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Latvia University of Life Sciences and Technologies

Faculty of Veterinary Medicine

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Doctoral thesis

PREVALENCE, GENETIC DIVERSITY, ENVIRONMENTAL SHEDDING AND ASSOCIATED FACTORS OF *GIARDIA DUODENALIS* IN CATTLE (*BOS TAURUS*) AND CANIDS (CANIDAE) IN LATVIA

GIARDIA DUODENALIS IZPLATĪBA, ĢENĒTISKĀ DAUDZVEIDĪBA, VIDES PIESĀRŅOJUMS UN TO IETEKMĒJOŠIE FAKTORI GOVĪM (*BOS TAURUS*) UN SUŅU DZIMTAS (CANIDAE) DZĪVNIEKIEM LATVIJĀ

Doctoral degree Doctor of Science (*Ph.D.)*

Veterinary Sciences

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The doctoral thesis was elaborated from 2019 to 2025 at the Institute of Food and Environmental Hygiene of the Faculty of Veterinary Medicine and at the Institute of Food Safety, Animal Health and Environment "BIOR", the Department of Microbiology and Pathology, Parasitology and Microbial Genomic groups.

To cover the main areas within the One Health concept, a total of 1642 animal samples, including productive animals, pets, and wildlife were collected. Among those, 972 cattle (*Bos taurus*), 373 domestic dog (*Canis familiaris*) samples were selected for assessment of the prevalence of *G. duodenalis* in domestic animals, while 219 red foxes (*Vulpes vulpes*) and 78 raccoon dogs (*Nyctereutes procyonoides*) were sampled to analyze the prevalence, cyst load, and genetic diversity of *Giardia duodenalis* in wildlife in Latvia.

Questionnaires for cattle and domestic dogs were developed to assess potential risk and protective factors for establishment of *G. duodenalis* infection. For cattle, the questionnaire was organized in sections covering individual animal information, general herd information, calves management, walking areas and pastures, herd management and feeding practices, and farm surrounding area. For domestic dogs, the questionnaire included sections on individual animal information, daily activities, health status, feeding practices, and contact with other animals. Data on age, hunting region, and forestry district was obtained for wild canids. Finally, cyst load intensity from all four animal species was estimated to determine which of the species contributes most to the environmental contamination with *G. duodenalis*.

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TRANSPAR

FLPP
FUNDAMENTĀLO UN LIETIŠĶO PĒTĪJUMU PROJEKTI

ANNOTATION

The doctoral thesis "Prevalence, genetic diversity, environmental shedding and associated factors of *Giardia duodenalis* in cattle (*Bos taurus*) and canids (Canidae) in Latvia" by Maira Mateusa was conducted between 2019 and 2025 at the Institute of Food and Environmental Hygiene of the Faculty of Veterinary Medicine and at the Institute of Food Safety, Animal Health and Environment "BIOR", the Laboratory of Microbiology and Pathology, Parasitology and Microbial Genomic groups. The study was done in three study periods. The first study period was focused on the prevalence, cyst load, genetic diversity, risk and protective factors associated with *G. duodenalis* prevalence in cattle in Latvia. The second study period covered the prevalence, cyst load, and genetic diversity of *G. duodenalis* in domestic dogs, and risk analysis was performed to identify factors that could influence the infection in domestic dogs. The third study period was focused on the prevalence, cyst load intensity, and genetic diversity of *G. duodenalis* in red foxes and raccoon dogs. Additionally, statistical analysis was performed to estimate the contribution of studied animal species to the contamination of the general environment with *G. duodenalis* cysts with the focus on zoonotic assemblage A.

The first study period was from March 2019 to March 2021. A total of 973 cattle fecal samples from 32 herds were collected. Feces were examined using immunofluorescence staining followed by microscopic examination. Microscopically positive samples were subjected to *G. duodenalis* assemblage differentiation targeting the *beta-giardin* gene with restriction length fragment polymorphism analysis (RFLP). A questionnaire was developed to gather data on herd management practices and to analyze potential factors affecting the prevalence of *G. duodenalis* in studied herds. Questionnaires were filled out by interviewing the herd owners or responsible veterinarians. Generalized linear mixed modeling (GLMM) fit by maximum likelihood (Laplace approximation) was performed to identify risk and protective factors associated with the prevalence of *G. duodenalis*. All factors were assessed at the herd level, excluding animal age, sex, breed, and diarrhea (present/absent). Age was expected to be an important effect-modifying variable; therefore, data on age in days were included in risk and protective factor calculation models.

The second study period lasted from April 2020 until May 2022. A total of 373 dog feces were tested. The same methodology as for cattle was applied for *G. duodenalis* cyst and assemblage detection. The dog owners filled out a questionnaire. The generalized linear model (GLM) of the binomial family was performed to identify risk and protective factors associated with *G. duodenalis* infection.

The third study period lasted from February 2020 to January 2025, where feces from red foxes and raccoon dogs were collected. A total of 219 red foxes and 78 raccoon dog feces were tested for *G. duodenalis* cysts using the same methods as for cattle and domestic dogs. Information about age, hunting parish, and forestry districts where animals were hunted had been collected from hunters.

Finally, the evaluation of the examined animal species across the three different ecosystems – rural, urban, and wildlife, contribution to the environmental contamination with *G. duodenalis* cysts was done. For this purpose, the cysts per gram (CPG) was compared between species, and then adjusted to the mean fecal/scat mass (grams per defecation), and number of defecations per day, to calculate the daily *G. duodenalis* cyst load to evaluate impact on the general environment. Additionally, the spatial distance between the zoonotic *G.*

duodenalis assemblage A-positive animals and surface waterbodies was assessed to estimate a possible environmental spill-off.

The doctoral thesis hypothesis: The prevalence, genetic diversity, and environmental cyst load of *G. duodenalis* differ among cattle (*Bos taurus*), domestic dogs (*Canis familiaris*), red foxes (*Vulpes vulpes*), and raccoon dogs (*Nyctereutes procyonoides*) in Latvia and are influenced by farming practices, housing conditions, and animal-specific factors.

The aim of the doctoral thesis was to analyze the prevalence, cyst load, and genetic diversity of *G. duodenalis* in cattle (*Bos taurus*), domestic dogs (*Canis familiaris*), red foxes (*Vulpes vulpes*), and raccoon dogs (*Nyctereutes procyonoides*) in Latvia, to identify factors associated with the increased prevalence of *G. duodenalis* in studied animal species and to assess their potential contribution to environmental contamination.

Tasks of the doctoral thesis:

1. to analyze the prevalence, cyst load, genetic diversity, animal-level and herd-level factors potentially associated with *G. duodenalis* in cattle;
2. to detect the prevalence, cyst load, genetic diversity, and animal-level and housing factors potentially associated with *G. duodenalis* in domestic dogs;
3. to establish the prevalence, cyst load, genetic diversity, and animal-level factors potentially associated with *G. duodenalis* in red foxes and raccoon dogs;
4. to assess and compare the cyst-shedding intensity of cattle and canids, to determine their contributions to environmental contamination with *G. duodenalis* zoonotic assemblage A.

Scientific novelty of the doctoral thesis:

1. the first study in Latvia on the prevalence of *G. duodenalis*, cyst load, genetic diversity in cattle, domestic dogs, red foxes and raccoon dogs in Latvia, revealing *G. duodenalis* assemblages C, D and E and the zoonotic assemblage A;
2. identified factors associated with the prevalence of *G. duodenalis* in cattle, domestic dogs, red foxes and raccoon dogs in Latvia, providing veterinarians and experts in the field with insight into how to limit the spread of this parasite;
3. provides insight into the potential dissemination of zoonotic *G. duodenalis* assemblage A in the environment within the One Health approach.

Authors' personal contributions:

1. collection of feces from cattle;
2. communication with the herd owners and dog owners to collect fecal samples and conduct questionnaires;
3. feces preparation using immunofluorescence staining technique for *G. duodenalis* cyst detection and microscopy;
4. molecular analyses, including genomic *G. duodenalis* DNA isolation, PCR amplification, and Restriction Length Fragment Polymorphism Analysis (RFLP), were done personally in most cases;
5. descriptive statistics and risk and protective factor analysis using generalized linear mixed and generalized linear models to identify risk and protective factors associated with the prevalence of *G. duodenalis* in animal species were done personally in most cases.

