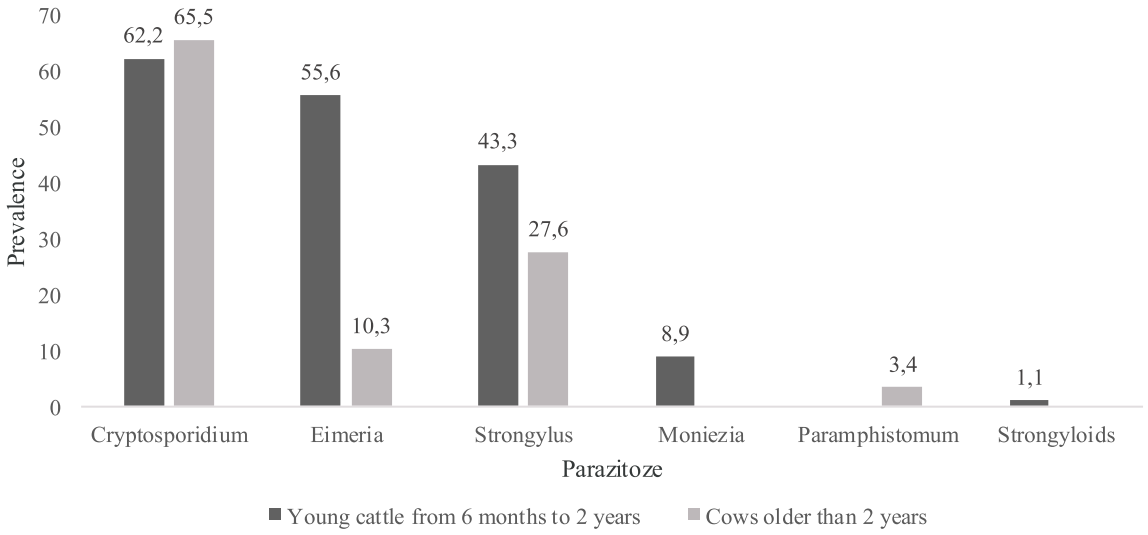

Figure 2. Prevalence (%) of parasite invasion in beef cattle.

much lower. The research in Spain reported about *Paramphistomum* spp. IE was 29% (Gonzales-Warleta *et al*., 2013), in Thailand IE was 6.4% in beef cattle under 1 year old, and IE was 23.3% of cattle from one to two years old (Lwin, 2011). Similar IE has been mentioned regarding *Moniezia* spp.: in Thailand *Moniezia* spp. IE in cattle under one year was 12.9%, from one to two years old it was 23.3% (Lwin, 2011). Relatively higher *Strongyloides* spp. IE was found in the Czech Republic - 4.3% (Kvač & Vitovec, 2007).

In general, younger animals are most likely to show signs of parasitism, while mature cows acquire a degree of immunity to parasites that reside in the gastrointestinal tract. Dairy cattle in a dry lot are less likely to have heavy worm infection than beef cattle on pastures (Gadberry & Powell, 2011).

The results show that we should pay more attention to studies of cryptosporidiosis in Latvia because this disease has not been thoroughly investigated. Our research showed a high *Cryptosporidium* spp. invasion both in dairy and beef cattle herds. According to the sources of scientific literature, cryptosporidiosis is a serious invasion that is registered not only in the tropical zone countries (Kounty *et al*., 2012; Уркхарт *et al*., 2000), but also in Europe and it is shown by the recent research conducted in Latvia (Keidāne, Krūklīte, & Medne, 2012; Lassen, 2011). We didn't include calves aged from the first to the tenth day of life in this study, which are in a high-risk group. Therefore, we will continue studies of the *Cryptosporidium* spp. invasion, treatment and prevention.

Conclusions

- In dairy cattle herds in the age group of six months to two years were diagnosed with low invasion of ascarides, *Dictyocaulus* spp. and *Trichuris* spp.
- Dairy cattle aged from six months to two years and cattle older than two years were more frequently infected with *Cryptosporidium* spp. IE 32.6% and 19%, *Eimeria* spp. IE 30% and 7.3% and strongylatoses of digestive system IE 17.6% and 3.7%.
- Beef cattle aged from six months to two years and cattle older than two years, were more frequently infected with *Cryptosporidium* spp. IE 62.2% and 65.5%, *Eimeria* spp. IE 55.6% and 10.3% and *Strongylus* spp. 43.3% and 27.6%.
- Both dairy and beef cattle herds were infected with *Moniezia* spp., *Paramphistomum* spp. and *Strongyloides* spp.

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Effect of immunoglobulin G concentration in dairy cow colostrum and calf blood serum on Cryptosporidium spp. invasion in calves

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RESEARCH ARTICLE
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Effect of immunoglobulin G concentration in dairy cow colostrum and calf blood serum on *Cryptosporidium* spp. invasion in calves

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Abstract

Aim: The research aimed to test the association between the level of immunoglobulin G (IgG) in bovine colostrum and calf blood serum and to evaluate its relation to *Cryptosporidium* spp. invasion in calves.

Materials and Methods: Fresh colostrum and fecal specimens from cows (n=114) as well as blood and fecal specimens from newborn calves (n=114) were collected in the dairy cattle farm. Investigated calves were separated from their mothers directly after birth and received 2 L of colostrum in two separate feedings within the first 24 h. Blood samples were taken from calves at the age of 2 days. Coprological samples were taken from calves at the age of 1, 10, and 15 days. Both colostrum and fecal samples from cows were taken on the 1st day after calf birth. Rectal fecal samples were collected separately from each calf and cow into plastic bags. The collected calf serum samples and bovine colostrum samples were tested for bovine IgG by competitive enzyme-linked immunosorbent assay kit bovine Ig. To record oocysts of *Cryptosporidium* spp. in feces, the flotation method was used. Binomial logistic regression was performed to ascertain the effects of IgG in bovine colostrum and calf blood serum on the likelihood of *Cryptosporidium* spp. infection in calves.

Results: The concentration of IgG in bovine colostrum was higher (70.7±26.6 g/L, mean±standard deviation) than that in calf blood serum (13.2±6.1 g/L); the statistically significant difference was 57.4 g/L (95% confidence interval, 52.4-62.4), t (124.872)=22.536, p<0.001. Mann–Whitney's U-test showed a significant difference between samples collected on days 10 and 15 of the experiment (U=1944, z=2.330, p=0.020). The higher number of oocysts in calf feces was recorded on day 15 (median=6.5) compared to day 10 (median=4). The prevalence of calf infection from days 10 to 15 increased from 26.3 to 45.6% and was at least 3 times higher than in cows. A statistically significant positive correlation was recorded between IgG concentration of cow colostrum and calf blood serum (r (114)=0.414, p=0.001), whereas a correlation between the concentration of IgG and the intensity of *Cryptosporidium* spp. infection was not recorded (p>0.05). The logistic regression model was not statistically significant ($\chi^2(2)=0.013$, p=0.99 (10 days) and $\chi^2(2)=0.100$, p=0.95 (15 days)).

Conclusion: Mother passive transfer of immunity to the offspring through colostrum does not influence the susceptibility of calves to *Cryptosporidium* infestation.

Keywords: calves, *Cryptosporidium*, dairy cows, immunoglobulins.

Introduction

Cryptosporidiosis is a frequent disease in neonatal dairy and beef cattle calves. *Cryptosporidium* spp. cause varying degrees of naturally occurring diarrhea in farm animals. For example, *Cryptosporidium parvum* is an economically important parasite that causes neonatal diarrhea in calves, lambs, and goat kids. Ignoring the presence of this parasite in farms may result in increased costs for the labor involved in supporting these calves during cryptosporidiosis or even in the death of infected calves [1]. The importance of *Cryptosporidium* spp. is also highlighted by their zoonotic and anthroponotic nature and by the numerous paths of parasite transmission: Water, food, clothes, and footwear. *Cryptosporidium* is a reason for the common cause of acute diarrhea in immunocompetent individuals [2] and recognized as a major waterborne parasite worldwide [3].

The parasites commonly act in concert with other enteropathogens to produce intestinal injury and diarrhea [4]. Colostrum is the first source of newborn food that not only feeds the animal but also provides necessary components for protection. Therefore, receiving adequate colostrum immediately after birth is needed to help prevent the invasion of opportunistic pathogens which can worsen or compound the severity of disease in calves with cryptosporidiosis. Such compounds necessary are immunoglobulins (Ig), which are glycoproteins that specifically recognize and bind to antigens present on pathogens. As Ig have a high

degree of specificity, they assist in the destruction of specific pathogens. In animals with the first and second placenta types, such as cows, and Ig are not transferred from maternal blood to the fetus. However, calves receive Ig through passive transfer from colostrum [5]. There are several classes of Ig, including IgA, IgD, IgE, IgG, and IgM [6]; however, most of them are present in colostrum at low concentrations. Of these, IgG are of particular interest because they are the primary Ig found in bovine colostrum and milk and play the main role in the development of humoral immunity [7]. The concentration of IgG in colostrum may reach up to 50–100 mg/ml, and by passive transfer, they provide effective prevention or treatment of several human or animal diseases caused by pathogens (*Yersinia enterocolitica*, *Campylobacter jejuni*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella typhimurium*, *Staphylococcus*, *Streptococcus*, *Cryptosporidium*, etc.) [8]. As Ig can prevent the adhesion of pathogens to intestinal epithelial cells, they act as the primal protection against most of the potential gastrointestinal pathogens. For example, IgG by adhesion to invasive stages of *Cryptosporidium* may play a role in preventing invasion into host cells.

The concentration and effect of IgG on calf health have repeatedly been studied [9-14]. For example, Johnsen *et al*. [9] attempted to develop a less invasive and easy method to measure IgG concentration, whereas Aydogdu and Guzelbektes [10] compared colostrum composition between primiparous and multiparous dairy cows. However, only a few investigations of Ig focused on parasitic infections [15,16]. The effects of cryptosporidiosis on calf growth in the long term have not yet been shown, but occurring diarrhea may be costly for farmers due to the loss of income from lower carcass weights. Petry *et al*. [17] noted that the protective role of antibodies is questionable because high titers of parasite-specific IgG can be found in AIDS patients with chronic cryptosporidiosis.

In light of this, this study aimed to test the association between the level of IgG in bovine colostrum and the calf's blood serum and to evaluate its relation to *Cryptosporidium* spp. invasion in calves. We expected to find an immunological link between cow immunity and the passive transfer of immunity to calves, related to *Cryptosporidium* spp. infection.

Materials and Methods

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards. The study was approved by the Animal Welfare and Ethical Council of the Faculty of Veterinary Medicine, Latvia, University of Life Sciences and Technologies, and complied with current laws in Latvia.

Sample collection

Fresh colostrum and fecal specimens from cows (n=114) as well as blood and fecal specimens from newborn calves (n=114) were collected in the dairy cattle farm between December 2018 and March 2019. Investigated calves were separated from their mothers directly after birth and received 2 L of colostrum in two separate feedings within the first 24 h. Blood samples were taken from 2-day-old calves. Coprological samples were taken from calves at the age of 1, 10, and 15 days. Both colostrum and fecal samples from cows were taken on the 1st day after calf birth. Rectal fecal samples were collected separately from each calf and cow into plastic bags, marked, and kept in a refrigerator at 4°C before examination. If the number of feces was too small (especially in the 1st days of the calves' life), native smears were made. Before laboratory investigation, blood samples were centrifuged to obtain blood serum and stored at −80°C. The colostrum was stored at the same temperature. During the research, no animals were subjected to unnecessary pain or distress.

Serological techniques

The collected calf serum samples and bovine colostrum samples were tested for bovine IgG, using the competitive enzyme-linked immunosorbent assay (ELISA) kit bovine Ig (Bio – X Diagnostics, Belgium) in the Institute of Food Safety, Animal Health, and Environment "BIOR," Serology Division, Latvia. Samples were tested according to the manufacturer's instructions, and the calibration curve for calf serum and bovine colostrum was established. Calf serum samples were diluted 1/100, and colostrum samples were diluted 1/1000. In the dilution microplate wells, 100 µL of the calibration curve dilutions and diluted samples were transferred, and diluted conjugate was added to each well, mixed, and 100 µL of the content were transferred to the kit's microplate wells. The microplate was incubated at +21±3°C for 1 h. Subsequently, the microplate was rinsed 3 times with a washing solution, and 100 µL of chromatogen solution were added to each followed by incubation at +21±3°C for 10 min in the dark. The reaction was stopped by adding 50 µL of stop solution to each well. The optical density of the investigated samples was determined using a monochromatic ELISA reader (Thermo Scientific Multiskan FC) with a 450-nm filter. The Ig concentrations were calculated using "Four Parameter Logistic Curve" online data analysis tool, MyAssays Ltd., 10th March 2017, http://www.myassays.com/four-parameter-logistic-curve.assay.

Coprological examination

All coprological samples were exanimated on the collection day. Laboratory examinations were made in the Laboratory of Parasitology, Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture. To record oocysts of *Cryptosporidium* spp. in feces, the flotation method was used according to Fujino *et al*. [18]. Slides were stained using the modified Ziehl-Neelsen method [19].

Statistical analysis

To test the difference between the means of two groups, we used the independent-samples t-test or the Mann–Whitney U-test. The assumption of normality was tested by Shapiro–Wilk's test and assumption of homogeneity of variances by Levene's test. To determine the strength and direction of a linear relationship between two continuous variables, we used Pearson's correlation. Binomial logistic regression was performed to ascertain the effects of IgG in bovine colostrum and calf's blood serum on the likelihood of *Cryptosporidium* spp. infection in calves. The linearity of the continuous variables with respect to the logit of the dependent variable was assessed through the Box and Tidwell procedure [20]. Bonferroni correction was applied using three terms in the model, resulting in statistical significance being accepted when $p<0.016667$ [21]. Based on this assessment, all continuous independent variables were found to be linearly related to the logit of the dependent variable.

Results

A Welch t-test was run to determine if there were differences in the concentrations of IgG between bovine colostrum and calf's blood serum due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances ($F=127.433$ $p<0.001$). Concentrations of IgG for bovine colostrum and calf blood serum were normally distributed ($p>0.05$). The concentration of IgG in bovine colostrum was higher (70.7±26.6 g/L, mean±standard deviation) than that in calf blood serum (13.2±6.1 g/L); the statistically significant difference was 57.4 g/L (95% confidence interval, 52.4 to 62.4), t (124.872)=22.536, $p<0.001$. The number of *Cryptosporidium* spp. oocysts in feces was not normally distributed ($p<0.05$). In the 1st day of the experiment, the prevalence of *Cryptosporidium* spp. was 0%. Mann–Whitney U-test showed a significant difference between samples collected on days 10 and 15 of the experiment; $U=1944$, $z=2.330$, $p=0.020$. The higher number of oocysts in calf feces was recorded on day 15 (median=6.5) compared to day 10 (median=4) of the experiment. The prevalence of calf infection from days 10 to 15 increased from 26.3 to 45.6% and was at least 3 times higher than in cows.

A statistically significant positive correlation was recorded between concentrations of IgG in cow colostrum and calf blood serum (r (114)=0.414, $p=0.001$), whereas a correlation between concentrations of IgG and intensity of *Cryptosporidium* spp. infection was not recorded ($p>0.05$; Figure-1). The logistic regression model was not statistically significant; $\chi^2(2)=0.013$, $p=0.99$ (10 days) and $\chi^2(2)=0.100$, $p=0.95$ (15 days), indicating the lack of relationships between the concentration of IgG (in bovine colostrum and calf blood serum) and calf infection with *Cryptosporidium* spp.