Giardia duodenalis prevalence in cattle in Latvia reached 8.4% (82/973) with the animals were shedding an average of 5756 CPG of feces. The highest prevalence of 16.4% (53/324) was observed in the 0–3-month-old cattle age group, followed by 6.8% (19/281) in the 4–24-

month-old and 2.7% (10/368) in the > 24-month-old age groups. Significantly higher *Giardia* cyst load was shed by cattle in the 0–3-month-old age group ($p = 0.0005$). The highest proportion of diarrhea was observed in the 0-3-month-old cattle age group; but no statistical significance was observed between diarrhea and the presence of *G. duodenalis* ($p > 0.05$). Cattle herd prevalence was up to 84.4% (27/32) with the highest prevalence of 100% in herds with 251–500 (7/7) and more than 500 cattle (8/8), and 90.0% (9/10) in the herds with less than 150 cattle. The lowest prevalence of *G. duodenalis* was observed in herds with 151–250 cattle (42.9%, 3/7). In individual cattle, *G. duodenalis* assemblage A was detected in 11.3% (7/62), but assemblage E was detected in 88.7% (55/62) of the positive cattle. *G. duodenalis* assemblage A was identified in five cattle from the 4–24-month-old age group (71.4%, 5/7) and two cattle from the >24-month age group (28.6%, 2/7). *G. duodenalis* assemblage E was detected in 76.4% (42/55) of the calves from 0–3-month-old age group, followed by 14.5% (8/55) in the 4–24-month-old age group, and 9.1% (5/55) in the >24-month-old age group. For *G. duodenalis*-associated factors in cattle, in the final model, one risk (ability to leave the herd premises) and five protective factors (age, pasture season beginning in May or no pastures; manure kept in open pit or pile) appeared to be associated with the reduction of *G. duodenalis* infection in cattle in Latvia.

The prevalence of *G. duodenalis* in domestic dogs was 10.7% (40/373), with the highest prevalence in the puppy age group (under one year old) (18.5%, 12/65). Significantly higher prevalence was observed in male dogs than female dogs ($p = 0.01$), but no differences were observed between sex and cyst load ($p = 0.05$). In domestic dogs, the zoonotic assemblage A was detected in 10.5% of the dogs (2/19), and dog-specific assemblages C and D were detected in 31.6% (6/19) and 42.1% (8/19) of the dogs, respectively. In the final GLM, *G. duodenalis* was associated with male dogs and co-infection with *Cryptosporidium* spp., but activity outside the city with a leash was a protective factor against *G. duodenalis* infection.

In red foxes, *G. duodenalis* prevalence was 27.4% (60/219), with the highest prevalence observed in animals around five years old (50.0%, ½). No significant differences were observed among the age groups and cyst shedding in red foxes ($p = 0.07$). In raccoon dogs, the prevalence reached 30.8% (24/78), and the highest prevalence was observed in the 1–1.5-year-old age group (46.7%, 7/15). Differences between age and *G. duodenalis* cyst load were not significant ($p = 0.7$) in raccoon dogs. In red foxes, assemblages C and D were detected in one animal each, but one raccoon dog was positive for assemblage D. In the final GLM, the increased prevalence of *G. duodenalis* was associated with older animals (OR 2.3, $p = 0.007$) and co-infection with *Cryptosporidium* spp. (OR 111.1, $p < 0.001$). In raccoon dogs, increased *G. duodenalis* infection was associated with younger animals (OR 0.1, $p = 0.005$) and with co-infection of *Cryptosporidium* spp. (OR 16.0, $p < 0.001$).

Regarding environmental contamination, *G. duodenalis* prevalence in both wild canids was significantly higher than in domestic dogs and cattle ($p < 0.0001$), and 3.5 times higher odds of infection were observed in red foxes compared to cattle. After adjusting the shed of *G. duodenalis* CPG to the weight of feces produced by animals, cattle shed significantly higher amounts of *G. duodenalis* cysts than other species ($p < 0.05$). While higher prevalence of the zoonotic *G. duodenalis* assemblage A (77.8%, 7/9) was in cattle, domestic dogs shed higher load of *G. duodenalis* assemblage A cysts in the environment (33,400-68,200). Three (42.7%) of the seven *G. duodenalis* assemblage A-positive cattle had access to pastures. Three out of the seven *G. duodenalis* assemblage A-positive cattle herds were located within 2 km from a major river – Bērze, Tērvete and Engure. From the questionnaires, 9 (28.1%) out of the 32 herd owners reported to have open waterbodies in the pastures (such as lakes and rivers). All interviewed herd owners used manure for field fertilization (32/32, 100.0%), out of which eight herd owners stored the manure in a pile next to the facilities (25.0%). Ten out of the 32 herd owners did not treat manure or slurry before field fertilization (31.2%). Manure from herds with

the zoonotic *G. duodenalis* assemblage A (3/7) was stored in open pits next to the farm facilities (71.4%). Despite differences between the prevalence, cyst load, and assemblage distribution among all four animal species, species-specific factors such as age, sex, co-infection status, and management practices influence the prevalence rates and intensity of cyst shedding. Although significantly higher *G. duodenalis* prevalence was observed in wild canids rather than in cattle or domestic dogs, cattle appear to be the most important contributors to the environmental contamination with *G. duodenalis* cysts.

Ten conclusions and **four practical recommendations** have been formulated at the end of the doctoral thesis. There are **142 pages**, **41 tables**, **32 figures,** and **seven appendices** attached. The bibliography contains **319 references**.

ANOTĀCIJA

Mairas Mateusas promocijas darbs "*Giardia duodenalis* izplatība, ģenētiskā daudzveidība, vides piesārņojums un to ietekmējošie faktori govīm (*Bos taurus*) un suņu dzimtas (Canidae) dzīvniekiem Latvijā" izstrādāts Veterinārmedicīnas fakultātes Pārtikas un vides higiēnas institūtā un Pārtikas drošības, dzīvnieku veselības un vides zinātniskajā institūtā "BIOR" Mikrobioloģijas un patoloģijas laboratorijas Parazitoloģijas grupā un Mikroorganismu genoma grupā no 2019. līdz 2025. gadam. Pētījums veikts trīs periodos. Pirmajā periodā tika noteikta *G. duodenalis* izplatība, intensitāte, ģenētiskā daudzveidība, kā arī veikta riska un aizsargājošo faktoru analīze govīm Latvijā. Otrajā periodā tika noteikta *G. duodenalis* izplatība, intensitāte, ģenētiskā daudzveidība mājas suņiem, kā arī veikta invāzijas risku faktoru analīze. Trešajā periodā tika noteikta *G. duodenalis* izplatība, intensitāte un ģenētiskā daudzveidība rudajām lapsām un jenotsuņiem. Pētījuma noslēgumā papildus tika veiktas statistiskās analīzes, lai noteiktu, kura no dzīvnieku sugām ir nozīmīgākais žiardiju cistu izplatītājs apkārtējā vidē, īpaši uzsverot zoonotisko *G. duodenalis* A apakštipu.

Pirmais periods norisinājās no 2019. gada marta līdz 2021. gada martam. Kopumā tika izmeklēti 973 govju fekālie paraugi no 32 ganāmpulkiem. Fekāliju paraugi tika analizēti, izmantojot tiešo imunofluorescences krāsošanu, kam sekoja mikroskopiskā izmeklēšana. Visi mikroskopiski pozitīvie dzīvnieku paraugi tika tālāk analizēti, lai noteiktu *G. duodenalis* apakštipus, nosakot bēta-giardīna gēnu ar restrikcijas fragmentu garuma polimorfisma (RFLP) analīzi. Tika izstrādāta anketa, lai iegūtu datus par ganāmpulka pārvaldības praksēm un analizētu iespējamos faktorus, kas varētu ietekmēt *G. duodenalis* izplatību. Anketas tika aizpildītas, intervējot ganāmpulka īpašniekus vai atbildīgos veterinārārstus. Riska un aizsargājošo faktoru identificēšanai tika veikta vispārinātā lineārā jaukto efektu modelēšana (GLMM), izmantojot maksimālās ticamības novērtējumu (Laplace aproksimācija). Visi faktori tika izvērtēti ganāmpulka līmenī, izņemot dzīvnieka vecumu, dzimumu, un šķirni. Tika sagaidīts, ka vecums būs svarīgs modificējošs, mainīgais lielums, tāpēc riska un aizsardzības faktoru aprēķinu modeļos tika iekļauti vecuma dati dienās.

Otrais pētījuma periods norisinājās no 2020. gada aprīļa līdz 2022. gada maijam. Kopumā izmeklēti 373 suņi. *G. duodenalis* cistu noteikšanai un ģenētiskās analīžu veikšanai izmantota govju fekālajiem paraugiem pielietotā metodoloģija. Suņu īpašnieki aizpildīja aptaujas anketu, lai noteiktu riska un aizsargājošos faktorus, kuru aprēķiniem pielietota ģeneralizētā lineārā modelēšana (GLM).

Trešais periods norisinājās no 2020. gada februāra līdz 2025. gada janvārim, lai ievāktu fekāliju paraugus no rudajām lapsām un jenotsuņiem. Kopumā ievākti fēkālie paraugi no 219 rudājām lapsām un 78 jenotsuņiem, kuru *G. duodenalis* noteikšanai pielietota govju un suņu paraugiem izmantotā metodoloģija. Papildus no medniekiem tika ievākta informācija par dzīvnieku vecumu, medību vietu – pagastu un reģionu, kā arī virsmežniecību.

Lai novērtētu, kuras no pētītajām dzīvnieku sugām trijos dažādos izplatības areālos – lauku, pilsētas vai dabiskā ekosistēmās, veicina vides piesārņojumu ar *G. duodenalis* cistām, tika salīdzināts cistu skaits vienā fekāliju gramā starp sugām, un, pēc tam šis skaits tika pielāgots vidējai fekāliju masai (gramos) un defekācijas skaitam dienā, lai aprēķinātu izdalīto *G. duodenalis* cistu skaitu. Papildus tam, tika novērtēts attālums starp *G. duodenalis* zoonotiskā A apakštipa pozitīvajiem dzīvniekiem un virszemes ūdenstilpēm, lai aprēķinātu iespējamo ietekmi uz virszemes ūdens kontamināciju.

Promocijas darba hipotēze: *Giardia duodenalis* izplatība, ģenētiskā daudzveidība un cistu izdalīšanas intensitāte vidē atšķiras starp govīm (*Bos taurus*), mājas suņiem (*Canis familiaris*), rudām lapsām (*Vulpes vulpes*) un jenotsuņiem (*Nyctereutes procyonoides*) Latvijā,

ko govīm ietekmē ganāmpulku un bet suņiem turēšanas apstākļi, kā arī dzīvnieku sugām piemītošie vai raksturīgie specifiskie faktori.

Promocijas darba mērķis: Noteikt žiardijas (*Giardia duodenalis*) izplatību, cistu izdalīšanās intensitāti, ģenētisko daudzveidību un to izplatību ietekmējošos faktorus govīm (*Bos taurus*), mājas suņiem (*Canis familiaris*), rudajām lapsām (*Vulpes vulpes*) un jenotsuņiem (*Nyctereutes procyonoides*) Latvijā, kā arī izvērtēt izmeklēto dzīvnieku ietekmi uz vides radīto piesārņojumu ar *Giardia duodenalis*.

Promocijas darba uzdevumi:

1. noteikt *G. duodenalis* izplatību, cistu izdalīšanas intensitāti, ģenētisko daudzveidību, individuālos un turēšanas faktorus, kas saistīti ar ierosinātāja izplatību govīm;
2. analizēt *G. duodenalis* izplatību, cistu izdalīšanas intensitāti, ģenētisko daudzveidību, individuālos un turēšanas faktorus, kas saistīti ar ierosinātāja izplatību mājas suņiem;
3. noskaidrot *G. duodenalis* izplatību, cistu izdalīšanas intensitāti, ģenētisko daudzveidību, un individuālos faktorus, kas saistīti ar ierosinātāja izplatību rudajām lapsām un jenotsuņiem;
4. izvērtēt govju un suņu dzimtas dzīvnieku radīto *G. duodenalis* vides piesārņojumu, lai noteiktu, kura dzīvnieku suga veicina ierosinātāja izplatību ārvidē.

Promocijas darba zinātniskā novitāte:

1. pirmais pētījums Latvijā par *G. duodenalis* izplatību, izdalīto cistu intensitāti, ģenētisko daudzveidību govīm, mājas suņiem rudajām lapsām un jenotsuņiem Latvijā, atklājot *G. duodenalis* C, D, E un zoonotisko A apaštipu;
2. identificēti faktori, kas saistīti ar *G. duodenalis* govīm, mājas suņiem, rudajām lapsām un jenotsuņiem Latvijā, dodot ieskatu veterinārārstiem un jomas ekspertiem, kā ierobežot šī parazīta izplatību;
3. izvērtēta potenciālā zoonotiskā *G. duodenalis* A apakštipa izplatība apkārtējā vidē Vienas veselības pieejas ietvaros.

Personīgais ieguldījums:

1. paraugu ievākšana no govīm;
2. saziņa ar govju ganāmpulku un suņu īpašniekiem, aptauju veikšana un dokumentēšana;
3. paraugu sagatavošana izmeklēšanai, izmantojot imunofluorescences krāsošanas metodi *G. duodenalis* cistu noteikšanai un mikroskopēšana;
4. genētisko analīžu veikšana, tostarp *G. duodenalis* DNS izdalīšana, polimerāzes ķēdes reakcijas (PĶR) un restrikcijas fragmentu garuma polimorfisma analīzes (RFLP) veikšana. Lielākā daļa no paraugu analīzes veikta personīgi;
5. aprakstošā statistika, riska un aizsargājošo faktoru analīze, izmantojot vispārināto lineāro jaukto efektu un vispārināto lineāro modeļu aprēķinu metodiku, lai identificētu ar *G. duodenalis* izplatību saistītos faktorus. Lielākā daļa aprēķinu veikta personīgi.

Giardia duodenalis izplatība govīm sasniedza 8,4 % (82/973), ar vidējo intensitāti 5757 cistas vienā gramā fekāliju. Augstākā izplatība (16,4 %, 53/324) novērota 0–3 mēnešu vecuma teļu grupā, kam sekoja 4–24 mēnešu vecuma teļu grupa ar 6,8 % (19/281) izplatību, taču 2,7 % (10/368) izplatība novērota govīm, kas bija vecākas par 24 mēnešiem. Būtiski augstāka cistu izdalīšanas intensitāte tika novērota teļiem no 0–3 mēnešu vecuma grupas (p = 0,0005). Visbiežāk caurejas novērotas 0–3 mēnešu vecuma grupā, bet rezultātiem nebija saistības (p > 0,05) ar *G. duodenalis* klātbūtni. Izplatība govju ganāmpulkos sasniedza 84,4 % (27/32), un 100,0 % izplatība tika novērota ganāmpulkos ar 251–500 (7/7) un vairāk nekā 500 govīm (8/8),

bet nedaudz zemāka izplatība novērota ganāmpulkos ar mazāk nekā 150 govīm (90,0 %, 9/10), taču, viszemākā izplatība bija ganāmpulkos ar 151–250 govīm (42,9 %, 3/7). Govīm *G. duodenalis* A apakštips konstatēts 11,3 % (7/62), bet E apakštips – 88,7 % (55/62). *G. duodenalis* A apakštips konstatēts piecām govīm (71,4 %) vecuma grupā no 4 līdz 24 mēnešiem un divām govīm (28,6 %), kas ir vecākas par 24 mēnešiem. Savukārt E apakštips tika izolēts no visu vecuma grupu govīm – 76,4 % (42/55) no govīm 0–3 mēnešu vecumā, kam sekoja 14,5 % (8/44) 4–24 mēnešu vecuma grupa, bet visretāk izolēts no govīm, kas bija vecākas par 24 mēnešiem (9,9%, 5/55). Analizējot ar *G. duodenalis* saistītos faktorus govīm, tika identificēts viens riska faktors (iespēja atstāt kūts/mītni) un pieci aizsargājošie faktori (vecums, ganību sezona sākums maijā, ganību trūkums; kūtsmēsli tiek uzglabāti atklātā bedrē vai kaudzē).