Discussion

The main results showed that calves are born with lower IgG concentrations in the blood compared to cow colostrum. The concentration of IgG in calf blood is directly related to feeding with cow colostrum. Although there is an immunological link between immunity of the mother and passive transfer of immunity to the offspring [22], this study showed the lack of a relationship between concentrations of IgG (both mother and offspring) and *Cryptosporidium* spp. infection. Surprisingly, there was an increase in the infection prevalence of *Cryptosporidium* spp. because colostrum and milk contain not only IgG but also a range of other components such as neutrophils, macrophages, lymphocytes, antimicrobial factors, and other molecules that provide energy for an effective immune response [23-25].

The immune responses to *Cryptosporidium* species infection involve both innate and adaptive immunity. Numerous studies have described the role of T and B cells, intestinal epithelial cells, interferon-gamma and natural killer cells, nitric oxide, antimicrobial peptides, prostaglandins, mannose-binding lectin, cytokines, chemokines, dendritic cells, and

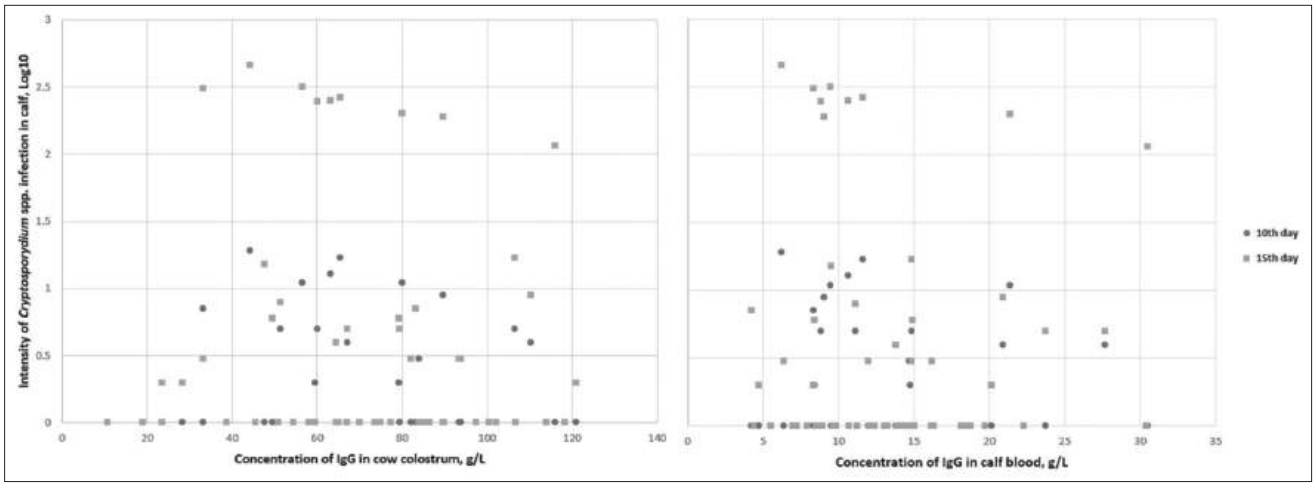

Figure-1: Association between the concentration of immunoglobulin G and intensity of *Cryptosporidium* spp. infection in calves.

macrophages in the formation of immune responses to *Cryptosporidium* spp.; a summary of these studies may be found in the reviews of Leitcha and Heb [26] and Vanathy *et al*. [27]. Our study indicates that innate and adaptive immunity play more significant roles in immune responses to *Cryptosporidium* species than mother passive transfers of immunity to the offspring. Furthermore, Siachos *et al*. [28] suggest that passive transfer of Ig is not protective against cryptosporidiosis. However, Ajjampur *et al*. [29] have studied antibody responses to specific antigens (gp40 and gp15) before and after the first episode of symptomatic cryptosporidiosis in children and found significant increases of IgG levels in response to *Cryptosporidium* antigens. Similar results have been obtained by Allison *et al*. [30], who studied antibody responses to the immunodominant gp15 antigen from *Cryptosporidium hominis* and *C. parvum*. They also recorded that IgM response occurs immediately after an acute infection and levels decrease within weeks, whereas IgG responses are slower to appear but persist for a longer period.

Previous studies of *Cryptosporidium* prevalence in the USA [31] and China [32] coincide with our findings that calves have a higher prevalence of infection than cows. For example, Santín *et al*. [33] concluded that the prevalence of *Cryptosporidium* species is age-related between pre-weaned and post-weaned calves. Harp *et al*. [34] demonstrated that initial exposure of *C. parvum* to calves (from birth to 3 months) and their recovery renders calves resistant to further challenge with the parasite. Most likely, these findings indirectly highlight the significance of adaptive immunity to *Cryptosporidium* infection. However, Gong *et al*. [32] have indicated that *Cryptosporidium* species/subtypes vary among the different age groups of cattle, suggesting that infection with a single species of *Cryptosporidium* and further recovery do not guarantee the inability of cryptosporidiosis caused by other species. Thomson *et al*. [35] have suggested that the ability of the parasite to infect the gut is linked to changes in the gut microflora during animal maturation, although there are no experimental trials demonstrating this in cattle.

Conclusion

We found that mother passive transfer of immunity to the offspring through colostrum does not influence the susceptibility of calves to *Cryptosporidium* infestation.

Authors' Contributions

AD developed the study design and carried out the experiment. ZS performed calf serum and bovine colostrum sample serological examination. DK supervised the research process. MZ performed the statistical analysis of all data. AD and MZ wrote the manuscript with input from all authors. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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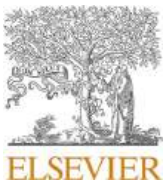

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Original Article

Prevalence, risk factor and diversity of *Cryptosporidium* in cattle in Latvia

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ABSTRACT

The epidemiology of *Cryptosporidium* spp. in Latvia was investigated by testing fecal samples from 926 animals aged from one day to 24 years for the presence of *Cryptosporidium* spp. oocysts. The samples were collected from 87 cattle farms and from four slaughterhouses, and analyzed by conventional and fluorescent microscopy, followed by *Cryptosporidium* species and *C. parvum* subtype differentiation. Moreover, using a questionnaire, we surveyed factors that could be relevant as risk factors of *Cryptosporidium* spp. infection on the farms. *Cryptosporidium* spp. were shed by 33.8% of the investigated cattle and at least one shedding animal was found on 77.8% of the farms. In the present study, all four *Cryptosporidium* species reported to commonly infect cattle and two additional *Cryptosporidium* species (*C. scrofarum* and *C. ubiquitum*) were identified. In addition, mix infections of *C. parvum/C. bovis*, *C. bovis/C. ryanae*, *C. parvum/C. ryanae*, *C. parvum/C. andersoni* and *C. bovis/C. andersoni* were observed.

C. parvum and *C. bovis* was mostly prevalent in young animals (0–3 months old) and in addition, diarrhea associated with *C. parvum* infection was observed only in very young animals. *Cryptosporidium andersoni* and *C. ryanae* in age group 0–3 months was observed in low prevalence, while a higher proportion of animals with diarrhea associated with *C. andersoni* infection was observed in very young animals and with *C. ryanae* in animals age group 4–24 months. Eight previously described *C. parvum* subtypes were observed. The majority of the subtypes were in the IIa subtype family, while one subtype was identified from the IId subtype family. The most common subtype was IIaA15G2R1, which was found in 34.2% of the *C. parvum* successfully subtyped samples. The probability of *Cryptosporidium* spp. associated diarrhea in cattle decreased significantly with the age of the animals and a prolonged period during which calves were fed with milk.

1. Introduction

Cryptosporidiosis is caused by coccidian parasites of the genus *Cryptosporidium*, of which there are more than 40 species and an equal number of *Cryptosporidium* genotypes of unknown species status (Feng et al., 2018; Bolland et al., 2020; Holubová et al., 2020). Worldwide surveys carried out in cattle (*Bos taurus*) have shown that nine species and at least two genotypes were responsible for *Cryptosporidium* infections in this host species (Fayer et al., 2008; Xiao and Fayer, 2008; Imre and Dărăbus, 2011; Razakandrainibe et al., 2018). Calves are most commonly infected with the zoonotic species *C. parvum*. While older cattle after weaning tend to be much more commonly infected with *C. andersoni* or *C. bovis* (Santín et al., 2004; Chako et al., 2010). In addition to *C. parvum*, *C. andersoni* is of minor public health significance (Šlapeta, 2013). *Cryptosporidium bovis* and *C. ryanae* are both cattle-adapted and they have not been found to be infectious to humans (Fayer et al., 2008; Nichols et al., 2014). Frequently *C. scrofarum* and *C. ubiquitum* genotypes are found in cattle (Xiao and Fayer, 2008; Diaz et al., 2010; Fayer et al., 2010; Imre and Dărăbus, 2011). *Cryptosporidium ubiquitum* it is of minor public health importance, however, it is a

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zoonotic species and person-to-person transmission has been discussed (Fayer et al., 2010).

Cryptosporidiosis most commonly affects neonatal calves (Xiao and Ryan, 2004). Young animals are at greater risk for both infection and disease, and the primary clinical sign in calves is a profuse watery diarrhea. Approximately half of dairy calves between ages of one to three weeks are shedding oocysts at any time (Sturdee et al., 2003; Maddox-Hyttel et al., 2006; Santín et al., 2008; Åberg et al., 2020). Young animals are more likely to be infected with *Cryptosporidium* spp. and develop cryptosporidiosis, and the species causing infection vary with host age (Sturdee et al., 2003; Santín et al., 2004; Santín et al., 2008; Åberg et al., 2020). Several studies have suggested that the immunity against one *Cryptosporidium* species does not extend to other species (Follet et al., 2011; Thomson et al., 2019). Moreover, numerous farm management characteristics (including herd size, type of herd, water sources and weaning modality) have been evaluated as potential risk factors for the transmission of these pathogens on farms (Ramirez et al., 2004; Kváč et al., 2006). For example, if the calves were bred in the cowshed together with dams, and contact between calves was limited, there was a statistically lower prevalence of *C. parvum* than in the individual box technology with direct contact among calves (Kváč et al., 2006).

Among food-borne diseases, the burden of cryptosporidiosis at regional and national levels is largely unknown (FAO/WHO, 2014; Lake et al., 2015). Although cryptosporidiosis is a leading cause of diarrhea morbidity and mortality in children younger than 5 years, the disease generally has an acute non-fatal outcome with few long-term consequences (Scallan et al., 2011; Khalil et al., 2018). The burden of disease in high-income countries due to zoonotic *Cryptosporidium* spp. is low (Torgerson and Macpherson, 2011). Nevertheless, cryptosporidiosis and *Cryptosporidium* spp. was recently ranked as the fifth most important foodborne parasite in Europe (Bouwknegt et al., 2018).

In Latvia, cryptosporidiosis in humans is a notifiable but most-likely under-reported disease (Plutzer et al., 2018; van der Giessen et al., 2021). Surveillance data do not provide a good overview of the epidemiology of cryptosporidiosis, and the need to fill the knowledge gaps with a One Health approach is evident as there is a lack of studies on cryptosporidiosis in humans and *Cryptosporidium* spp. presence in animals in Latvia. Previous study has shown that 69% of the studied Latvian cattle farms had cattle that were shedding *Cryptosporidium* spp. (Lassen, 2011). However, data on the epidemiology and the zoonotic potential of *Cryptosporidium* spp. shed by cattle and the circulating *C. parvum* subtypes in Latvia are scarce. Studies from neighboring countries showed that *Cryptosporidium* spp. herd prevalence ranged from 66% in Estonia to 100% in Lithuania (Lassen and Järvis, 2009; Santoro et al., 2019).

To fill the existing gaps of knowledge, the aims of this study were to study the prevalence of *Cryptosporidium* spp., including species and subtype determination as well as to investigate the risk factors that could be relevant for cryptosporidiosis in Latvian cattle.

2. Materials and methods

2.1. Study design

A sampling was conducted in the Latvian cattle with a population size of 395,320 cattle (Agricultural Data Centre Republic of Latvia, www.ldc.gov.lv, accessed on 1st of April 2020), with the aim to obtain baseline data on the *Cryptosporidium* spp. prevalence and to analyze *Cryptosporidium* species and sub-type diversity. The study population consisted of 926 individual samples (1–36 samples per farm) from 87 farms. To decrease a regional bias, the sampling was proportionally stratified to the counties of Latvia. Sampling of farms was biased by volunteering for investigation upon selection and contact. The main inclusion criterion was dairy farming, including farms with different management and size, thus ranging from small family farms with one animal to large farms with more than thousand animals. Farmers or veterinarians volunteering to participate were asked to collect individual fecal samples. Following this instruction, up to 15 animals from each animal age groups were collected (0–3 months, 4–24 months, older than 24 months) per each farm in total up to 45 samples per farm. In case where less than 15 animals from specific age group were present, samples from all animals of this group were collected. To obtain a better regional distribution, additional samplings were done once in four slaughterhouses and samples from all slaughtered animals at the day of visit were collected (8–62 samples per slaughterhouse). Information from the animals sampled in slaughterhouses were collected from Agricultural Data Centre Republic of Latvia (www.ldc.gov.lv, accessed on 1st of April 2020) and used on farm level. Visits to farms and slaughterhouses were completed in the period between July 2018 and June 2019. In most cases, individual fecal samples were collected from the rectum. In rare cases, samples were collected during defecation or from the ground, immediately after an individual animal had defecated. In the slaughterhouses, samples were collected from all slaughtered animals at the day of visit from the large intestine after intestines had been eviscerated. Samples were collected in disposable gloves and stored in a transportable cooler during transport to the laboratory where stored at +4 °C until examined.

Overall, 926 individual samples were collected from all territory of Latvia (Fig. 1).

2.2. Questionnaire

A questionnaire was designed to collect information on diarrhea in cattle on the farms as well as on factors with potential relevance of infection of *Cryptosporidium* spp. on farms. The questionnaire in Latvian language was filled in by the farmer during sampling. In case of the samples collected in slaughterhouses, only information available in the official database was collected (Agricultural Data Centre Republic of Latvia, www.ldc.gov.lv). The questionnaire covered feed-related (feed and feed preparation/storage), breeding and housing-related, hygiene and general health-related variables and further used for statistical analysis (Supplementary material 1).

2.3. Conventional and fluorescent microscopy analyses

Samples for conventional microscopy were processed by a saturated NaCl flotation method which had previously been described as highly efficient in recovering oocysts from fecal samples (Kuczynska and Shelton, 1999). For the flotation, 1 g of fecal sample was used and after flotation and centrifugation steps, 2 ml of purified material were available for subsequent analyses.

In the first step all samples of purified material were examined. A 10 µl drop of purified oocysts was applied to a microscopic slide, dried at

Fig. 1. Distribution of sampled farms and presence of cattle shedding *Cryptosporidium* spp. oocysts.

room temperature and stained with a modified Ziehl-Neelsen technique using the TB Stain kit (BD, Ireland). Positive controls had previously been established and were included in all batches of analysis. For enumeration, all dark red to pink oocysts with a typical morphology were counted for each of the 10 µl drops using a 200× magnification in microscopy. Thus, each microscopically detected oocyst represented 200 oocysts per one gram of feces (OPG).