Mājas suņiem *G. duodenalis* izplatība sasniedza 10,7 % (40/373), ar visaugstāko izplatību kucēnu vecuma grupā (18,5 %, 12/65). Tika konstatēta būtiski augstāka ierosinātāja izplatība suņu tēviņiem nekā mātītēm (p = 0,01), taču netika novērotas būtiskas atšķirības starp cistu izdalīšanas intensitāti un dzimumu (p > 0,05). Zoonotiskais A apakštips konstatēts 10,5% no suņiem (2/19), bet sugai specifiskie C un D apakštipi konstatēti attiecīgi 31,6% (6/19) un 41,1% (8/19) no izmeklētajiem suņiem. Mājas suņiem *G. duodenalis* izplatības risks bija augsts tēviņiem un vienlaicīgas *Cryptosporidium* spp. invāzijas gadījumā, savukārt aktivitāte ārpus pilsētas ar pavadu bija aizsargājošais faktors.

Rudajām lapsām, *G. duodenalis* izplatība sasniedza 27,4 % (60/219), un augstāka izplatība novērota aptuveni piecus gadus veciem dzīvniekiem – 50,0 % (1/2). Starp vecuma grupām un cistu izdalīšanu nenovēroja būtiskas atšķirības (p = 0,07). Jenotsuņiem *G. duodenalis* izplatība sasniedza 30,8 %, (24/78) ar augstāko izplatību 1–1,5 gadus vecu dzīvnieku grupā – 46,7 % (7/15). Divām rudajām lapsām konstatēja C un D apakštipus, pa vienam apakštipam katram dzīvniekam. Savukārt tikai vienam jenotsunim konstatēja D apakštipu. Rudajām lapsām *G. duodenalis* izplatība bija saistīta ar dzīvnieku vecumu, tā vecākiem dzīvniekiem ar vienlaicīgu *Cryptosporidium* spp. invāziju, ierosinātāju novēroja biežāk. Savukārt jenotsuņiem *G. duodenalis* izplatība bija augstāka jaunākiem dzīvniekiem. Arī jenotsuņiem *Cryptosporidium* spp. invāzija bija saistīta ar *G. duodenalis* izplatību.

Salīdzinot *G. duodenalis* izplatību starp izmeklētajām dzīvnieku sugām, rudajām lapsām un jenotsuņiem novēroja būtiski augstāku ierosinātāja izplatību nekā mājas suņiem un govīm (p < 0,0001); turklāt rudajām lapsām invāzijas iespējamība bija 3,5 reizes augstāka nekā govīm. Tomēr, pielāgojot izdalīto *Giardia* cistu skaitu uz gramu fekāliju attiecībā pret izdalīto fekāliju daudzumu vienā reizē, tika konstatēts, ka govis izdala būtiski lielāku *G. duodenalis* cistu daudzumu nekā pārējās sugas (p < 0,05). Lai arī govīm novēroja augstāku zoonotiskā *G. duodenalis* A apakštipa izplatību (77,8 %), suņi ārvidē izdalīja lielāku cistu intensitāti (33 400 – 68 200). Trim no septiņām *G. duodenalis* A apakštipa pozitīvām govīm bija pieeja ganībām. Trīs no septiņām *G. duodenalis* A apakštipa pozitīvām govju novietnēm, atradās divu kilometru attālumā no nozīmīgām upēm – Bērzes, Tērvetes, Engures. Deviņi no 32 (28,1 %) aptaujātiem govju novietņu īpašniekiem atzīmēja, ka ganībās atrodas pie atvērtas ūdenstilpes kā ezers vai upe. Visi aptaujātie govju novietņu īpašnieki izmantoja kūtsmēslus lauku mēslošanai (32/32, 100,0 %), no kuriem, 8 no 32 (25,0 %) glabāja kūtsmēslus kaudzē pie novietnes ēkām. Desmit no 32 aptaujāto govju novietņu īpašnieku neveica nekādu kūtsmēslu vai vircas apstrādi pirms lietošanas mēslojumam (31,2 %). Trīs no septiņām (71,4 %) novietnēm, kur tika konstatēts zoonotiskais *G. duodenalis* A apakštips, uzglabāja kūtsmēslus atvērtā tipa bedrē.

Starp visām četrām pētījumā iekļautājām dzīvnieku sugām pastāv būtiskas atšķirības *G. duodenalis* sastopamībā, cistu izdalīšanas intensitātē un ģenētisko apakštipu sadalījumā. Tādi dzīvnieka faktori kā vecums, dzimums, vienlaicīgas invāzijas statuss ar *Cryptosporidium* spp. un turēšanas prakse, ietekmē izplatību un cistu izdalīšanās intensitāti. Lai arī rudajām lapsām un jenotsuņiem tika konstatēta būtiski augstāka *G. duodenalis* izplatība nekā govīm vai mājas suņiem, govis ir nozīmīgākais *G. duodenalis* cistu avots vidē.

Promocijas darba noslēgumā ir formulēti **desmit secinājumi** un **četras praktiskās rekomendācijas**. Promocijas darbs noformēts **142 lapaspusēs**, darbā ir **41 tabula**, **32 attēli**, kā arī pievienoti **septiņi pielikumi**. Literatūras sarakstā ietverti **319 informācijas avoti**.

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Abbreviation	Meaning in English	Meaning in Latvian
spp.	species	sugas
GLMM	generalized linear mixed model	ģeneralizēts lineārais miksētais modelis
Mm	Micron	mikrons
ECDC	European Centre for Disease Prevention and Control	Eiropas Slimību profilakses un kontroles centrs
FITC	fluorescein isothiocyanate	fluoresceīna izotiocianāts
CWP	cyst wall protein	cistas sienas proteīns
EIA	enzyme immunoassays	enzīmu imūntesti
ELISA	enzyme-linked immunosorbent assays	cietfāzes enzīmu imunosorbences tests
ICT	immunochromatographic tests	imūnhromatogrāfiskie testi
tRNA	transport ribonucleic acid	transporta ribonukleīnskābe
IFM	immunofluorescence	imūnfluorescence
SSU	small subunit	mazā apakšvienība
Ghd	glutamate dehydrogenase	glutamāta dehidrogenāze
Bg	β-giardin	β -giardīns
Tpi	triose-phosphate isomerase	triozes-fosfāta izomerāze
The UK	the United Kingdom	Apvienotā karaliste
NaCl	sodium chloride	nātrija hlorīds
x g	times gravity	gravitates spēks
USA	United States of America	Amerikas Savienotās valstis
RTE	ready to eat	gatavi lietošanai
DALY	disability-adjusted life-years	invaliditātes koriģētie dzīves gadi
mAbs	monoclonal antibody solution	monoklonālo antivielu šķīdums
PBS	phosphate-buffered saline	fosfāta-buffera šķīdums

RFLP	restriction fragment length polymorphism	restrikcijas fragmentu garuma polimorfisms
bp	base pairs	bāzes pāri
CPG	cysts per gram	cistas vienā gramā
N	number	skaits
NA	not applicable	nav attiecināms
NR	not reported	nav ziņots
Min	Minimum	minimālais
Max	Maximum	maksimālais
χ^2	Chi squared	Hī kvadrātā
OR	odds ratio	izredžu attiecība
Ref.	Reference	reference
AIC	Akaike information criterion	Akaikes informācijas kritērijs
VIF	variance inflation factor	vērtības inflācijas koeficients
DS	Danish Red	Dānijas sarkanā
XX	mixed breed	Krustojums
LB	Latvian brown	Latvijas brūnā
HS	Holstein Red and White	Holšteinas sarkanraibā
HM	Holstein Friesian	Holšteinas melnraibā
LI	Limousin	Limuzīnas

INTRODUCTION

Giardia duodenalis (syn. *G. lamblia*, *G. intestinalis*) is a food and water-borne protozoan parasite that can cause giardiasis in susceptible animal species and humans (Dixon, 2021). *G. duodenalis* can infect a wide range of hosts, including cattle, canids – dogs, foxes, raccoon dogs, and humans, which may become asymptomatic carriers of *G. duodenalis* (Dixon, 2021). Assemblages A and B are zoonotic and had caused several *Giardia*-associated outbreaks in humans (Dixon, 2021). In individuals with underlying health conditions the protozoan may cause clinical infection which manifestations vary from mild to severe or acute to chronic diarrhea, resulting in fluid, electrolyte, or nutrient malabsorption (O'Handley et al., 2003; Geurden et al., 2010; Dixon, 2021). Even though no long-term studies have been done on impact of giardiasis on calves and dogs, the infection causes severe weight loss and reduction in weight in lambs (Aloisio et al., 2011; Sweeny et al., 2011; Šmit et al., 2023). Since 2004, *G. duodenalis* has been included in the Neglected Disease initiative of the World Health Organization (WHO) and is currently ranked as the 6th and 8th the most important foodborne parasite in Europe and Eastern Europe, respectively (Savioli et al., 2006; Bouwknegt et al., 2018). *G. duodenalis* cysts are small (7-10 μm) with thick walls that make them highly robust in the environment and enhance the survival of the pathogen under harsh environmental conditions. The infectious dose can be as low as 10 cysts to cause clinical signs and this parasite is well adapted to be transferred via food, feed, and especially water (Dixon, 2021).