Further, all samples, negative by conventional microscopy were re-analyzed by fluorescent microscopy using the AquaGlo kit (Waterborne INC, USA) for labelling *Giardia* spp. cysts or *Cryptosporidium* spp. oocysts by specific antibodies. The same concentrated samples previously used for Ziehl-Neelsen technique were further processed for the fluorescent microscopy as follows: 10 µl of the thoroughly suspended purified oocysts was added to the well of a Teflon slide (i.e. a Teflon slide with three 12 mm-wells; Immuno-Cell, Mechelen, Germany), dried on the well and fixed by submerging the slide in methanol. Subsequently, the material was stained with FITC-labeled anti-*Cryptosporidium/Giardia* mAbs (AquaGlo, Waterborne, Inc., USA) for 30 min in moisture chamber, before rinsing the antibody-solution off with PBS. After the slide was completely air-dried, seven microliters of mounting fluid were added to each well and covered with cover slide. For enumeration, brightly stained oocysts with typical morphology were counted in all wells at 200× magnification. Each detected oocyst represents 200 OPG.

2.4. DNA extraction, polymerase chain reaction and sequencing

Genomic DNA was extracted from the pellets obtained after centrifugation of the 2 ml purified fecal sample using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Elution was done with 80 µl of Solution C6 (i.e. the elution buffer of the DNeasy PowerSoil Kit). Two microliters of each DNA sample were submitted to polymerase chain reaction (PCR) amplification targeting the 18S rDNA as previously described (Xiao et al., 1999; Åberg et al., 2019). Nuclease-free water and *C. parvum* genomic DNA were used as negative and positive controls. The first amplification mixture contained 1× of KAPA2G Buffer (KAPA Biosystems), 200 µM each of deoxynucleoside triphosphate, 0.5 µM each of primary primers, 2 µl DNA solution in total volume of 25 µl. After initial denaturation at 95 °C for 3 min, 40 cycles followed, consisting of 95 °C for 30 s, 61 °C for 30 s, 72 °C for 1 min and a final extension of 72 °C for 2 min. For the second amplification, 2 µl from the first reaction were added to the reaction mixture as above, except containing secondary primers. The nested PCR reaction conditions was 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 63 °C for 30 s, 72 °C for 1 min and a final extension of 72 °C for 2 min. The PCR products were run on capillary electrophoresis (QIAxel Advances, QIAGEN, Germany). Products of the expected size (approximately 820 bp) were submitted to sequencing for species identification.

The samples that differentiated as *C. parvum* were submitted to PCR amplification targeting the 60 kDa glycoprotein (*gp60*) gene for subtype identification (Peng et al., 2001) using the reaction mixture as above. Products of approximately 490 bp were selected for subsequent sequencing.

The PCR products were cleaned up and sequenced in both directions with the Applied Biosystems® 3130xl Genetic Analyzer. Forward and reverse sequences were aligned with the BioEdit v7.2.5 software (Hall, 1999) to generate single consensus sequences and correct mismatches. The resulting sequences were compared with nucleotide sequences deposited in GenBank using BLASTn (nucleotide Basic Local Alignment Search Tool, Altschul et al., 1990). *Gp60* subtypes were named in agreement with the system proposed by Sulaiman et al. (2005) based on the number of serine-coding trinucleotide repeats. To identify mixed infections the CryptoGenotyper tool on Galaxy was used (Afgan et al., 2016; Yanta et al., 2021). All sequences were analyzed using the 18S contig workflow.

2.5. Statistics

For the counted OPGs, means and medians were calculated to summarize data for particular age groups and farm size levels. For calculating confidence limits for point estimated proportions (e.g. proportion of *Cryptosporidium* positive animals) we assumed a binomial distribution and 95% confidence intervals were calculated according to Wilson (1927) using the Mid-p Exact of the open source software OpenEpi v.2.3.1 (Dean et al., 2015). Two-tailed $p < 0.05$ was considered statistically significant.

For statistical analysis a farm was considered positive if at least one animal of the investigated animals within the farm excreted *Cryptosporidium* spp. oocysts.

An animal was considered *Cryptosporidium* spp.-positive if its sample tested positive at least with one of the microscopy methods. In risk factor analysis, a variable combining the microscopy results for *Cryptosporidium* spp. of cattle and the record of diarrhea in the individual animal ("Crypto+Diarrhea") was considered as the dependent variable and the animals with *Cryptosporidium* spp. and diarrhea positive records were later called "animals with *Cryptosporidium* spp.-associated diarrhea".

For the identification of potential risk factors (including individual animal and herd level factors), multilevel-modelling [generalized linear mixed modelling fit by maximum likelihood (Laplace approximation)] was performed using R (http://www.R-proje ct.org) version 3.3.1, by applying the package lme4, i.e. using the glmer function and assuming a binomial distribution (Fig. 2), including individual farm ID identification number ("FarmID") as a random effects variable. With the exception of age, animal gender, animal breed and origin (moved or bought), as well as body temperature (high, low, normal) all factors assessed were herd level data (Supplementary material 2). Because no detailed farm level data were available for slaughterhouse animals, results on these animals were not assessed during risk factor analysis. Because age was expected to be an important effect-modifying explanatory variable, as confirmed in the present study, data on age (in months) of individual animals were included into each of the models calculated to identify putative risk or protective factors for *Cryptosporidium* spp.-associated diarrhea.

To select from those explanatory variables (ExplVar) which were significant ($P < 0.05$) or tended to be significant ($0.05 \leq P < 0.1$) in the

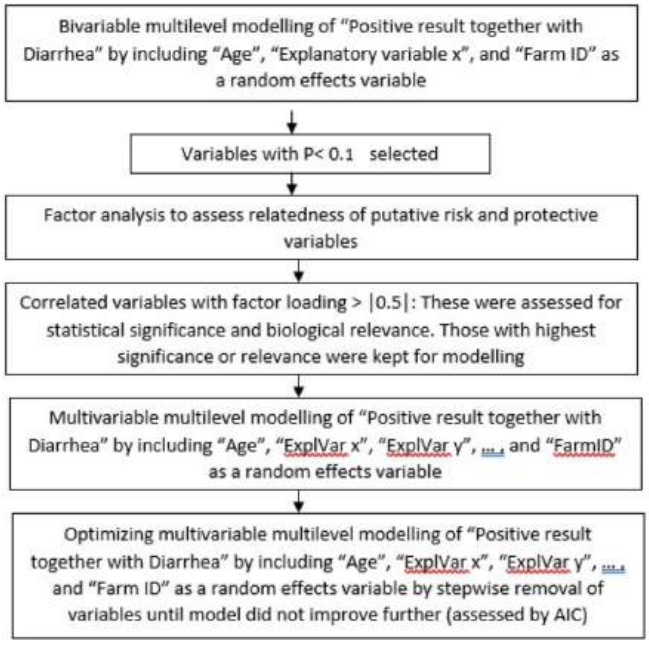

Fig. 2. Flow chart on data analysis to assess potential risk factors for cryptosporidiosis in cattle.

initial analysis step (Fig. 2; bivariable multilevel modelling) those which were independent of each other in the dataset, a factor analysis (assuming a maximum number of possible factors) was performed using the command "factanal" (scores = 'Bartlett'). Absolute factor loadings of >0.5 were regarded as an indication of dependence between ExplVar's (Fig. 2). In case factor analysis models indicated that some of the tested ExplVar's were dependent from each other, two strategies were followed: i. The number of ExplVar's was reduced to one per factor by choosing among these variables the ExplVar which returned the best (i.e. lowest) Akaike information criterion (AIC) in the bivariable multilevel-modelling (Fig. 2). ii. In two cases those ExplVar's were excluded which were regarded as less relevant to have a biological effect on the occurrence of *Cryptosporidium* spp.-associated diarrhea. With the remaining variables, factor analysis was repeated until no further dependence between ExplVar's was observed.

In the last step, only independent ExplVar's were included into a generalized linear mixed model (Fig. 2, multivariable-multilevel-model) to determine potential risk or protective factors for *Cryptosporidium* spp. associated diarrhea in cattle. After optimization by a stepwise elimination of those variables that, if removed, did not cause an increase in AIC, a final linear mixed model was generated.

3. Results

3.1. Overall prevalence of Cryptosporidium spp.

The age of sampled animals varied from one day to 24 years. Because there was a high variability of numbers of collected samples per animal age months, we divided the samples in three age groups. The highest number of samples were collected from animals older than 24 months (Table 1). A total *Cryptosporidium* spp. oocyst prevalence of 33.8% (95% CI 31.0–36.9%) was observed. Out of the 626 samples that tested negative with the Ziehl-Neelsen technique, additional 25 (4.0%; 95%CI 2.7–5.8%) samples were tested positive with fluorescent microscopy using the AquaGlo kit. The proportion of infected cattle and proportion of *Cryptosporidium* spp.-associated diarrhea significantly ($p < 0.05$) decreased with the age (Table 1). The highest prevalence (46.2%) and the highest mean number of excreted oocysts (369,147 OPG) was observed in animals from the age group 0–3 months. In addition, the significantly highest proportion of diarrhea in oocyst shedding animals (52.3%) was observed in the same group.

At least one of the investigated animals was *Cryptosporidium* spp.-infected on 63 (72.4%; 95%CI 62.3–81.0%) of the 87 herds and at least one shedding animal was found 58.9% (95%CI 45.8–70.8%) of herds with less than 50 animals, 94.4% (95%CI 72.4–100%) of herds with 50–200 animals and 100% (95%CI 79.4–100%) of herds with more than 200 animals. While the animal prevalence ranged from 43.5% in farms with more than 200 animals ($n = 13$, 8–36 samples per farm) and 39.9% in farms with 50–200 animals ($n = 18$, 5–30 samples per farm) to 67.4% in farms with less than 50 animals ($n = 56$, 1–14 samples per farm). In addition, a significantly ($p < 0.01$) higher proportion of *Cryptosporidium* spp. shedding animals was observed in farms with more than 200 animals (Table 1). The highest mean OPG (725015) was observed on farms with more than 200 animals in the age group 0–3 months. Whereas, the second highest mean OPG (115620) was found on farms with less than 50 animals in animals older than 24 months (Table 2).

3.2. Presence of Cryptosporidium species and C. parvum sub-types

Cryptosporidium spp. DNA was successfully amplified and sequenced from 135 (41.4%) of the 326 fecal samples from 54 farms which were microscopically positive. Overall, six *Cryptosporidium* species were detected – *C. parvum*, *C. bovis*, *C. andersoni* and *C. ryanae* (Table 3). *Cryptosporidium scrofarum* and *C. ubiquitum* were detected each in a single individual sample.

Of the 135 samples with successful species determination, 31 samples flagged for mixed species/variants by the CryptoGenotyper tool. Of these, 20 represented chromatograms of *C. andersoni* type A and type B copy variants and two samples were *C. parvum* type A and type B variant mixes. For the remaining nine samples three were mix infection of *C. parvum/C. bovis*, three mix of *C. bovis/C. ryanae*, one a mix of *C. parvum/C. ryanae*, one a mix of *C. parvum/C. andersoni* and one a mix of *C. bovis/C. andersoni*.

In 55.6% (95%CI 42.2–68.4%) of the sampled farms at least one species, in 31.5% (95%CI 20.2–77.7%) of the sampled farms at least two species and in 13.0% (95%CI 5.9–24.0%) of the sampled farms three or more *Cryptosporidium* species were observed in infected animals. *Cryptosporidium parvum* was detected in 63.0% (95%CI 49.6–75.0%) of farms followed by *C. bovis* (37.0%, 95%CI 25.0–50.4%), *C. andersoni* (37.0%, 95%CI 25.0–50.4) and *C. ryanae* (26.0%, 95%CI 15.6–38.8%). In all animal age groups, *C. parvum* was found to be the most prevalent, while diarrhea associated with *C. parvum* infection was observed only in very young animals (Table 4). *C. bovis* was prevalent in all age groups and the highest proportion of infected animals was in age group 0–3 months as well as the highest proportion of diarrhea associated with *C. bovis* infection was observed in the same age group. *Cryptosporidium andersoni* and *C. ryanae* in age group 0–3 months was observed in low prevalence, while a higher proportion of animals with diarrhea associated with *C. andersoni* infection was observed in very young animals and with *C. ryanae* in animals age group 4–24 months.

Of the 62*C. parvum*-positive fecal samples, 58 were successfully sequenced and typed by *gp60* analysis. A total of eight different subtypes were identified. The majority of the subtypes were in the IIa subtype family, while one subtype was identified from the IId subtype family. The most common subtype was IIaA15G2R1, which was found in 30.7% of the *C. parvum* successfully subtyped samples. A single *C. parvum* subtype per farm was found on all except two farms. Two *C. parvum* subtypes (IIaA15G2R1 and IIaA16G1R1) were identified on a farm in the Vidzeme region with 35 animals on the farm. While two other *C. parvum* subtypes (IIaA14G2R1 and IIaA15G1R1) were identified on a farm in the Kurzeme region with 260 animals on the farm.

Table 1
Cryptosporidium spp. prevalence, proportion of diarrhea in oocyst shedding animals and oocysts per g of feces (OPG) per different cattle age groups and farm size. Confidence intervals for proportions were calculated assuming a binomial distribution.

Factor		Total no. analyzed / infected animals	Prevalence (95%CI)	Mean OPG	Median OPG	Min-Max OPG	Proportion of diarrhea (95%CI)
Age group*	0–3 months	260/122	46.2 (41.0–53.0)	396,147	900	200–45,423,600	52.3 (46.3–58.3)
	4–24 months	248/86	34.7 (29.0–40.8)	35,391	800	200–1,203,700	13.3 (9.6–18.3)
	> 24 months	418/118	28.2 (24.1–32.7)	83,495	500	200–203,640	8.6 (6.3–11.7)
Farm size**	1–50 animals	302/93	30.8 (25.8–36.2)	76,067	500	200–841,200	14.2 (10.7–18.7)
	50–200 animals	295/91	30.9 (25.8–36.3)	26,369	500	200–1,043,600	22.7 (18.3–27.8)
	> 200 animals	329/142	43.2 (37.9–48.6)	364,462	300	200–45,423,600	28.9 (24.2–34.0)

* Significant difference ($P < 0.05$, Fisher exact test) for the prevalence of *Cryptosporidium* spp. and proportion of diarrhea of shedding animals between age group 0–3 months and other two age groups.

** Significant difference ($P < 0.05$, Fisher exact test) for the prevalence of *Cryptosporidium* spp. in farms with more than 200 animals other farm size groups.

Table 2
Cryptosporidium spp. prevalence and proportion of diarrhea of oocyst shedding animals per different cattle age groups in different farms. Confidence intervals for proportions were calculated assuming a binomial distribution.

Age group		0–3 months			4–24 months			> 24 months		
	Farm size, number of animals	Total no. analyzed / Prevalence (95%CI)	Proportion of positive findings in animals with diarrhea (95%CI)	Mean OPG	Total no. analyzed / Prevalence (95%CI)	Proportion of positive findings in animals with diarrhea (95%CI)	Mean OPG	Total no. analyzed / Prevalence (95%CI)	Proportion of positive findings in animals with diarrhea (95%CI)	Mean OPG
	1–50	52/44.2 (31.6–57.6)	39.1 (22.2–59.2)	10,535	83/29.0 (20.3–39.4)	12.5 (4.3–31.0)	63,054	167/27.5 (21.3–24.7)	52.3 (46.3–58.3)	115,620
	50–200	66/51.5 (39.7–63.2)	73.5 (56.9–85.4)	28,281	102/31.4 (23.2–40.9)	12.5 (5.0–28.1)	3403	127/19.7 (13.7–27.5)	13.3 (9.6–18.3)	53,120
	> 200	142/45.7 (37.8–54.0)	53.9 (41.2–65.4)	725,015	63/47.6 (35.8–59.7)	36.7 (21.9–54.5)	47,388	124/37.9 (29.9–46.7)	8.6 (6.3–11.7)	68,212
TOTAL		260/47.0 (41.0–53.0)	56.6 (47.7–65.0)	396,147	248/34.7 (29.0–41.0)	20.9 (13.7–30.7)	35,393	418/28.2 (24.1–32.7)	21.3 (18.8–24.0)	83,469

Table 3
Proportion of isolates, number of oocysts per gram (OPG) of *Cryptosporidium* spp. and subtypes, age (months) and proportion of isolates in diarrheic oocyst shedding animals. Confidence intervals for proportions were calculated assuming a binomial distribution.