Within the One Health concept, *G. duodenalis* is an important human and animal pathogen (Geurden et al., 2010). *G. duodenalis* prevalence in cattle in Europe reached 31.1%, with the highest prevalence observed in calves (Taghipour et al., 2022). *G. duodenalis* usually affects one-month-old calves, and out of eight *G. duodenalis* assemblages, three were found in cattle – assemblage E, which is cattle-specific, and zoonotic assemblages A and B (Dixon, 2021). One infected cattle can excrete from 10^5 to 10^6 cysts with one gram of feces, and due to asymptomatic or chronic nature of infection, infected animals are hard to identify and isolate to prevent further spread of the pathogen (Fayer et al., 2000; Dixon et al., 2021). Additionally, cattle could be potential reservoirs for the zoonotic *G. duodenalis* assemblages A and B (Coklin et al., 2007; Bartley et al., 2018; Zahedi et al., 2020). Chronic giardiasis in cattle can last up to several months and together with the release of large amounts of feces (up to 30 kg) ensures prolonged environmental contamination (Aland et al., 2002). If untreated cattle manure containing zoonotic *G. duodenalis* assemblages is used in field and garden fertilization, it can contaminate not only crops but also the surface and underground water sources (Martinez et al., 2009). There have been concerns on a role of domestic and wild canids in the spread of *G. duodenalis* with domestic dogs and red foxes being expected to serve as potential source of *G. duodenalis* for humans (Traub et al., 2004; Onac et al., 2015; Adell-Aledón et al., 2018; Moghaddasi et al., 2024). However, scarce information is available about *G. duodenalis* assemblages in wild raccoon dogs, with dog-specific assemblage D being reported in farmed raccoon dogs in Poland (Solarczyk et al., 2016).

Dogs are among the most popular pets and may become an infection source for owners (Sun et al., 2023). In dogs, *G. duodenalis* is one of the most detected parasites, especially in animals from shelters and kennels, with the worldwide prevalence of 15.2% (n = 4,309,451) (Claerebout et al., 2009; Ferreira et al., 2011; Bouzid et al., 2015). Giardiasis in dogs, similar to cattle, is often asymptomatic, but in puppies, mild to severe intermittent diarrhea can be observed (Feng & Xiao, 2011). Red foxes and raccoon dogs are often seen in various environments – forests, countryside, cities, and suburban territories without direct contact with humans. However, their broad environmental habitat creates a risk of contamination of larger areas with zoonotic *G. duodenalis* assemblages (Debenham et al., 2017).

In humans, *G. duodenalis* causes acute or chronic diarrhea accompanied by bloating, nausea, and abdominal pain and can lead to lower cognitive functions, allergies, vitamin and mineral deficiencies (Akkaub & Buret, 2020). The pathogen has been associated with pancreatic cancer (Furukawa et al., 2011; Akkaub & Buret, 2020). Additional long-term effects such as failure to thrive and stunted growth have been observed in children when infected in the first years of their lives (Bergman et al., 2005; Botero-Garcés et al., 2009).

Cattle farming has been listed as one of the main sources for the environmental contamination with zoonotic pathogens, mostly due to usage of untreated manure for crop fertilization (Koyun et al., 2023). This subsequently can increase the risk of water and food contamination, therefore increasing the probability of pathogen-associated outbreaks (Koyun et al., 2023). Using untreated manure for field fertilization has resulted in increased food and water contamination with the zoonotic protozoa due to run-offs and the sale of contaminated food directly from the fields (Lewerin et al., 2020). The rise of miniature or petting zoos and open farms could increase contact of humans, pets and wildlife with potentially infected cattle and other livestock (Dunn et al., 2015; Conrad et al., 2017). Also recreational water systems and open water sources may promote further spread of the pathogen (Brunn et al., 2018).

To the best of our knowledge, no studies about *G. duodenalis* in cattle and cattle herds, domestic dogs, red foxes, and raccoon dogs have been published in Latvia. Data about factors that affect the prevalence of *Giardia* in cattle, domestic dogs, and wild canids, their genetic diversity and zoonotic potential are largely missing. Since there is a lack of studies to tackle the environmental transmission of *G. duodenalis*, especially within One Health approach, to prevent foodborne and waterborne transmission of the pathogen, research on the epidemiology of *G. duodenalis* is needed.

The **following theses** were put forward:

1. *G. duodenalis* prevalence, cyst load, and assemblage distribution differs among cattle, domestic dogs, red foxes, and raccoon dogs in Latvia;
2. animal-level factors, including age and sex, as well as management, and housing practices, influence risk of *G. duodenalis* infection in cattle, domestic dogs, red foxes, and raccoon dogs in Latvia;
3. zoonotic *G. duodenalis* assemblages are present in cattle, domestic dogs, red foxes and raccoon dogs in Latvia;
4. cattle contribute the largest *G. duodenalis* cyst load to the environmental contamination;

Problem analysis:

There is a lack of knowledge regarding *G. duodenalis* prevalence, cyst load, and genetic diversity of *G. duodenalis*, particularly the zoonotic assemblages A and B in cattle, domestic dogs, red foxes, and raccoon dogs in Latvia. Limited information is available on animal-level and management and housing factors affecting *G. duodenalis* prevalence in these animals. Understanding of factors affecting the prevalence of the pathogen is crucial for assessing the risk of environmental contamination and potential zoonotic transmission, especially within the One Health framework. Filling knowledge gaps could further determine factors such as whether a zoonotic outbreak in humans in Latvia may occur and whether the source could be of animal origin.

The aim of the doctoral thesis was to analyze the prevalence, cyst load, and genetic diversity of *G. duodenalis* in cattle (*Bos taurus*), domestic dogs (*Canis familiaris*), red foxes (*Vulpes vulpes*), and raccoon dogs (*Nyctereutes procyonoides*) in Latvia, to identify factors associated with the increased prevalence of *G. duodenalis* in studied animal species and to assess their potential contribution to environmental contamination.

Tasks of the doctoral thesis:

1. to analyze the prevalence, cyst load, genetic diversity, animal-level and herd-level factors potentially associated with *G. duodenalis* in cattle;
2. to detect the prevalence, cyst load, genetic diversity, and animal-level and housing factors potentially associated with *G. duodenalis* in domestic dogs;
3. to establish the prevalence, cyst load, genetic diversity, and animal-level factors potentially associated with *G. duodenalis* in red foxes and raccoon dogs;
4. to assess and compare the cyst-shedding intensity of cattle and canids, to determine their contributions to environmental contamination with *G. duodenalis* zoonotic assemblage A.

Scientific novelty of the doctoral thesis:

1. the first study in Latvia on the prevalence of *G. duodenalis*, cyst load, genetic diversity in cattle, domestic dogs, red foxes and raccoon dogs in Latvia, revealing *G. duodenalis* assemblages C, D and E and the zoonotic assemblage A;
2. identified factors associated with the prevalence of *G. duodenalis* in cattle, domestic dogs, red foxes and raccoon dogs in Latvia, providing veterinarians and experts in the field with insight into how to limit the spread of this parasite;
3. provides insight into the potential dissemination of zoonotic *G. duodenalis* assemblage A in the environment within the One Health approach.