Species	Subtype*	Total no. of isolates	Proportion (95%CI)	Median OPG	OPG range	Median age (months)	Age range (months)	Proportion of isolates in diarrheic animals (95%CI)
C. parvum		62	45.9 (37.8–54.3)	1000	200–476,600	3.0	0.03–111	41.9 (30.4–54.3)
	IIaA14G2R1	6	9.6 (4.2–19.9)	8700	200–242,200	3.1	0.5–9.0	33.3 (9.3–70.4)
	IIaA15G1R1	7	11.3 (5.3–21.8)	700	200–475,200	36.0	0.8–89.0	0.0 (0.0–40.4)
	IIaA15G2R1	19	30.7 (20.5–43.0)	1200	200–63,000	4.0	0.03–66.0	36.8 (19.1–59.1)
	IIaA16G1R1	5	8.1 (3.1–17.9)	2000	200–8000	1.5	0.3–36.0	80.0 (36.0–98.0)
	IIaA17G2R1	13	20.9 (12.6–32.8)	2200	200–476,600	2.5	1.0–41.0	53.9 (29.1–76.8)
	IIaA19G1	1	1.6 (0.0–9.4)	N/A	200	N/A	0.8	100.0 (16.8–1000.)
	IIaA20G3R1	4	6.5 (2.1–15.9)	1400	400–8800	1.1	0.2–5.0	75.0 (28.9–96.6)
	IIdA24G1	3	4.8 (1.1–13.8)	5400	4800–174,800	13.0	1.5–61.0	33.3 (5.6–79.8)
C. bovis		29	21.5 (15.4–29.2)	400	200–1,043,600	3.5	0.2–172	41.4 (25.5–59.3)
C. andersoni		22	16.3 (11.0–23.5)	900	200–2,036,400	17.5	0.09–197	22.7 (9.7–43.9)
C. ryanae		11	8.1 (4.5–14.1)	800	200–49,600	6.0	0.2–70	18.2 (4.0–48.9)
C. scrofarum		1	0.7 (0.0–4.5)	N/A	3600	N/A	20.0	0.0
C. ubiquitum		1	0.7 (0.0–4.5)	N/A	1000	N/A	84.0	100.0
C. parvum / C. bovis		3	2.2 (0.5–6.6)	400	200–1400	0.06	0.03–3	100.0
C. parvum / C. andersoni		1	0.7 (0.0–4.5)	N/A	63,000	N/A	16	0.0
C. parvum / C. ryanae		1	0.7 (0.0–4.5)	N/A	1400	N/A	1.2	100.0
C. bovis / C. andersoni		1	0.7 (0.0–4.5)	N/A	200	N/A	147	100.0
C. bovis / C. ryanae		3	2.2 (0.5–6.6)	400	200–400	11	1–55	33.3 (1.7–86.8)
All species		135	41.4 (36.5–46.8)	800	200–2,036,400	4.5	0.03–197	38.5 (30.6–46.9)

N/A – Not Applicable.
* *Cryptosporidium parvum* subtype data for 41 samples.

Overall, 182 sequences were deposited in the GeneBank database under accession numbers OK429136 - OK429317.

3.3. Models

Generalized linear mixed modelling fit by maximum likelihood (Laplace approximation) revealed a statistically significant effect of age ($P < 0.01$, Table 3) on the likelihood of *Cryptosporidium* spp.-associated diarrhea ("Crypto+Diarrhea") in cattle. Based on this finding, we concluded that the likelihood of an animal to become a case, i.e. an animal with diarrhea and *Cryptosporidium* spp. infection was influenced, i. e. decreased by the age of the cattle. Thus, univariable statistics was avoided and data on further risk or protective factors was thus exclusively analyzed by multilevel modelling, with age (in months) as an effect-modifying variable and the farm as a random effects variable. First generalized linear mixed models (including, in addition each factor in question, always age in month [in the following referred to as "Age"] as effect modifying variable and the individual farm identification number [in the following referred to as "FarmID"] as random effects variable; Table 5) revealed that a low body temperature in the individual animal, the presence of other than Holstein black and red, and Latvian brown breeds in the farm, prolonged periods of feeding calves with milk (i.e. periods longer than 1 week; Table 5), using cats for rodent control, presence of wild birds, presence of other houses nearby, keeping manure as pile were statistically significant ($P < 0.05$) protective factors. In contrast, a high body temperature in the individual animal, high proportion of calf-deaths, disinfection of calf box, regular deworming, and traps used for rodent control were statistically significant ($P < 0.05$) risk

Table 4
The prevalence and proportion of oocyst isolates in animals with diarrhea per different cattle age groups and per *Cryptosporidium* species. Confidence intervals for proportions were calculated assuming a binomial distribution.

Age group		0–3 months		4–24 months		> 24 months	
	Cryptosporidium spp.	Total no. isolates / Proportion (95%CI)	Proportion of isolates in animals with diarrhea (95%CI)	Total no. isolates / Proportion (95%CI)	Proportion of isolates in animals with diarrhea (95%CI)	Total no. isolates / Proportion (95%CI)	Proportion of isolates in animals with diarrhea (95%CI)
	C. parvum	30/52.6 (39.9–65.0)	70.0 (52.1–83.3)	14/38.9 (24.8–55.1)	0.0 (0.0–21.5)	13/41.9 (26.4–59.2)	0.0 (0.0–22.8)
	C. bovis	20/35.1 (24.0–48.1)	65.0 (43.3–81.9)	8/22.9 (12.1–39.0)	25.0 (7.2–59.1)	6/19.4 (9.2–36.3)	16.7 (3.0–56.4)
	C. andersoni	5/8.8 (3.8–18.9)	40.0 (11.8–76.9)	8/22.9 (12.1–39.0)	25.0 (7.2–59.1)	8/25.8 (13.7–43.3)	12.5 (2.2–47.1)
	C. ryanae	2/3.5 (1.0–11.9)	0.0 (0.0–65.8)	5/14.3 (6.3–29.4)	40.0 (11.8–76.9)	4/12.9 (5.1–28.9)	0.0 (0.0–49.0)
TOTAL		57/45.6 (37.1–54.3)	63.2 (50.2–74.5)	35/28.0 (20.9–36.4)	17.1 (8.1–32.7)	31/24.8 (18.1–33.1)	6.5 (1.8–20.7)

factors for *Cryptosporidium* spp.-associated diarrhea in cattle associated with *Cryptosporidium* spp. infection (Table 5).

Because abnormal body temperature and a high proportion of calf-deaths on farm are rather consequences of diarrhea related to *Cryptosporidium* spp. than its cause, these two variables were excluded from further modelling putative risk or protective factors causing *Cryptosporidium* spp.-associated diarrhea on farms.

To find out whether the input variables were independent of each other in the dataset, factor analyses were done for all risk or protective factors that were significant in addition to "Age" at a level of $P < 0.1$ (Table 5) in bivariable generalized linear mixed models including age ("Age") as effect modifier and "FarmID" as a random effects variable modelling presence of *Cryptosporidium* spp.-associated diarrhea (Supplementary file 2, Table Y1-Y5; Table 6).

After factor analyses all variables regarded as independent (Table 6) were included into a full model. The full model (including "Age", "Farm size", "Calf separation", "Pet animal", "Period feeding milk to calf', "Disinfection of calf boxes") had an AIC of 355.1. In a final step, this model was optimized by a stepwise elimination of those variables that, if removed, did not cause a decrease in AIC. The final linear mixed model had an AIC of 346.8 and comprised the three variables "Age", "Period feeding milk to calves" and "Disinfection of calf boxes" (Table 7). In this final model, the probability of *Cryptosporidium* spp.-associated diarrhea decreased statistically significantly with the age of the animals. In addition, feeding milk to a calves longer than three weeks relative to feeding milk to calves only one week had a protective effect. Reporting disinfection of calf boxes turned out as a putative risk factor in modelling.

4. Discussion

Within the present study, when we used *Cryptosporidium* spp. specific detection methods, there were 33.8% *Cryptosporidium* spp.-infected cattle in Latvia. While previous microscopy-based *Cryptosporidium* spp. prevalence estimates were 30% (95%CI 27–33%) in Estonia, 32% in Denmark (95%CI 28–36%) and 41% (95%CI 34–48%) in small-scale study in Latvia (Maddox-Hyttel et al., 2006; Lassen and Talvik, 2009; Lassen, 2011). However, the herd prevalence in the present study was 77.8% which is comparable to that observed in a small-scale study from Lithuania, in which a herd prevalence in cattle of 67% had been reported (Lassen and Talvik, 2009).

In the present study we identified all four *Cryptosporidium* species reported to commonly infect cattle and two additional *Cryptosporidium* species (*C. scrofarum* and *C. ubiquitum*) which also have been detected in cattle in Europe (Imre and Dărăbus, 2011). In individual animals few, this study revealed in total nine, mixed infections of several *Cryptosporidium* species. It should be noted that PCR methods targeting the 18S rDNA and direct sequencing are likely to detect the most abundant species and genotype in the specimen and underestimate the occurrence of mixed infection (Silverlås et al., 2010; Hadfield et al., 2011; Mercado et al., 2015). However, the newly released CryptoGenotyper tool identified some samples as mixed which could be verified by manual interpretation of the chromatograms. Previous studies show that the genetic diversity within an individual host, in the form of mixed species or intra-species diversity, has been identified in a large number of epidemiological surveys of cryptosporidiosis in variable proportions, but has often been treated as a secondary finding and not analyzed (Grinberg and Widmer, 2016).

It has previously been shown, that the occurrence of *Cryptosporidium* species in cattle is age related (Silverlås et al., 2010). Our study partially confirms previous studies on age-related patterns of *Cryptosporidium* spp. in cattle. All of the main four *Cryptosporidium* species were observed in very young calves and old cattle and in very young animals *C. parvum* and *C. bovis* were observed at higher rates while *C. andersoni* and *C. ryanae* were rarely observed in the same age group. Interestingly, *C. parvum* and *C. bovis* were observed in calves as early as two days after parturition, which is in contrast to the prepatent periods reported for *C. parvum* (5–12 days) and *C. bovis* (10–12 days) previously (Faubert and Litvinsky, 2000; Fayer et al., 2005; Silverlås et al., 2009). However, there are previous studies showing results which are similar to our observation, suggesting that infection with *Cryptosporidium* spp. occurs at the first hours after birth (Björkman et al., 2015; Garro et al., 2016). In the present study, *C. parvum* was the predominant *Cryptosporidium* species detected and it was found in a two-day young calf (13,600 OPG) as well as in 9.3-years old cattle (1400 OPG). One of the oldest cattle infected with *C. parvum* in this study was 7.4 years old and was shedding high number of oocysts (128,800 OPG). It had been observed on a farm with biological farm management. *C. parvum* can cause high morbidity in calves, and typically, profuse diarrhea can result in high morbidity (Abeywardena et al., 2015). Outbreaks with a high mortality in calves due to *C. parvum* have been also described in Estonia (Lassen and Talvik, 2009; Niine et al., 2018). In previous studies, *C. parvum* was frequently identified in pre-weaned calves, *C. bovis* and *C. ryanae* in post-weaned calves and *C. andersoni* in adult cattle (Santín et al., 2008). However, as the lengths of the weaning period of calves in Latvia can vary from farm to farm depending on cattle breed and number of animals housed, it cannot be assumed that there is a conjunction between the occurrence of other species and relation to the weaning period. However, *C. andersoni* was more prevalent in older animals with a median age of 17.5 months, but still the youngest infected calf with *C. andersoni* was only three days old and it was shedding 400 OPG. The presence of *C. andersoni* in pre-weaned cattle was previously reported and it is evident that calves could acquire *C. andersoni* infection at an early stage of life (Kváč et al., 2006; Silverlås et al., 2010). However, *C. andersoni* has always been associated more with post-weaned or mature cattle (Huetink et al., 2001; Enemark et al., 2002). The majority of infections

Table 5
Fixed effects in generalized linear mixed models to determine potential risk factors for *Cryptosporidium* spp.-associated diarrhea in Latvian cattle. Data were analyzed by bivariable generalized linear mixed modelling including age in months ("Age") as effect modifier and farm identification number ("FarmID") as random effects variable in modelling "Crypto+Diarrhea" (i.e. *Cryptosporidium* spp.-positive during fecal examination in diarrheic cattle). The Akaike information criterion (AIC) was used to characterize the relative model quality. Only models with statistically significant explanatory variables ($P < 0.05$) or variables tending to be significant ($0.05 \leq P < 0.1$) in addition to "Age" are displayed.