Authors' personal contributions:

1. collection of feces from cattle;
2. communication with the herd owners and dog owners to collect fecal samples and conduct questionnaires;
3. feces preparation using immunofluorescence staining technique for *G. duodenalis* cyst detection and microscopy;
4. molecular analyses, including genomic *G. duodenalis* DNA isolation, Polimeraze Chain Reaction (PCR) amplification, and Restriction Length Fragment Polymorphism Analysis (RFLP), were done personally in most cases;
5. descriptive statistics and risk and protective factor analysis using generalized linear mixed and generalized linear models to identify risk and protective factors associated with the prevalence of *G. duodenalis* in animal species were done personally in most cases.

Approbation of scientific work:

List of original publications in journals that are indexed in SCOPUS and Web of Science databases and are included in the thesis:

1. **Mateusa, M**., Ozoliņa, Z., Terentjeva, M., & Deksne, G. (2023). *Giardia duodenalis* Styles, 1902 prevalence in cattle (*Bos taurus* Linnaeus, 1758) in Europe: A systematic review. *Microorganisms*, 11(2), 309. IF 4.926. https://doi.org/10.3390/microorganisms11020309
2. **Mateusa, M**., Selezņova, M., Terentjeva, M., & Deksne, G. (2023). *Giardia duodenalis* (Styles, 1902) in cattle: isolation of calves with diarrhoea and manure treatment in the lagoon presented as protective factors in Latvian herds. *Microorganisms*, 11(9), 2338, IF 4.926, https://doi.org/10.3390/microorganisms11092338
3. **Mateusa, M**., Cīrulis, A., Selezņova, M., Šveisberga, D.P., Terentjeva, M., & Deksne, G. (2024). *Cryptosporidium* spp. are associated with *Giardia duodenalis* co-infection in wild and domestic canids. *Animals*, 14(23), 3484, IF 2.7, https://doi.org/10.3390/ani14233484

Result reports at international conferences on the topic of the doctoral thesis:

1. Deksne, G., **Mateusa, M**., & Krūmiņa, A. (2021). Occurrence of important foodborne zoonotic pathogens in Latvia – *Cryptosporidium* spp. and *Giardia duodenali*s. *RSU International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice"*. Riga, Latvia. 24.03.–26.03.2021.
2. **Mateusa, M**., Terentjeva, M., & Deksne, G. (2021). *Giardia duodenalis* and *Cryptosporidium* spp. prevalence in cattle in Latvia", *9th Conference of the Scandinavian-Baltic Society for Parasitology (SBSP)*, Vilnius, Lithuania, 21.04.2021.–23.04.2021.
3. **Mateusa, M**., Terentjeva, M., & Deksne, G. (2021). *Giardia duodenalis* prevalence in cattle in Latvia", *13th European Multicolloquium of Parasitology*, Belgrad, Serbia, 12.10.2021.–16.10.2021.
4. **Mateusa, M**., Selezņova, M., & Deksne, G. (2022). Zoonotic parasites in red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*), *80th International Scientific Conference of the University of Latvia*, Riga, Latvia, 03.02.2022.
5. **Mateusa, M**., Rozenfelde, M., Upeniece, M., Terentjeva, M., & Deksne, G. (2023). Prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in domestic dogs (*Canis familiaris*). *81st International Scientific Conference of the University of Latvia*, Riga, Latvia, 25.01.2023.
6. **Mateusa, M.**, Šveisberga, D. P., Terentjeva, M., & Deksne, G. (2023). *Cryptosporidium* spp. and *Giardia duodenalis* in untreated wastewaters in Latvia: Preliminary results. *10th Conference of the Scandinavian-Baltic Society for Parasitology*, Tartu, Estonia, 05.06.2023.–07.06.2023.
7. **Mateusa, M**., Šveisberga, D. P., Rozenfelde, M., Selezņova, M., Terentjeva, M., Krūmiņa, A., & Deksne, G. (2024). *Cryptosporidium* spp. and *Giardia* spp. from One Health perspective in Latvia. *14th European Multicolloquium of Parasitology*, Wroclaw, Poland, 26.08.2024.–30.08.2024.

Publication associated with the topic of the doctoral thesis that is indexed in the SCOPUS and Web of Science databases but not included in the doctoral thesis:

1. Deksne, G., Krūmiņš, A., **Mateusa, M**., Morozovs, V., Šveisberga, D.P., Korotinska, R., Bormane, A., Vīksna, L., & Krūmiņa, A. (2022). Occurrence of *Cryptosporidium* spp. and *Giardia* spp. infection in humans in Latvia: Evidence of underdiagnosed and underreported cases. *Medicina* (Kaunas). 24;58(4):471. IF 2.948. https://doi.org/10.3390/medicina58040471

List of popular science publications associated with the topic of the doctoral thesis that are not indexed in the SCOPUS databases and/or are not included in the doctoral thesis:

1. Deksne, G., **Mateusa, M**., & Ozoliņa, Z. (2020). Kriptosporidioze un žiardioze liellopos Latvijā Vienas Veselības kontekstā (Cryptosporidiosis and giardiasis in cattle in Latvia, from One Health context). *Veterinārais žurnāls*, Latvijas Veterinārārstu biedrības informatīvs biļetens, Riga, Latvia, Autumn, 2020.
2. **Mateusa, M**., Selezņova, M., Terentjeva, M., & Deksne, G. (2023). Kriptosporīdijas un žiardijas suņos (*Cryptosporidium* and *Giardia* in dogs), *Veterinārais žurnāls*, Latvijas Veterinārārstu biedrības informatīvs biļetens, Riga, Latvia, Winter, 2023.
3. Deksne, G., Šveisberga, D. P., **Mateusa, M**., & Krūmiņa, A. (2024). Kriptosporidioze un žiardiāzes no "Vienas veselības" skatpunkta (Cryptosporidiosis and giardiasis from "One Health" perspective). *Latvijas Ārsts, nacionālais medicīnas žurnāls*, Riga, Latvia, March–April, 2024.

4. **Mateusa, M**., Selezņova, M., Cīrulis, A., & Deksne, G. (2024). Kriptosporīdijas un žiardijas savvaļas suņu dzimtas dzīvniekos (*Cryptosporidium* and *Giardia* in wild canids). *Veterinārais žurnāls,* Latvijas Veterinārārstu biedrības informatīvs biļetens, Riga, Latvia, Summer, 2024.

1. LITERATURE REVIEW

1.1. *Giardia* genus taxonomy and characteristics of *Giardia duodenalis* assemblages

Giardia spp. was first described by A. van Leeuwenhoek in 1681 during microscopical examination of his own diarrheal stool, however W. Lambl was the first who proposed a name for the newly described species – *Cercomonas intestinalis* in 1859 (Lambl, 1859; Ankarklev et al., 2010). Later, in 1888, R. Blanchard proposed renaming the species to *Lamblia intestinalis* in honor of W. Lamb (Blanchard, 1888). The genus *Giardia* was originally proposed by Kunstler in 1882 as a tribute to Belgian taxonomist A. M. Giard (Kunstler, 1882). Later, the species name *Lamblia intestinalis* was revised to *Giardia duodenalis* by W. C. Stiles in 1902 (Blanchard, 1888; Stiles, 1902). In 1915, the name *Giardia lamblia* was proposed by A. C. Kofoid and B. E. Christensen (Kofoid & Christensen, 1920), and since then, both names of *G. duodenalis* and *G. lamblia* have been used interchangeably in scientific literature.

Giardia duodenalis classification (Schoch et al., 2020):
Phylum: Metamonada, Grassé 1952
Class: Fornicata, Leuckart 1850
Order: Diplomonadida, Kofoid & Barber 1925
Family: Hexamitidae, Kent 1880
Subfamily: Giardiinae, Kulda & Nohynkova 1978
Genus: *Giardia*, Künstler 1882
Species: *Giardia duodenalis* (syn. *G. lamblia*, *G. intestinalis),* Stiles, 1902.

The genus *Giardia* currently consists of nine recognized species, out of which *G. duodenalis* is considered zoonotic. The pathogen has been found in humans and livestock, including cattle, goats, and sheep. Other domestic and wild animals may include domestic dogs, beavers, coyotes, rodents, primates, and other mammals (Table 1.1) (Cacciò et al., 2018).