Model (AIC, model fit)	Variable	Odds ratio (95% CI)	*z*-value	*P*-value
1 (621.4)	(Intercept)	0.180 (0.125–0.261)	−9.103	< 2e-16 ***
	Age	0.979 (0.970–0.988)	−4.658	3.2e-06 ***
2 (391.8)	(Intercept)	0.461 (0.458–0.464)	−262.12	<2e-16 ***
	Age	0.962 (0.956–0.967)	−14.09	<2e-16 ***
	Body temperature: normal (ref.)			
	Body temperature: high	0.474 (0.471–0.477)	252.79	<2e-16 ***
	Body temperature: low	2.359 (2.346–2.373)	290.72	<2e-16 ***
3 (352.9)	(Intercept)	0.149 (0.0808–0.276)	−6.070	1.28e-09 ***
	Age	0.963 (0.9482–0.979)	−4.561	5.08e-06 ***
	Proportion of calf-deaths on farm	1.445 (1.0501–1.990)	2.260	0.0238 *
4 (351.6)	(Intercept)	0.407 (0.259–0.641)	−3.876	0.000106 ***
	Age	0.962 (0.947–0.978)	−4.661	3.14e-06 ***
	Other breeds[a]: no (ref.)			
	Other breeds: yes	0.468 (0.260–0.841)	−2.536	0.011204 *
5 (350)	(Intercept)	0.914 (0.4265–1.958)	−0.232	0.81664
	Age	0.965 (0.9497–0.980)	−4.506	6.61e-06 ***
	Feeding milk to calves: 1 week (ref.)			
	Feeding milk to calves: 2 weeks	0.297 (0.1216–0.724)	−2.669	0.00761 **
	Feeding milk to calves: 3 weeks	0.274 (0.1031–0.729)	−2.594	0.00949 **
	Feeding milk to calves: >3 weeks	0.186 (0.0753–0.458)	−3.652	0.00026 ***
6 (351.6)	(Intercept)	0.196 (0.126–0.305)	−7.257	3.95e-13 ***
	Age	0.963 (0.948–0.979)	−4.568	4.93e-06 ***
	Disinfection calf box: no (ref.)			
	Disinfection calf box: yes	2.010 (1.094–3.693)	2.249	0.0245 *
7 (351.6)	(Intercept)	0.236 (0.166–0.337)	−7.945	1.94e-15 ***
	Age	0.963 (0.948–0.979)	−4.614	3.95e-06 ***
	Deworming regular: no (ref.)			
	Deworming regular: yes	4.510 (1.453–14.001)	2.606	0.00916 **
8 (353.7)	(Intercept)	0.347 (0.230–0.522)	-5.071	3.96e-07 ***
	Age	0.963 (0.948–0.978)	−4.654	3.26e-06 ***
	Cats for rodent control: no (ref.)			
	Cats for rodent control: yes	0.531 (0.296–0.954)	−2.118	0.0342 *
9 (352.3)	(Intercept)	0.222 (0.156–0.316)	−8.340	< 2e-16 ***
	Age	0.963 (0.947–0.978)	−4.674	2.95e-06 ***
	Traps for rodent control: no (ref.)			
	Traps for rodent control: yes	2.378 (1.246–4.539)	2.626	0.00864 **
10 (347.4)	(Intercept)	0.389 (0.268–0.564)	−4.970	6.70e-07 ***
	Age	0.963 (0.948–0.978)	−4.687	2.77e-06 ***
	Wild birds present: no (ref.)			
	Wild birds present: yes	0.382 (0.213–0.683)	−3.242	0.00119 **
11 (352.3)	(Intercept)	0.565 (0.299–1.070)	−1.753	0.0796 .
	Age	0.962 (0.947–0.978)	−4.667	3.06e-06 ***
	Other houses: no (ref.)			
	Other houses: yes	0.398 (0.200–0.793)	−2.620	0.0088 **
12 (352.6)	(Intercept)	0.150 0.0821 0.275	−6.146	7.94e-10 ***
	Age	0.963 0.9475 0.978	−4.668	3.05e-06 ***
	Distance to next farm, meter	1.001 1.0002 1.002	2.392	0.0168 *
13 (356.2)	(Intercept)	0.339 0.224 0.513	−5.106	3.29e-07 ***
	Age	0.963 (0.948–0.978)	−4.739	2.15e-06 ***
	Keeping manure: no (ref.)			
	Keeping manure: Open pit	0.771 (0.413–1.442)	−0.813	0.4160
	Keeping manure: Pile	0.466 (0.224–0.971)	−2.039	0.0415 *
14 (621.0)	(Intercept)	0.105 (0.0535–0.206)	−6.562	5.30e-11 ***
	Age	0.980 (0.9709–0.989)	−4.482	7.41e-06 ***
	Farm size: <21 (ref.)			
	Farm size: 21–100	2.057 (0.9570–4.421)	1.847	0.0647 .
	Farm size: >100	1.950 (0.9380–4.052)	1.789	0.0737 .
15 (356.8)	(Intercept)	0.289 (0.209–0.400)	−7.491	6.84e-14 ***
	Age	0.962 (0.946–0.977)	−4.813	1.49e-06 ***
	Calf separation: early (ref.)			
	Calf separation: late	0.351 (0.105–1.177)	−1.696	0.0899 .
	Calf separation: other	1.49e-12 (0.000-Inf.)	0.000	1.0000
16 (356.9)	(Intercept)	0.432 (0.230–0.811)	−2.614	0.00895 **
	Age	0.963 (0.947–0.978)	−4.663	3.12e-06 ***
	Pet animals: no (ref.)			
	Pet animals: cat	0.518 (0.253–1.061)	−1.798	0.07222 .
	Pet animals: dog	0.479 (0.087–2.631)	−0.847	0.39676
17 (357.3)	(Intercept)		−5.706	

(*continued on next page*)

Table 5 (*continued*)

Model (AIC, model fit)	Variable	Odds ratio (95% CI)	*z*-value	*P*-value
		0.172 (0.0941–0.315)		1.16e-08 ***
	Age	0.963 (0.9475–0.978)	−4.727	2.28e-06 ***
	Restroom for workers: no (ref.)			
	Restroom for workers: on farm	1.794 (0.9131–3.526)	1.696	0.0898 .
	Restroom for workers: outside farm	1.655 (0.6193–4.421)	1.004	0.3152

Abbreviation: ref., reference; Inf., Infinite.
$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[a] Other breeds: other breeds on farm than Holstein black, Holstein red, or Latvian brown.

Table 6
Summary of a factor analysis to assess the dependency of explanatory variables that were statistically significant ($P < 0.05$) or tended to be statistically significant ($0.05 \leq P < 0.1$) in the bivariable generalized linear mixed modelling including age in months ("Age") as effect modifier and farm identification number ("FarmID") as random effects variable in modelling diarrhea associated to *Cryptosporidium* spp.-positivity in cattle from Latvia (detailed information on factor analysis in Supplementary file 2, Tables Y1-Y5).

Factor analysis models (phase of analysis)	Factor	Not excluded from further analysis	Excluded from further analysis (reason for exclusion)
Model 1 (initial)	1	Period feeding milk to calves	Cats for rodent control (no (bio-)logical explanation for protective effect), Keeping manure (lower statistical significance in bivariable risk factor analysis)
	2	Disinfection calf box	Other breed[a] (no logical explanation for protective effect)
	3	Calf separation	Regular deworming (no logical explanation for risk effect)
Model 2 (subsequent)	1	Period feeding milk to calf	Workers rest room, Other houses, Distance to next farm (no (bio-)logic explanation for effects)
Model 3 (subsequent)	1	Period feeding milk to calf	Wild birds present (no (bio-) logic explanation for protective effect)
Model 4 (subsequent)	1	Period feeding milk to calf	Traps used for rodent control (no (bio-)logic explanation for risk effect)

Notes: Variables with absolute loadings >0.5 were regarded as dependent. The initial model (Model 1) included all variables that were statistically significant or tended to be statistically significant in the bivariable generalized linear mixed modelling. The subsequent models (Models 2, 3) included only variables that were not excluded on the basis of the results obtained in the initial factor analysis model.
[a] Other breeds: other breeds on farm than Holstein black, Holstein red, or Latvian brown.

caused by *C. ryanae* were asymptomatic which is in concordance with the observations of Fayer et al. (2008). However, in the present study the highest proportion of diarrheic animals shedding *Cryptosporidium* spp. oocysts was infected with *C. parvum* and *C. bovis* (Table 3).

All eight of the *C. parvum* subtypes identified in present study, including the most common one (IIaA15G2R1), have been found in humans (Plutzer and Karanis, 2009) highlighting the zoonotic potential of *C. parvum* shed by cattle in Latvia. Previous studies had emphasized that zoonotic *C. parvum* infections are caused by IIa subtype families,

Table 7
Fixed effects in generalized linear mixed models to determine potential risk factors for diarrhea associated to *Cryptosporidium* spp.-positivity in Latvian cattle. Data were analyzed by bivariable generalized linear mixed modelling including age in months ("Age") as effect modifier and farm identification number ("FarmID") as random effects variable in modelling "Crypto+Diarrhea" (i.e. *Cryptosporidium* spp.-positive during fecal examination in diarrheic cattle). The Akaike information criterion (AIC) was used to characterize the relative model quality.

Model (AIC, model fit)	Variable	Odds ratio (95% CI)	*z*-value	*P*-value
Final (346.8)	(Intercept)	0.563 (0.2340–1.353)	−1.285	0.198929
	Age	0.966 (0.9508–0.981)	−4.406	1.05e-05 ***
	Feeding milk to calves: 1 week (ref.)			
	Feeding milk to calves: 2 weeks	0.403 (0.1586–1.024)	−1.910	0.056181
	Feeding milk to calves: 3 weeks	0.377 (0.1363–1.045)	−1.875	0.060757
	Feeding milk to calves: >3 weeks	0.203 (0.0818–0.505)	−3.431	0.000602 ***
	Disinfection of calf box: no (ref.)			
	Disinfection of calf box: yes	2.034 (1.1080–3.735)	2.291	0.021971 *

Abbreviation: ref., reference.
$P < 0.1$, *$P \leq 0.05$, ***$P < 0.001$.

particularly the dominant IIaA15G2R1 subtype (Chalmers et al., 2011; Xiao and Feng, 2017; Feng et al., 2018). There was a low variation in the *gp60* gene and in the majority of farms only a single subtype was identified per farm. This can be explained by the closed herd management with no or minimal movement of calves among herds (Silverlås et al., 2010; Kváč et al., 2011).

The evidence of mixed species and intra-specific genetic diversity within *Cryptosporidium* spp. population was observed. Out of the 31 samples, in which mixed species/variants were observed, *C. andersoni* type A and type B copy variants (Nagano et al., 2007; Ikarashi et al., 2013) and *C. parvum* type A and type B variant mixes (Carraway et al., 1996; Le Blancq et al., 1997; Gharieb et al., 2019) were identified.

In the present study, several of the risk and protective factors were found to be statistically significant when evaluated separately including age as effect modifying variable. Two of the statistically significant variables (i.e. body temperature, proportion of calf-deaths on farm) were not related to the risk of infection or risk of disease but related to possible clinical consequences of *Cryptosporidium* spp.-associated diarrhea.

The animals of the age group 0–3 months showed the highest rates of *Cryptosporidium* spp. oocysts shedding and a significantly higher proportion of these shedding animals had diarrhea. And also, during modelling the risk of animals of being diarrheic and *Cryptosporidium* positive ("Crypto+diarrhea") age in month ("Age") had a protective effect (Table 1, Model 1). This corresponds with findings in several previous studies (Maddox-Hyttel et al., 2006; Abeywardena et al., 2015; Santoro et al., 2019). However, in the present study, older cattle were frequently shedding *Cryptosporidium* spp. but oocyst counts were generally lower (200–203,640 OPG) than in younger animals (200–45,423,600 OPG). These results are in agreement with the widely accepted view that young animals are usually more susceptible and may act as amplifiers and infection source to other animals (Geurden et al., 2010). In addition, it was assumed that self-limiting *Cryptosporidium* spp. infections in early life protect older cattle due to acquired immunity (Wyatt, 2000). Adult cattle are generally considered refractory to heavy infections by *Cryptosporidium* spp. and associated clinical diseases because of the strong immune response that they produce. Nevertheless, these animals can act as a source of infection for younger animals,

especially during the periparturient period (Fayer et al., 2000; Ralston et al., 2003). Previous studies have shown that in dairy farms with a long history of cryptosporidiosis and diarrhea in young calves, calves as early as 2–7 days after parturition were excreting oocysts, indicating an infection very short after birth (Santín et al., 2004). The percentage of animals excreting oocysts declined after the third week but peaked again at six months of age in a previous study (Huetink et al., 2001). The second peak around six months of age was observed also in several additional studies in Europe for *C. parvum* mainly (Huetink et al., 2001; Santín et al., 2004; Maddox-Hyttel et al., 2006).

The final multivariable model (Table 7) in the present study showed that animal age and a prolonged period of feeding calves with milk (Feeding milk to calves: >3 weeks) were putative protective factors. In the initial model including in addition to "Age" only farm level data on milk feeding also "Feeding milk to calves: 2 weeks", "Feeding milk to calves: 3 weeks" turned out as protective but based on the Odds ratios with a lower effect than "Feeding milk to calves: >3 weeks" (Table 5). There is a common practice in Latvia to feed calves with dairy cattle milk for a prolonged period of 2–3 months while the calves start to feed on hay and other feed in small amounts. This is in line with the results of previous studies, which reported that significant risk factors favoring *C. parvum* infection are housing of calves separated from their dams, whereas the practice of calves being nursed by dams was observed of being protective (Duranti et al., 2009). The protective effects for calves staying with a dam (more that for 72 h) might result from the continuous and longer intake of maternal antibodies with colostrum in the first days of life and with maternal milk before weaning (Duranti et al., 2009). Earlier studies from Denmark, however, reported an increased probability that calves excrete higher levels of *Cryptosporidium* spp. oocysts for organically managed dairy herds. In such herds the newborn calf remained with the dam in the maternity pen at least for 24 h after calving (Maddox-Hyttel et al., 2006). In the parturient period, cows are shedding increased numbers of oocysts, probably because of immunological reasons (Faubert and Litvinsky, 2000). Thus, calves staying with the dam after parturition may become exposed to higher doses of oocysts as compared to calves which were moved away (Faubert and Litvinsky, 2000). In addition, the cleanliness of such calving pens is essential because a contaminated pen will in general allow exposure of calves to significant levels of *Cryptosporidium* spp. oocysts due to the parturient rise in oocyst excretion of dams (Faubert and Litvinsky, 2000; Huetink et al., 2001).

One of the factors predominantly observed in animals with *Cryptosporidium* ssp.-associated diarrhea was the observation of a low body temperature in individual animals. This low body temperature could be a result of the diarrhea, absence of appetite, lethargy and dehydration reported for animals suffering from cryptosporidiosis (Thompson et al., 2008). Interestingly, animals with fever appeared to be less likely affected by *Cryptosporidium* spp.-associated diarrhea which could be an indication that fever should not be regarded as a clinical sign of cryptosporidiosis in calves.

A second factor related to the clinical consequences of bovine cryptosporidiosis was an increased proportion of calf-death on farm. This could be an indication, that in Latvian herds, an important indicator and risk factor for diarrhea associated cryptosporidiosis is a high proportion of calf-death in the farm.. Indeed, also in other countries infections with *Cryptosporidium* spp. are very often associated with diarrhea in calves; e.g. in UK in more than 50% of the diagnosed causes of calf diarrhea were associated with *Cryptosporidium* spp. within the time period of 2007–2011 (Thomson et al., 2017). However, besides *Cryptosporidium* spp., other etiological agents, e.g. other parasites, viruses, bacteria or dietetic disorders could cause diarrhea. There is good evidence that rotavirus is a primary pathogen causing acute diarrhea in neonatal calves and that it can be detected more frequently in the feces of diarrheic than of non-diarrheic calves, and there are studies showing that rotavirus is the infectious agent excreted most commonly by calves with diarrhea, not only alone but also in mixed infections with other pathogens, including *Cryptosporidium* spp. indicating that rotavirus has a predominant role in the pathogenesis of neonatal calf diarrhea (Lanz Uhde et al., 2008). While there is a study showing that *Cryptosporidium* spp. is often (76%) present together with *Giardia duodenalis* (Hamnes et al., 2006), we did not observe this association in the present study.

Obviously, some of the farmers with diarrheic animals in our study tended to deworm animals. This may have caused that in our study "regular deworming" turned out as a statistically significant risk factor in initial modelling, which of course was regarded as a biologically non-plausible variable. A previous study from Estonia also reported that calves from farms with high calf-mortality, which had received veterinary treatment had higher odds to shed *Cryptosporidium* spp. or *C. parvum* (Santoro et al., 2019). Whether this veterinary treatment was cryptosporidiosis specific or was not suitable to treat cryptosporidiosis (e.g. by deworming) was not reported by Santoro et al. (2019).

A number of other factors appeared to have a putative risk or protective effects during the initial statistical modelling of *Cryptosporidium*-associated diarrhea. However, several of these factors were not independent from each other as shown by factor analysis and were removed from further analysis, mainly based on lack of (bio-)logical plausibility. Such factors included e.g. presence of other breeds on farm than Holstein black, Holstein Red or Latvian brown, presence of wild birds, presence of other houses near the farm. There is no clear support in previous studies for significant differences in susceptibility to *C. parvum* between the major breeds of dairy cattle, however, there are studies indicating that pure-bred animals were at higher risk than cross breeds (Imre et al., 2015; Brainard et al., 2020).