Table 1.1. **List of species in the *Giardia* genus, major hosts, and reports in humans**

Species name	Major host(s)	Reports in humans	Reference
G. agilis	Amphibians	No	Feely & Erlandsen, 1985
G. ardea	Birds	No	Ebani et al., 2021
G. cricetidarum	Hamsters	No	Lyu et al., 2018
G. duodenalis	Humans, cattle, canids, and other mammals	Yes	Dixon, 2021
G. microti	Muskrats and voles	No	Van Keulen et al., 1998
G. muris	Rodents	No	Friend, 1966
G. peramelis	Quenda	No	Hillman et al., 2016
G. psittaci	Birds	No	Ebani et al., 2021
G. varani	Lizards	No	Upton & Zien, 1997

G. duodenalis is the only *Giardia* species consisting of the eight *G. duodenalis* assemblages, out of which C–H are primarily host-specific, but A and B have a wide range of potential hosts, including livestock, domestic animals, and wildlife, and are considered to be zoonotic (Heyworth, 2016). Recently, due to the expansion of molecular methods and genomic sequencing, it has been proposed to revise the taxonomy of some of the *G. duodenalis* assemblages by giving species names (Wielinga et al., 2023). The proposed names are *G.*

duodenalis for assemblage AI, *G. intestinalis* for assemblage AII, *G. enterica* for assemblage B, *G. canis* for assemblage C, *G. lupus* for assemblage D, *G. bovis* for assemblage E, *G. cati* for assemblage F, and *G. simoni* for assemblage G (Wielinga et al., 2023). These taxonomic names are still not official, and the summary of the main hosts and other hosts of *G. duodenalis* assemblages are summarized in Table 1.2.

Table 1.2. **Primary and secondary hosts of *Giardia duodenalis* assemblages (Heyworth, 2016)**

Assemblage	Primary hosts	Secondary hosts
A	Humans	Cattle, dogs, cats, pigs, beavers, alpacas, deer, horses, sheep, goats, chickens, gulls, non-human primates
B	Humans	Cattle, dogs, deer, horses, beavers, chickens, sheep, seals, rabbits, ferrets, non-human primates
C	Canines	Cattle, pigs
D	Canines	Cattle
E	Cattle	Sheep, pigs, goats, horses, foxes, deer, cats
F	Felines	Cetaceans, pigs
G	Rodents	N/A*
H	Pinnipeds	Sea gulls

**N/A: not available*

Multilocus genotyping has revealed different sub-assemblages within *G. duodenalis* assemblages A and B (Cacciò et al., 2008; Wielinga et al., 2023). Assemblage A is considered to consist of three sub-assemblages AI, AII, and AIII, out of which AI is found in humans and other non-human primates, livestock, such as cattle and sheep, cats, dogs, pigs, and water buffaloes, AII has been reported in humans, non-human primates, cattle, horses, beavers, dogs, and cats, but AIII has been reported in wild boars, moose, cats, raccoons, and fellow deer (Wielinga et al., 2023). Similarly to assemblage A, assemblage B is also divided into four sub-assemblages BI, BII, BIII, and BIV, with BI and BII mostly reported from animal hosts but assemblages BIII and BIV are considered zoonotic, with reported mainly in humans, non-human primates, dogs, cats, cattle, and pigs (Ryan & Cacciò, 2013; Zajaczkowski et al., 2021). Even though assemblage E is livestock, especially cattle-specific, previously it has been identified in humans with diarrhea (Fantinatti et al., 2016; Zahedi et al., 2017). Rare cases of humans being infected with canine-specific assemblages C and D have also been reported (Wielinga et al., 2023).

1.2. *Giardia duodenalis* transmission routes and life cycle in cattle, canids and humans

Giardia duodenalis is transmitted by direct contact with infected animal or human feces or indirectly with contaminated food, feed, and water by ingesting cysts via the fecal-oral route (Dixon, 2021).

Giardia is characterized by a monoxenous life cycle and reproduces only asexually by binary fission in all hosts equally (Fink et al., 2021).

After ingestion, cysts pass through the stomach, where the low pH of gastric acid of 1.3-2.7 induces the beginning of excystation (Bingham & Meyer, 1979). Full excystation occurs in the duodenum upon full exposure to bile, trypsin, and sudden pH changes to a more alkaline environment (Bingham & Meyer, 1979; Rice & Schaefer, 1981).

First, a flagellum appears through an opening in the poles of the cyst, and an excyzoite body forms (Buchel et al., 1987). The excyzoite body goes through cytokinesis twice, forms the attachment organelle – adhesive disc, segregates the rest of the motility flagellates, and finally produces four trophozoites, which are important in the disease-initiating stage (Bernander et al., 2001). Trophozoites attach to the enterocytes of the upper small intestines with the adhesive disc, and their heightened mobility allows them to avoid being eliminated via the digestion movements (Adam, 2001). In the upper intestines, trophozoites multiply by binary fission (Ankarklev et al., 2010).

By moving to the lower part of the mammal small intestine, trophozoites start to encyst due to environmental changes – changes in pH, elevated levels of bile, and low levels of cholesterol (Ankarklev et al., 2010). First, the flagella start to internalize; afterward, the adhesive disc starts to fragment, and the trophozoites progressively round up and enter a metabolically slow, thick-walled cyst with four nuclei (Erlandsen et al., 1996). Cysts are immediately infective upon excretion (Figure 1.1.) (Geurden et al., 2010).

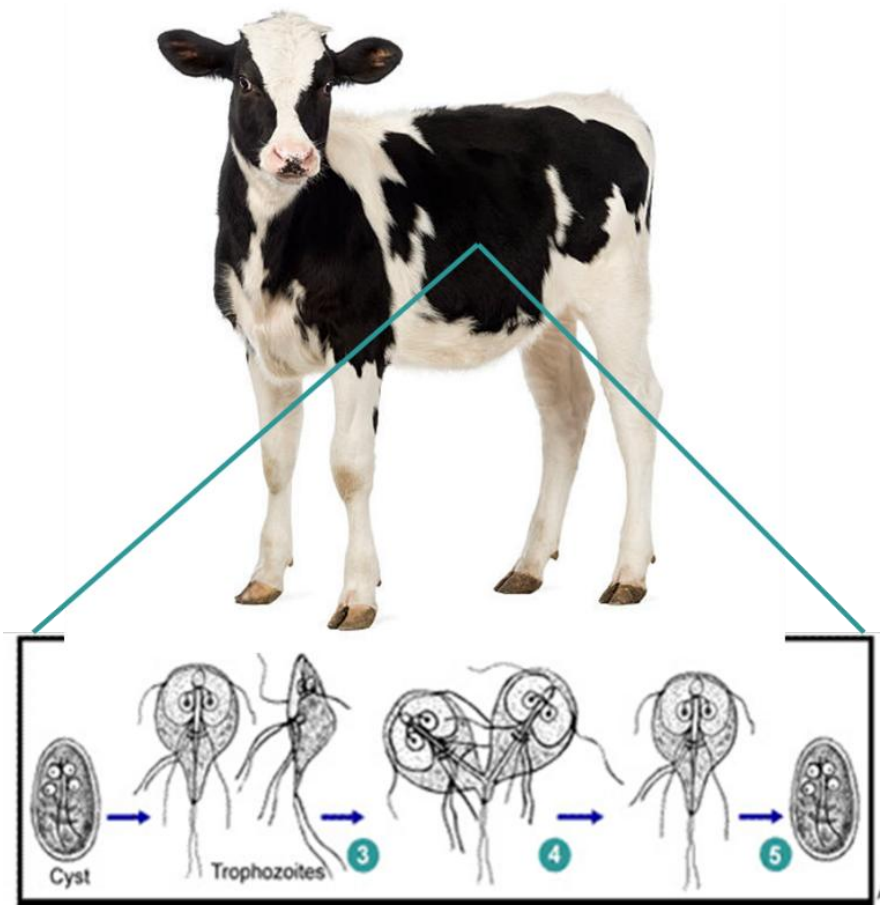

Figure 1.1. ***Giardia duodenalis*** **life cycle**

1 – *Giardia* cysts and trophozoites are passed with stools; 2 – Cysts are swallowed via contaminated food, water, or other means; 3 – Trophozoites in the intestines; 4 – Binary fission of trophozoites, 5 – Cyst (CDC, 2021)

1.3. Morphology and pathogenesis of *Giardia duodenalis* infection

Giardia has two forms – inactive cyst form, which is oval and 7–10 μm in diameter, and active, pear-shaped, disease-causing trophozoite form, which is 5–9 μm wide, and 12–15 μm long (Adam, 2001; Ankarklev et al., 2010).