In our study on Latvian farms, calf box disinfection turned out to be a risk factor in the final model. This appears not to be logic in the light of the studies mentioned earlier; however, we lack information on the type of disinfectants used and on the proper use of such disinfectants. The use of box disinfection might only be an indication for the farm having a problem of calf-diarrhea. Disinfectants used on impure materials may not be able to eliminate oocysts of *Cryptosporidium* spp. even if this disinfectant contains components able to inactivate oocysts (Bogan, 2018). Cleaning in most Latvian farms depends heavily on frequent but incomplete removal of feces by tractor or scraping systems and this level of cleaning is not likely to be sufficient to allow an efficient inactivation by a disinfectant. Because the oocysts of *Cryptosporidium* are very difficult to eliminate from the environment an alternative control measure is to try and reduce the environmental contamination in the first place (Thomson et al., 2017; Innes et al., 2020). Disinfection with recommended products can help to reduce build-up of contaminated feces on the farm, where disinfectants containing hydrogen peroxide are the most effective (Casemore and Watkins, 1998; Thomson et al., 2017). While deep and clean straw bedding would also help minimize contact with contaminated feces and regular cleaning out of calf pens along with steam cleaning, as oocysts are susceptible to extremes of temperature (down to −20 °C and up to 60 °C) and desiccation (Robertson et al., 1992).

However, not only calving pens and calf boxes might be sources of infection. Many Latvian farmers use loose housing barns. In such barns infected cows are able to move around and spread oocysts in the dairy herd, especially in common access areas such as drinking troughs. But even in herds with tied housing, several of the observed herds practiced releasing sick cows to roam freely. Even, if such animals are not infected with *Cryptosporidium* spp., it can mechanically transfer oocysts to other cows. Crowding could increase contact between animals, increasing the possibility of direct transfer of pathogens from one animal to another (Ramirez et al., 2004; Hamnes et al., 2006).

In the present study, a significantly higher proportion of shedding animal was observed in larger farms with more than 200 animals and also a slightly higher proportion of animals with diarrhea among shedding animals was observed on these farms. The answers in the questionnaires of those farms indicated rarely having problems with parasitic diseases previously, and almost none reported on problems with

coccidian parasites. However, 61.9% of those farms with more than 200 animals observed diarrhea problems in calves periodically. Reasons for this may lie in management practices of larger farms. These results correspond to the consistent evidence that risk of *C. parvum* infection increased when calves had more contact with other calves, were in larger herds or in organic production (Brainard et al., 2020). In contrast, previous studies in the Baltic states showed that larger herds had less severe *Cryptosporidium* spp. infections (Lassen and Järvis, 2009; Lassen, 2011).

In summary, the present study suggests that *Cryptosporidium* spp. do cause major problems in Latvian dairy herds and seems to cause *Cryptosporidium*-associated diarrhea. Thus, bovine cryptosporidiosis requires higher awareness in Latvian cattle herds. A recent study showed that cryptosporidiosis does not only causes economic losses due to increased calf mortality (Olson et al., 2003) but also due to long-term effects on productivity of individual animals (Shaw et al., 2020). Due to our observation, that the majority of cryptosporidiosis was related to *C. parvum*, it is likely that also farmers and veterinarians may become infected. Therefore, zoonotic aspects of this parasite need to be taken into account, too, and need to be addressed in future studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Research Article

Parity of Calving Influences the Likelihood of Calves Having *Cryptosporidium* spp.

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The effect of colostrum on calves' health status was intensively studied, while the role of transition milk was left underestimated. The common practice is to feed calves with an adequate amount of colostrum immediately after calving and soon after feeding calves are weaned from dams. In this research, calves were not weaned from dams for at least 2 weeks receiving both colostrum and transition milk on demand. Thus, we have recreated natural feeding conditions for calves' development. We used a stratified sample method to test whether the size of the dairy cattle farms, breed, parity number, season of calving, and length of the dry period affect the likelihood of calves' infection with *Cryptosporidium* spp. considering these factors influence both colostrum and transition milk quality. The main results showed that 26.1% of calves were positive for the presence of *Cryptosporidium* spp. oocysts. The presence of clinical signs of diarrhea was recorded in 15% of the positive animals. Regression analysis showed that multiparous cows decrease the chance of calves to have *Cryptosporidium* spp. by 82%–89%, while cows calved on small farms decrease the chance of calves to have *Cryptosporidium* spp. by 80%. We suggest that primiparous cows are spending inner resources primarily on their maturation, thereby leaving the prerequisites for the infection of their offspring, while intense farming just increases the chance of unprotected calves to obtain infections.

1. Introduction

Colostrum is an exceptionally complex secretion that contains more than 250 various active chemical compounds [1]. For example, it contains major nutrients (fat, lactose, proteins, minerals, and vitamins) and various growth factors [2, 3] and immune factors (live maternal immune cells, antimicrobial, antiviral, and antifungal matter) [4]. Because immunoglobulin G (IgG) is the predominant antibody present in cow colostrum and because calves are born without protective antibodies and must consume colostrum immediately after birth, immunoglobulins are the best-studied components of cow colostrum [5, 6]. The ingestion of an adequate volume of high-quality colostrum is one of the most important factors influencing the health and survival of dairy calves because it provides passive transmission of immunity from cow to calf. Although, IgG is reported as a protective substance [7] against various pathogens (*Yersinia enterocolitica, Campylobacter jejuni, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Salmonella typhimurium, Staphylococcus, Streptococcus,* and *Cryptosporidium*), Derbakova et al. [8] have not recorded a relationship between the level of IgG in bovine colostrum and the likelihood of *Cryptosporidium* spp. infection in calves. *Cryptosporidium* is a microscopic parasite that causes neonatal diarrhea in calves, resulting in a substantial economic loss to animal husbandry [9].

Because, in addition to IgG, there are many other factors in colostrum that may potentially influence infection with *Cryptosporidium* spp. [10], it seems reasonable to evaluate

factors that affect the quality of colostrum and their relationship to *Cryptosporidium* spp. infection. Moreover, the quality of colostrum depends on many factors such as cow age [11], breed [12, 13], parity number [13], calendar season [14], and length of dry period [15, 16]. Soon after colostrum secretion, cows produce transition milk for 1–2 days whose properties are lower than colostrum but higher than mature milk [17, 18]. However, Kargar et al. [19] suggest that extended transition milk feeding for 3 weeks improves growth performance and reduces the susceptibility to diarrhea in calves.

In light of this, this study aimed to test the association between *Cryptosporidium* spp. infection in calves and such factors as the size of the dairy cattle farms, breed, parity number, season of calving, and length of the dry period. The research objects were calves not weaned from dams for at least 2 weeks receiving both colostrum and transition milk on demand.

2. Materials and Methods

2.1. Sample Collection and Examination. Because many dairy cattle farms separate calves from cows soon after birth, we calculated the required minimum sample size for regression analysis with multiple factors according to Green's [20] recommendation: 90 calves was the minimum sample size needed for this study. A stratified random sampling method was used to collect the data. The fecal samples were collected by veterinarians from the rectums of calves between December 2018 and December 2020. All coprological samples were exanimated on the collection day. Laboratory examinations were conducted in the Laboratory of Parasitology, Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies.

Totally, fecal samples were obtained from 153 calves. Fecal samples were collected from 15 ± 2 day old calves who received colostrum of ~2.5 L within the first 4 hours of life (supervised or assisted where necessary), ~4 L within the first 12 hours of life and then continued receiving transition milk within 2 weeks. Samples were collected in disposable polyethylene packages and stored in a transportable cooler during transport to the laboratory until examined. To detect oocysts of *Cryptosporidium* spp. in feces, the flotation method was used according to Fujino et al. [21]. Slides were stained using the modified Ziehl-Neelsen method [22]. All procedures performed in studies involving animals were in accordance with the ethical standards. The study was approved by the Animal Welfare and Ethical Council of the Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, and complied with current laws in Latvia.

2.2. Questionnaire. Before fecal samples of calves were collected, the dairy farm owner was asked to fill in the anonymous questionnaire. The questionnaire did not contain questions about personal data, as this information was not collected in any other form. There were the following questions with classified answers: (1) size of dairy cattle farm: small (≤10 cows), medium (11–50 cows), and large farm (>50 cows); (2) cow breed; (3) parity number: 1, 2, and ≥3; (4) calendar season of calving: winter (December, January, and February), spring (March, April, and May), summer (June, July, and August), and autumn (September, October, and November); (5) dry period length: ≤45, 46–64, and ≥65 days. The obtained data was used to build regression model.

2.3. Statistical Analysis. Generalized linear mixed modelling was conducted to determine whether explanatory variables (size of dairy cattle farm, breed, parity number, calendar season of calving, and dry period length) are related to the probability of occurring calves' infection with *Cryptosporidium* spp. where farm identification number ("FarmID") was set as a random effects variable. Akaike's information criteria (AIC) were used to evaluate which model better fits the data. The prevalence of parasites was calculated as the percentage of hosts infected by *Cryptosporidium* spp. Statistical data analysis was conducted using Jamovi version 2.0.0 [23].

3. Results

Out of all the fecal samples analyzed, 26.1% of calves were positive for the presence of *Cryptosporidium* spp. oocysts. The presence of clinical signs of diarrhea was recorded in 15% of the positive animals. The proportion of categories of explanatory variables was summarized and visualized in Figure 1. Generalized linear mixed modelling revealed a statistically significant effect of parity ($X^2(2) = 15.83$ $p < 0.001$) and farm size ($X^2(2) = 8.68$ $p = 0.013$) on the likelihood of *Cryptosporidium* spp. infection in calf. The second cow calving significantly predicted the chance of infection of *Cryptosporidium* spp. ($B = -1.723$, $z = -3.073$, $p = 0.002$, OR = 0.18). This indicates that cows having their second calving decrease calves' chances of having *Cryptosporidium* spp. by 0.18 times (or by 82%) on average, 95% CI [0.05–0.54] compared to cows having their first calving. The third cow calving significantly predicted the chance to occur infection of *Cryptosporidium* spp. ($B = -2.181$, $z = -3.71$, $p < 0.001$, OR = 0.11). This indicates that cows having their third calving decrease calves' chances of having *Cryptosporidium* spp. by 0.11 times (or by 89%) on average, 95% CI [0.03–0.36] compared to cows having their first calving. The small farm size significantly predicted the likelihood of *Cryptosporidium* spp. infection ($B = -1.624$, $z = -2.843$, $p = 0.004$, OR = 0.20). This indicates that cows having their calving on small farms decrease calves' chances of having *Cryptosporidium* spp. by 0.20 times (or by 80%) on average, 95% CI [0.06–0.60] compared to large farm size. Other factors did not show significant effect on the chance to occur infection of *Cryptosporidium* spp. in calves.

4. Discussion

The main results showed that the parity number, as well as farm size markedly affect the chance of calves having *Cryptosporidium* spp. infection even, they immediately

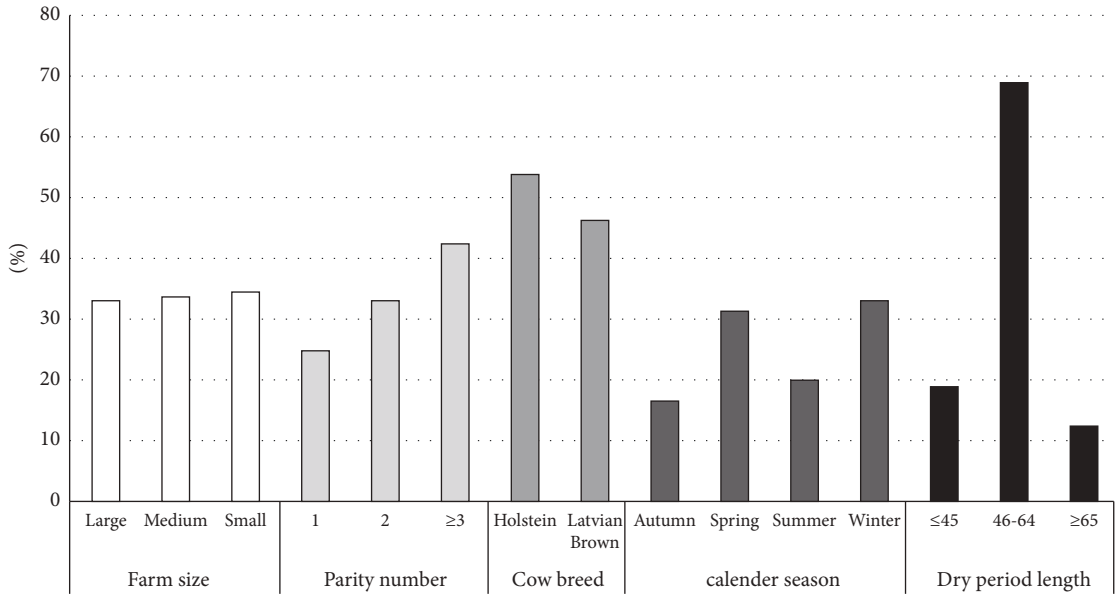

Figure 1: The proportion of categories of explanatory variables describing examined fecal samples of calves.

received colostrum and were fed by transition milk within two weeks, whereas cow breed, calendar season, and dry period length have no effect.

Our result of *Cryptosporidium* oocyst shedding (prevalence = 26.1%) is slightly higher than the reported result from neighboring Estonia (average prevalence = 23%) [24]. They also show that prevalence is markedly higher (52.03%) for calves aged between 8 and 14 days; however, nothing is known about intake of colostrum and transition milk by calves, suggesting that young animals are more susceptible to *Cryptosporidium* spp.

Although farm size cannot directly influence colostrum or transitional milk quality, there are many indirect factors that distinguish between large and small farms. For example, farm management differs between small and large farms. Usually, large farms work as a business by employing professionals to keep animals in perfect conditions to receive the maximum outcome, whereas small farms belong to families that keep animals where some specialists such as veterinarians are outsourced. Therefore, animals receive different conditions of keeping. For example, in large farms, cows have a regimented dry period length, whereas in small farms, the dry period may be set individually depending on cow health, behavior, and other factors. On small farms, calves are born mostly in the winter and spring seasons, whereas on large farms calves are born throughout the whole year. Results of this research show that in large farms calves have higher probability to have *Cryptosporidium* spp. infection compared to small farms. It may be explained by the high animal density kept in one place, since an increase in the number of hosts affects the probability for parasite transmission stages to contact new hosts. We suggest that the high density of hosts and the specificity of large farm management play a significant role in parasite transmission. For example, Mennerat et al. [25] have also discussed in detail the evolutionary implications for parasites in the frame of intense farming.

Parity numbers have been suggested to influence colostrum composition. For example, Morill et al. [13] found that with increasing parity number, the IgG concentration increased, and somatic cell count (SCC) decreased. Gulliksen et al. [26] suggest that older cows, being exposed to antigens for a longer time during their life than younger cows, produce colostrum with higher antibody levels; however, this is not always the case [27, 28]. Colostrum is the essential source of minerals (Ca, P, Mg, Na, Fe, Zn, Cu, and Mn) for newborn calves. Its concentration is significantly higher within the first hours after parturition and markedly differs between primiparous and multiparous cows [29]. The parity number also influences the mineral status of newborn calves. For example, Kume and Tanabe [29] showed that the hematocrit (Hct) and hemoglobin (Hb) of newborn calves increased as the parity number increased, and they suggested that the low Hb of primiparous cows is related to the high Fe demands of growing cows. Also, parity number is negatively associated with the cow gestation period and positively associated with the amount of milk production and calf birth weight [30].

The quality of colostrum may vary between different cow breeds [31, 32]; however, no evidence of a breed effect on infection with *Cryptosporidium* spp. Our study also showed no relationship between *Cryptosporidium* spp. infection and cow breed. Perhaps this is because there is no obvious difference in defensiveness against pathogens between many breeds of cows [33].

Seasonal variation in infectious disease transmission plays an important role, for example, high ambient temperature and high rainfall is associated with the risk of *Cryptosporidium* infection [34]. However, we did not find the effect of seasonality on the likelihood of *Cryptosporidium* spp. infection in calves. We suggest that the conditions of keeping animals on farms are the key to the lack of such a relationship. Perhaps, animals become infected in calves' pens rather than in pasture fields where ambient factor

fluctuation is common and cyclic. In addition, oocyst robustness plays an important role in infection by eliminating the negative impact of the environment on the survival of the pathogen [35], which leads to year-round infection regardless of the changing seasons.

The length of the dry period influences the following properties: the amount of milk and colostrum production, IgG concentrations in colostrum, the risk of mastitis, postpartum metabolic disorders of the cow, ruminal flora development of the cow, and the energy balance of the cow [15, 16, 36–38]. There is no evidence of the effect of dry period length on the health status of calves [39], although colostrum from cows with a short dry period has a lower IgG concentration compared with colostrum from cows having a long dry period [15]. We did not find a relationship between the length of the dry period and the likelihood of *Cryptosporidium* infection. However, we do not exclude that our result was influenced by the fact that 69% of dams had ~8-week dry period. In Latvia, farmers rarely shorten or extend the dry period of the cow.

5. Conclusion

In conclusion, evidence of parity relation to IgG, somatic cell count, source of minerals in colostrum, produced amount of mature milk, calves' birth weight, hematocrit and hemoglobin of newborn calves [13, 26, 29, 30], as well as the chance of calves having *Cryptosporidium* spp. infection seems to indicate that primiparous cows are spending inner resources primarily on their maturation, thereby leaving the prerequisites for the infection of their offspring, while intense farming just increases the chance of unprotected calves to obtain infection.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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RESEARCH ARTICLE
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Prevalence of susceptibility to *Cryptosporidium* spp. among dairy calves with different feeding regimens with an emphasis on the feeding of transition milk

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Abstract

Background and Aim: Colostrum composition and importance for newborn organisms were repeatedly studied. However, the interest in transitional milk usefulness is weak and recommendations concerning transition milk intake are not developed. The aim of this study was to evaluate whether transition milk intake after colostrum consumption affects the chances of calf infection with *Cryptosporidium* spp.

Materials and Methods: We collected data for *Cryptosporidium* spp. infection from calves (n=425) divided into three groups: The first group – supervised colostrum and transition milk intake; the second group – supervised colostrum and whole milk intake; and the third group – not supervised colostrum and whole milk intake. To detect oocysts of *Cryptosporidium* spp. in feces, the flotation method was used, and slides were stained using the modified Ziehl-Neelsen method. Generalized linear mixed modeling was conducted to determine whether the explanatory variable – the management of colostrum and transition milk feeding with three categories (three research groups) – was related to the probability of calves incurring infection with *Cryptosporidium* spp.

Results: In the first group, 26.1% of calves were positive for the presence of *Cryptosporidium* spp. oocysts, in the second – 37.2%, and in the third – 44.1%. Statistical data analysis showed that calves who did not receive transition milk after colostrum consumption had increased chances of having *Cryptosporidium* spp. (by 1.90-2.47 times on average). The main results showed that the management of colostrum and transition milk feeding is related to *Cryptosporidium* spp. infection, indicating that both colostrum and transitional milk play a significant role in controlling pathogenic infections.

Conclusion: The most effective management of colostrum and transition milk feeding against *Cryptosporidium* spp. infection is the timely intake of an adequate amount of colostrum followed by transitional milk consumption for at least 2 weeks before weaning from the dam.

Keywords: calves, colostrum, *Cryptosporidium*, neonatal diarrhea, transition milk.

Introduction

Neonatal diarrhea is a hazardous condition responsible for calf mortality worldwide. Several pathogens (Cryptosporidium, *Escherichia coli*, Rotavirus, Coronavirus, and Coccidia) cause diarrhea in young calves, with *Cryptosporidium* being the most common pathogen found in calves. It is a microscopic parasite that can cause a disease called cryptosporidiosis. The disease decreases the absorption of essential nutrients from milk and results in weight loss, dehydration, or even calf death [1-3]. The treatment of cryptosporidiosis is difficult as there are no vaccines available to prevent the disease, while available drugs often focus only on symptomatic (such as dehydration) treatment [4,5]. Therefore, effective farm management has become an alternative instrument for controlling or preventing cryptosporidiosis in livestock. For example, frequent removal of feces from cowsheds and calves' pens, as well as applying disinfectants, hot water, and desiccation may help to markedly reduce the number of oocysts in the environment [6,7].

Another potential method of cryptosporidiosis control is the management of colostrum and transition milk feeding. Since calves are born naively immune, they need the support that cows provide by producing colostrum and transitional milk. Colostrum is the first milk that cows produce after calving. It is a complex secretion that contains markedly elevated amounts of essential chemical compounds (nutrients, growth factors, and immune factors), which aim to contribute to the immune system and feed the newborn calf [8-10]. Following the first milking, cows produce transition milk that may stimulate development of the

gastrointestinal tract [11]. In practice, no clear line can be drawn regarding when colostrum is transformed into transitional milk and then into whole milk.

Based on recent research regarding evidence of colostrum composition and importance for newborn organisms, some recommendations for colostrum allowances and timely feeding of newborn calves were developed [12,13]. However, the interest in transitional milk usefulness is weak and recommendations concerning transition milk intake are not developed. For example, on large dairy farms, the calves are weaned from the cows and kept separately in the calves' pen soon after the first feeding, while the transitional milk is placed in a common storage tank where it is diluted with other milk.

This study aimed to test the association between *Cryptosporidium* spp. infection and the management type of colostrum and transition milk feeding in calves.

Materials and Methods

Ethical approval

All procedures performed in the study involving animals were in accordance with ethical standards. The study was approved (No. DzAĒP/2017/2) by the Animal Welfare and Ethical Council of the Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, and complied with current laws in Latvia.

Study period and location

The study was conducted from December 2018 to December 2020. All coprological samples were examined on the collection day. Laboratory examinations were made in the Laboratory of Parasitology, Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies.

Sample collection and examination

Fecal samples of calves were collected in disposable polyethylene packages and stored in a transportable cooler during transport to the laboratory until examined. To detect oocysts of *Cryptosporidium* spp. in feces (Figure-1), the flotation method was used according to Fujino *et al.* [14]. Slides were stained using the modified Ziehl-Neelsen method [15]. Samples for microscopy were previously prepared using the saturated NaCl flotation method. For the flotation, 1 g of fecal sample was used and after continuous flotation and centrifugation steps, it resulted in 2 mL of concentrated material, which was used for further analyses.

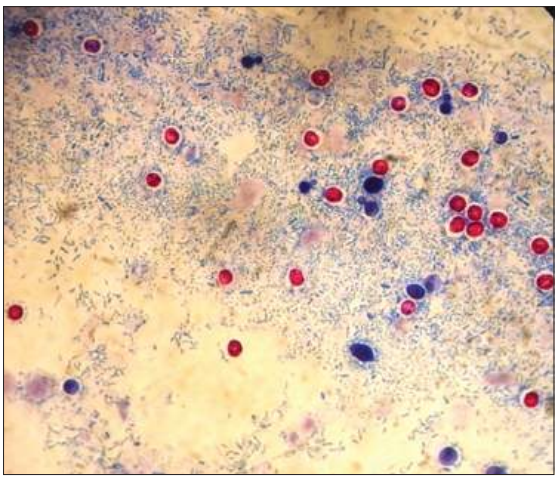

Figure-1: Oocysts of *Cryptosporidium* spp. stained with the modified Ziehl-Neelsen method.

Calves feeding regimens

Overall, 425 calves (15±2 days old) from 39 farms were examined in this study: The first group – calves (n=153) received colostrum of ~2.5 L within the first 0-4 h of life (supervised or assisted where necessary – calves were fed immediately after they were born), ~4 L within the first 12 h of life and then continued receiving transition milk within 2 weeks from their dams; the second group – calves (n=145) received colostrum of ~2.5 L within the first 0-4 h of life (supervised or assisted where necessary – calves were fed immediately after they were born), ~4 L within the first 12 h of life and then weaned from the dams and kept separately by feeding milk from a common storage tank where the dams' transition milk was diluted with other milk; and the third group – calves (n=127) were born at night and there was no certainty that a sufficient amount of colostrum was ingested on time; these calves were weaned from the dams and kept separately by feeding milk from a common storage tank where the dams' transition milk was diluted with other milk.

The number of cows on one farm ranged from 3 to 300 head. The sample was not limited by calving season, the length of the dry period, cow breed, or age. No dry cow vaccination against rotavirus-, coronavirus-, and *E. coli* bacteria was done. Calves were housed in individual pens. The first research group received milk from cows on demand, whereas the second and third groups received milk from a bucket with 4 feeding times during the 1st week and 3 feeding times during the 2nd week.

Statistical analysis

Generalized linear mixed modeling was conducted to determine whether the explanatory variable – the management of colostrum and transition milk feeding with three categories (three research groups) – was related to the probability of calves' incurring infection with *Cryptosporidium* spp. where the farm identification number ("FarmID") was set as a random effect variable. The prevalence of parasites was calculated as the percentage of hosts infected by *Cryptosporidium* spp. Statistical data analysis was conducted using Jamovi, version 2.0.0 [16].

Results

Out of all the fecal samples analyzed (n=425), 35.3% of calves were positive for the presence of *Cryptosporidium* spp. oocysts. The presence of clinical

signs of diarrhea was recorded in 20.6% of the positive animals**.** The percentage of calves positive for the presence of *Cryptosporidium* spp. oocysts and the percentage of calves with the presence of clinical signs of diarrhea in each research group are summarized in Table-1. The regression model was significant (χ^2 (2)=8.62, p=0.0013), indicating the variance in the chance of incurring one of the two outcomes of *Cryptosporidium* spp. infection is explainable by the management of colostrum and transition milk feeding. The second research group significantly predicted the chance of incurring infection by *Cryptosporidium* spp. (B=0.643, z=2.00, p=0.046, OR=1.901). This indicates that calves belonging to the second research group had an increased chance of having *Cryptosporidium* spp. by 1.90 times on average (95% CI [1.012-3.574]) compared to those of the first research group. The third research group significantly predicted the chance of infection with *Cryptosporidium* spp. (B=0.901, z=2.93, p=0.003, OR=2.469). This indicates that calves belonging to the third research group had increased chances of having *Cryptosporidium* spp. by 2.47 times on average (95% CI [1.35-4.52]) compared to those of the first research group.

Discussion

The results showed that the management of colostrum and transition milk feeding is related to *Cryptosporidium* spp. infection, indicating that both colostrum and transitional milk play a significant role in controlling pathogenic infection. The most effective combination against *Cryptosporidium* spp. infection is the timely intake of an adequate amount of colostrum followed by transitional milk consumption for at least 2 weeks before weaning from the dam.

Our results for *Cryptosporidium* oocyst shedding (average prevalence=35.3%, from 26.1% to 44.1%) were similar to reported results from Estonia, prevalence=30% [17] and prevalence=23% [18]; however, markedly different compared to Lithuania (prevalence=67%) [19], which was explained mainly by the small sample size in Lithuania.

The protective properties of colostrum were repeatedly studied and are mainly explained by transfer of immunoglobulins (such as IgG) from the dam to the calf through the ingestion of colostrum [20]. However, Derbakova *et al*. [21] did not record a relationship between the level of IgG in bovine colostrum and the likelihood of *Cryptosporidium* spp. infection in calves and suggested that innate and adaptive immunity play more significant roles in immune responses to *Cryptosporidium* species than mother passive transfers of immunity to the offspring. Our research findings show that the timely ingestion of an adequate volume of colostrum plays a significant role in the likelihood of *Cryptosporidium* spp. infection in calves, suggesting that the role of other bioactive compounds of colostrum (growth factors, hormones, cytokines, enzymes, polyamines, nucleotides, antimicrobial components, white blood cells, etc.) should not be underestimated. Moreover, the IgG and other immune compounds should be considered as one complex system where several elements cooperate with each other to create one universal barrier against pathogens, as there is evidence of coinfection with other viral and bacterial pathogens [3]. In addition, the results of our study (the increased percentage of diarrhea) indirectly showed that colostrum intake should be monitored so that it is ingested immediately after birth, which significantly reduces the chances of pathogenic infection. An explanation for these findings may be found in Fischer *et al*. [22], who demonstrated that calves fed immediately after birth (0 h) had greater serum IgG concentrations compared with calves fed at 6 and 12 h after birth, as well as influencing the establishment of the calf gut microbiome [23].

The results of our research show that calves fed transition milk for at least 2 weeks following the colostrum meal have significantly lower chances of having *Cryptosporidium* and experiencing diarrhea compared to calves receiving colostrum and then whole milk. These results are supported by Conneeley *et al*. [24] and Kargar *et al*. [25], who investigated the health status of calves fed transitional milk. For example, Kargar *et al*. [25] showed that extending the duration of feeding transitional milk positively influenced calf weight gain and decreased the chance of having diarrhea. This is explained by the greater concentrations of some bioactive compounds in transitional milk compared with whole milk [9,22,26].

The skipping of transitional milk ingestion is always associated with the weaning process, where the calf undergoes multiple stressful situations. For example, a calf not weaned from the dam receives milk on demand in unlimited quantities. In contrast, a weaned calf undergoes a specific feeding program that may not match the individual physiological needs of the calf. This influences the development of the gastrointestinal system, which serves as the first barrier to infections [27]. The other stressful factor is moving the animal to a calf pen. It will live in a limited area exposed to other different aged calves

Table-1: The percent of positive calves for the presence of *Cryptosporidium* spp. oocysts and the percent of calves with the presence of clinical signs of diarrhea.

Research groups	Diarrhea (%)	Positive (%)	Negative (%)
The first research group (timely colostrum+transition milk)	15	26.1	73.9
The second research group (timely colostrum+whole milk)	46.3	37.2	62.8
The third research group (not supervised intake of colostrum+whole milk)	48.2	44.1	55.9

(with different health statuses and infections), while its immune system remains naïve. Intensive farming management has some evolutionary implications for parasites and pathogens, as discussed by Mennerat *et al*. [28]. For example, the high density of hosts in a limited territory affects the probability of parasite transmission stages to contact new hosts and faster parasite development and increases the probability of coinfections [29,30]. In addition, the gut microbiota undergoes changes [31] during the weaning process, which may affect the predisposition to diarrhea. The weaning strategy can result in great differences in growth performance, gastrointestinal development, and health status; therefore, it should be chosen carefully to minimize economic losses.

Conclusion

The most effective management of colostrum and transition milk feeding against *Cryptosporidium* spp. infection is the timely intake of an adequate amount of colostrum followed by transitional milk consumption for at least 2 weeks before weaning from the dam. Colostrum feeding can be cross-checked by estimating the serum refractometry to identify the phenomenon of failure of passive transfer.

Data Availability

The supplementary data can be available from the corresponding author on a reasonable request.

Authors' Contributions

AZ: Study design and collected and examined the samples. DK: Supervised the research process. MZ: Collected samples and performed statistical analysis. AZ and MZ: Wrote the manuscript with input from all authors. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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A SEVEN-YEAR STUDY ON THE PREVALENCE AND INTENSITY OF *CRYPTOSPORIDIUM* SPP. INFECTIONS IN DAIRY CATTLE IN LATVIA: REGIONAL AND AGE-RELATED VARIATIONS

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Zolova A., Keidane D., Zolovs M. 2024. A seven-year study on the prevalence and intensity of *Cryptosporidium* spp. infections in dairy cattle in Latvia: regional and age-related variations. *Acta Biol. Univ. Daugavp., 2024(2): 239-246.*

Abstract
This study investigates the prevalence and intensity of *Cryptosporidium* spp. infections in dairy cattle across Latvia, focusing on regional and age-related variations. Over the period from 2013 to 2020, fecal samples from 2,655 dairy cattle were analyzed using Ziehl-Neelsen staining technique and flotation methods. The overall prevalence of *Cryptosporidium* spp. was found to be 27%, with significant regional differences, the highest prevalence observed in the Vidzeme region (31%) and the highest oocyst counts in the Kurzeme region (median = 600 OPG). Age-related susceptibility was evident, with calves aged 0 to 3 months showing the highest infection rates (39.4%) and oocyst counts (median = 800 OPG). Diarrhea was significantly more common in infected calves (56.6%) compared to older cattle. The findings highlight the need for targeted interventions in young calves and region-specific control strategies to mitigate the impact of cryptosporidiosis on the dairy industry. This comprehensive study provides valuable insights into the epidemiology of *Cryptosporidium* spp. in Latvian dairy cattle, emphasizing the importance of age and regional factors in infection dynamics.

Keywords: *Cryptosporidium* spp., Latvia, epidemiology, regional variation, age-related.

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INTRODUCTION

The study of *Cryptosporidium* spp. in dairy cattle has garnered significant attention due to its implications for both human health and animal productivity. *Cryptosporidium* spp. are protozoan parasites that infect the gastrointestinal tract of various hosts, leading to cryptosporidiosis, a disease characterized by diarrhea and other gastrointestinal symptoms (Fayer et al. 2000). The prevalence and intensity of *Cryptosporidium* infections in livestock, particularly dairy cattle, have been extensively documented, highlighting the economic and health burdens associated with these infections (Santín 2013). Therefore, understanding the epidemiology of *Cryptosporidium* spp. in dairy cattle is crucial for developing effective control and prevention strategies, which can mitigate the impact of this parasite on the dairy industry.

Previous research has demonstrated that the prevalence of *Cryptosporidium* spp. in dairy cattle varies widely across different regions and management practices (Xiao & Fayer 2008, Thomson et al. 2017). Factors such as age, environmental conditions, and herd management practices significantly influence the infection rates and oocyst shedding in cattle (Thomson et al. 2017). Young calves are particularly susceptible to infection, often exhibiting higher prevalence rates and more severe clinical symptoms compared to older cattle. Zambriski et al. (2013) showed that 17 oocysts were sufficient to cause diarrhea and oocyst shedding. Research shows that the infection rate in calves can reach up to 79.5%, significantly higher than in adult cattle (Olson et al. 2004, Bartley et al. 2023). This age-related susceptibility underscores the need for targeted interventions in young animals to reduce the overall burden of cryptosporidiosis in dairy herds.

The detection and quantification of *Cryptosporidium* oocysts in fecal samples are critical for assessing infection dynamics and implementing control measures. Various diagnostic techniques, including microscopy, immunoassays, and molecular methods, have been employed to identify and quantify *Cryptosporidium* oocysts in cattle feces (Smith et al. 2007, Vanathy et al. 2017). Among these, the modified Ziehl-Neelsen staining technique and flotation methods are commonly used due to their reliability and cost-effectiveness (Kuczynska & Shelton 1999). Accurate detection and quantification are essential for epidemiological studies, which provide insights into the distribution and intensity of infections within and between herds.

The present study aims to investigate the prevalence and intensity of *Cryptosporidium* spp. infections in dairy cattle in Latvia, with a focus on regional variations and age-related differences. Previous studies in Latvia have been limited in sample size, making this research the most comprehensive investigation of *Cryptosporidium* spp. prevalence and intensity of infection. By employing established diagnostic methods and statistical analyses, this research seeks to contribute to the existing body of knowledge on *Cryptosporidium* epidemiology in dairy cattle.

MATERIAL AND METHODS

The investigation was conducted over the period from 2013 to 2020. A total of 2655 dairy cattle were subjected to testing for the presence of *Cryptosporidium* spp. parasites through the analysis of fecal specimens collected during routine health assessments. In most cases, individual fecal specimens were obtained directly from the rectum. In exceptional circumstances, specimens were procured during the defecation process or retrieved from the ground immediately following the excretion by the animal. Specimens were gathered utilizing disposable gloves and subsequently maintained in a portable cooler during transit to the laboratory, where they were stored at a temperature of +4 ^{0}C until they underwent examination.

Samples intended for conventional microscopy

were subjected to a saturated NaCl flotation methodology (Kuczynska & Shelton 1999). In the flotation procedure, a quantity of 1 g of fecal sample was utilized, and following the flotation and centrifugation procedures, 2 ml of purified material was acquired for subsequent analytical assessments. Initially, all samples of the purified material underwent an examination. A 10 µl droplet of the purified oocysts was placed upon a microscopic slide, allowed to air dry at ambient temperature, and subsequently stained utilizing a modified Ziehl-Neelsen technique. All oocysts exhibiting a dark red to pink coloration with characteristic morphology were counted for each of the 10 µl droplets employing a microscopy magnification of 200x. Consequently, each oocyst identified microscopically corresponded to an equivalent of 200 oocysts per gram of feces (OPG) (Åberg et al. 2019).

The prevalence was calculated as the proportion of dairy cattle individual in a population infected with a *Cryptosporidium* spp. parasites. The comparison of prevalence and diarrhea between age groups was conducted by using the chi-squared test of homogeneity. The comparison of OPG between age groups was processed by the Kruskal – Wallis H test. Statistical analyses were conducted using Jamovi (v 2.5.5). Results were considered statistically significant at a p-value of less than 0.05.

RESULTS

The total prevalence of *Cryptosporidium* spp. infection in Latvia was determined to be 27% and a 95% confidence interval ranging from 26% to 29% with a median oocyst count per gram measured at 1000 (ranging from Q1 = 400 to Q3 = 3000). Diarrhea was observed in 30.1% of cows infected with *Cryptosporidium* spp., and it was noted in 24.5% of cows that were not infected with *Cryptosporidium* spp.

The minimum oocyst count per gram identified in bovine feces was 200, while the maximum count reached 476,500. The recorded oocyst counts per gram adhere to a negative binomial distribution, exhibiting a substantial degree of clustering or overdispersion, which suggests that the distribution of oocysts is not entirely stochastic. The majority of the analyzed fecal samples contained between 200 and 2000 oocysts per gram, with only a limited number of samples exceeding 15,000 oocysts, thereby underscoring a significant variation in infection intensity (Fig. 1).

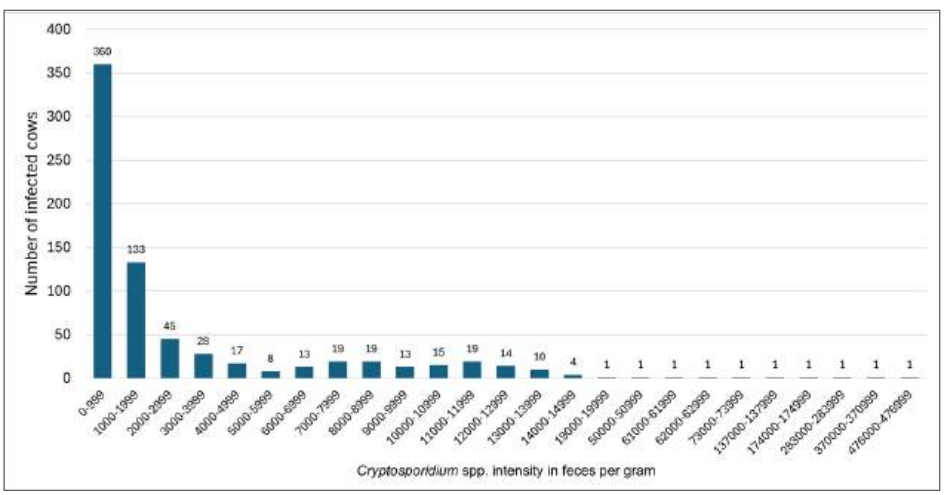

Figure 1. Distribution of *Cryptosporidium* spp. oocysts of bovine feces in Latvia.

The prevalence and oocyst count per gram of bovine feces exhibited a non-random distribution across planning regions of Latvia, showing the highest prevalence in Vidzeme region (31%) and highest oocyst count in Kurzeme region (median = 600, Q1-Q3 300 – 1200). The distribution of *Cryptosporidium* spp. with intensity of infection in Latvia by planning regions has been summarized in the Fig. 2.

The comparison of *Cryptosporidium* spp. infection revealed a statistically significant difference of prevalence between age groups ($p < 0.001$) and demonstrated a considerable reduction in infection rates as bovines mature (Tab. 1). Furthermore, the examination of diarrhea in cows infected with *Cryptosporidium* spp. parasites revealed a statistically significant difference among age groups ($p < 0.001$), with the highest percentage observed in calves aged 0 to 3 months.

Table 1. Mean prevalence, diarrhea and intensity of *Cryptosporidium* spp. infection in cows by age groups.

Parameter	Prevalence, % (95% CI)	Median (Q1 – Q3)	Diarrhea with *Cryptosporidium* spp., % (95% CI)
From 0 to 3 months	39.4 (32.6 – 46.5)	800 (200 – 2400)	56.6 (44.7 – 67.9)
From 4 to 24 months	20.3 (17.0 – 23.9)	400 (400 – 650)	4.2 (0.1 – 21.1)
More than 24 months	19.2 (16.7 – 21.9)	600 (400 – 1000)	7.0 (1.9 – 17.0)
p-value	<0.001	0.118	<0.001

Figure 2. Mean prevalence (%) and median (Q1 – Q3) of *Cryptosporidium* spp. oocyst per gram of bovine feces (OPG) by planning regions of Latvia (according to the Cabinet of Ministers' regulations of June 22, 2021, No. 418). Source: https://www.varam.gov.lv/lv/planosanas-regioni.

DISCUSSION

This study represents the first extensive, long-term investigation of *Cryptosporidium* spp. infections in Latvian dairy cattle, encompassing a large sample size of over 2,500 animals. The findings of this study reveal a significant prevalence of *Cryptosporidium* spp. infections in dairy cattle across Latvia, with notable regional and age-related variations. The reported overall *Cryptosporidium* prevalence rate of 27% in dairy herds is consistent with findings from various studies, which indicate similar infection rates across different regions. For instance, a meta-analysis revealed a global prevalence of 25.5% in cattle, with significant variations depending on geographical location and management practices (Buchanan et al. 2024). Specific studies have shown rates of 28.7% in India (Agrawal et al. 2023), 30% in France (Certad et al. 2024), and even higher rates in certain populations of calves, such as 91.6% in a Slovakian dairy farm (Kaduková et al. 2024). These findings underscore the nature of *Cryptosporidium* infections in dairy cattle and highlight the importance of monitoring and management practices to mitigate the economic and public health impacts associated with this zoonotic pathogen. Moreover, the highest prevalence observed in the Vidzeme region and the highest oocyst counts in the Kurzeme region suggest that environmental and management factors may play a crucial role in the distribution and intensity of infections.

The age-related differences in *Cryptosporidium* infection rates and oocyst shedding observed in this study are particularly noteworthy. Calves aged 0 to 3 months exhibited the highest prevalence and oocyst counts, which is in line with previous research indicating that young calves are more susceptible to *Cryptosporidium* infections. For example, Aguilar reported a prevalence of 26.6% in calves from dairy herds in Colombia, with the highest infection rates observed in calves aged 8-14 days, indicating a critical vulnerability during this early life stage (Aguilar 2023). Similarly, Urie et al. found that younger calves were more likely to test positive for *Cryptosporidium*, with peak prevalence occurring around two weeks of age (Urie et al. 2018). This aligns with findings from Garro et al., who noted that the frequency of oocyst shedding was highest in calves under 20 days of age (Garro et al. 2016). Doungmala et al. highlighted that oocyst shedding was particularly pronounced in calves aged 1-3 weeks, reinforcing the notion that early exposure is critical for infection (Doungmala et al. 2019). Additionally, Ebiyo and Haile demonstrated that calves under six months had a significantly higher risk of infection, with an odds ratio of 2.7, emphasizing the importance of age as a risk factor (Ebiyo & Haile 2022). The significant reduction in infection rates and oocyst shedding in older cattle underscores the importance of implementing targeted interventions in young calves to mitigate the impact of cryptosporidiosis in dairy herds.

The association between *Cryptosporidium* infections and diarrhea in dairy cattle observed in this study further underscores the clinical significance of this parasite. The higher incidence of diarrhea in infected animals, particularly in young calves, aligns with the pathogenic potential of *Cryptosporidium* spp. to cause gastrointestinal disturbances (Fayer et al. 2000). For example, Wells et al. reported a high prevalence of *Cryptosporidium* in cattle during the calving season, suggesting that the timing of calving significantly influences infection rates and, consequently, the incidence of diarrhea (Wells et al. 2015). Thomson et al. reported that *Cryptosporidium* was the most commonly detected pathogen causing diarrhea in calves less than one month of age, further highlighting its impact on animal health (Thomson et al. 2017). This age-related susceptibility is critical for understanding the dynamics of *Cryptosporidium* transmission within herds and for developing effective management strategies. Moreover, the economic implications of *Cryptosporidium* infections are substantial. Diarrhea in calves can

lead to dehydration, weight loss, and increased veterinary costs, ultimately affecting the productivity of dairy operations. As highlighted by Berhanu et al., adult cattle can serve as reservoirs for *Cryptosporidium*, shedding oocysts that contaminate the environment and pose a risk to younger animals (Berhanu et al. 2022). This emphasizes the need for comprehensive herd management practices that address both adult and juvenile cattle to mitigate the risk of outbreaks.

The detection of *Cryptosporidium* oocysts using the modified Ziehl-Neelsen staining technique and flotation methods proved effective in this study, showing the reliability of these diagnostic approaches (Kuczynska & Shelton 1999). The negative binomial distribution of oocyst counts highlights the overdispersion and clustering of infections within the population, which has been observed in other parasitological studies. For example, the negative binomial distribution is widely recognized for its ability to model the aggregated distribution of parasites among hosts, where a few hosts harbor many parasites while most hosts have few or none (Ieshko et al. 2024). This distribution is often attributed to variations in host susceptibility and parasite exposure (Gourbière et al. 2015). This suggests that a small proportion of highly infected animals may contribute disproportionately to environmental contamination and transmission dynamics, emphasizing the need for targeted control measures.

CONCLUSIONS

In conclusion, this study provides valuable insights into the epidemiology of *Cryptosporidium* spp. infections in dairy cattle in Latvia. The regional and age-related variations in prevalence and oocyst shedding underscore the need for tailored control strategies that consider local environmental conditions and the specific vulnerabilities of different age groups. Future research should focus on elucidating the specific environmental and management factors that contribute to the observed variations in infection rates, as well as developing and implementing effective intervention strategies to reduce the burden of cryptosporidiosis in dairy herds.

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