Compared to other eukaryotes, *Giardia* trophozoites are distinct, because they have two nuclei, lack mitochondria, peroxisomes and a typical Golgi apparatus (Ankarklev et al., 2010). Additionally, trophozoites have four flagella pairs (anterior, ventral, posterior/lateral, and caudal), a ventral side, that is slightly concaved, has a lateral crest and a flange, which helps the *Giardia* attach to the intestinal epithelium (also called the ventral/adhesive disc), and a convex dorsal side (Adam, 2021). Median body, which divides the trophozoite in half and gives it its distinguishable "smile", is responsible for the formation of the ventral disc (Woessner & Dawson, 2012). The flagella and ventral adhesive disc are made of unique proteins which are only found in the *Giardia* genus – α-giardin, β-giardin, γ-giardin and δ-giardin, out of which the β-giardin protein is used for molecular species detection (Figure 1.2) (Ankarklev et al., 2010).

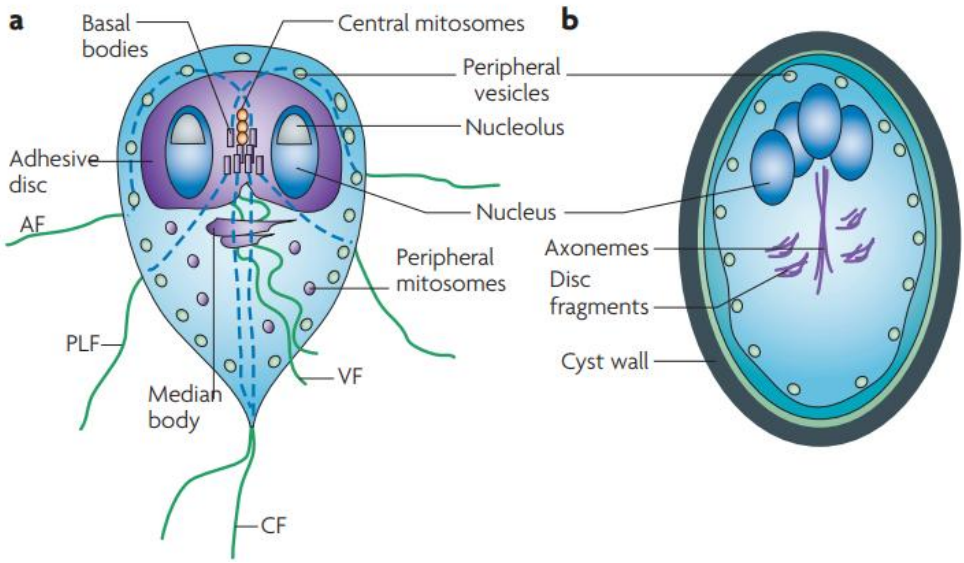

Figure 1.2. **Morphological structure of *Giardia duodenalis* trophozoite and cyst**
a – dorsal view of the trophozoite of *G. duodenalis*. AF – anterior flagella. VF – ventral flagella. PLF – posterior/lateral flagella. CF – caudal flagella. Blue dashed lines show internal structure. b – *G. duodenalis* cyst (Ankarklev et al., 2010)

Mitosomes are considered to date before the development of mitochondria and were only discovered in *Giardia* in 2003 (Tovar et al., 2003). There are two mitosome types – central and peripheral, and one cell can have from 25 to 100 mitosomes, and they are involved in protein biosynthesis and biogenesis of organelles (Tovar et al., 2003; Regoes et al., 2005). There are eight basal bodies that are positioned between the two nuclei, and they are the origin of the flagellas (McInally & Dawson, 2016).

The cyst forms when the environment becomes unlivable for the trophozoite. The cyst is thick-walled and consists of four nuclei, axonemes, disc fragments, and peripheral vesicles (Figure 1.2, b) (Sheffield et al., 1977; Erlandsen et al., 1996; Chávez-Munguía et al., 2007). The cyst wall is 0.3–0.5 μm thick, has a double membrane, and consists of carbohydrate chains, wall sugars and cyst wall proteins (CWP 1-3) (Sheffield et al., 1977; Erlandsen et al., 1996). The cyst vesicles are responsible for the formation of the CWP (Marti et al., 2003).

Several virulence factors are vital for *Giardia* pathogenesis and ensure not only the survival of this parasite outside the host, but also the ability to avoid the hosts immune system, which prolongs the duration of giardiasis (Ankarklev et al., 2010). In the acute phase of giardiasis, the trophozoite load can exceed one million cells per centimeter of the intestines, however, the host usually does not show any signs of inflammation or infection (Hardin et al., 1997; Buret et al., 2015). The ventral disc with the flagellas is responsible for the attachment of

the parasite to the microvillus boarder of the intestinal epithelium, and, after the attachment, the trophozoite secretes toxin-like proteins (CRP136, ESP58), which ensure the water and electrolyte leakage, and the intestinal hypermotility (Chen et al., 1995**;** Buret et al., 2015). The flagellar motility ensures colonization to new endothelial cells and the trophozoite surface cysteine proteins help protect the *Giardia* cell against the host luminal proteases and free radicals (Prucca et al., 2008; Ankarklev et al., 2010). Furthermore, the cysteine surface proteins help degrade the villin, defensins and immunoglobulins (especially immunoglobulin A), and alter the host microbiota composition, causing dysbiosis (Buret et al., 2015; Argüello-García & Ortega-Pierres, 2021). It has also been suggested that during the acute phase of the infection, *Giardia* may increase the virulence of commensal microbiota (Chen et al., 2013; Buret et al., 2015).

1.4. *Giardia duodenalis* in cattle

1.4.1. Epidemiology of *Giardia duodenalis* and factors associated with the prevalence of *Giardia* in cattle in Europe

Giardia is widespread in cattle and cattle herds in Europe and usually represented by three assemblages – A, B, and E (Bartley et al., 2019). In Europe, giardiasis has been commonly observed in cattle, with the mean prevalence, depending on the diagnostic method used, varied from 13.7% using direct or indirect microscopic methods (1011/5579) to 31.1% with molecular methods (456/1542) (Taghipour et al., 2022). The lowest prevalence in cattle has been reported from Spain (1.4%; n = 277), but the highest from the United Kingdom (54.9%; n = 556) (Table 1.3)

Table 1.3. **The prevalence of *Giardia duodenalis* in cattle, cattle herds, and genetic diversity in Europe**

Country	**Prevalence in cattle, % (Total no. of examined cattle)**	**Prevalence in cattle herds, % (Total no. of farms visited)**	***G. duodenalis* assemblages**	**Cattle production type**	**Reference**
Belgium	22.0 (499)	48 (100)	A, E	Dairy	Geurden et al., 2008
	45.0 (333)	64 (50)	A, E	Beef	
Denmark	20.0 (1150)	NR	A, E	Dairy	Langkjaer et al., 2007
	44.0 (1150)	100.0 (50)	NR*	Dairy	Maddox-Hyttel et al., 2006
Estonia	27.0 (240)	NR	NR	NR	Plutzer et al., 2018
France	39.8 (477)	82.4 (101)	A, E	Dairy	Geurden et al., 2012
Germany	NR	100 (1–31)	A, E	Dairy	Geurden et al., 2012
Greece	41.3 (254)	NR (15)	A, E	NR	Ligda et al., 2020

Country	Prevalence in cattle, % (Total no. of examined cattle)	Prevalence in cattle herds, % (Total no. of farms visited)	*G. duodenalis* assemblages	Cattle production type	Reference
Italy	32.2 (503)	88.6 (44)	A, E	Dairy	Geurden et al., 2012
Norway	49.0 (1386)	93.0 (136)	NR	Dairy	Hamnes et al., 2006
Scotland	10.1 (128)	NR (19)	A, B, E	Beef	Bartley et al., 2019
	44.7 (253)	NR (19)	A, B, E	Dairy	
Spain	1.4 (277)	42.9 (21)	NR	NR	Cardona et al., 2011
	11.7 (554)	53.3 (30)	NR	Beef and dairy	Quílez et al., 1996
	26.6 (379)	96.6 (60)	NR	Dairy	Castro-Hermida et al., 2007
	16.0 (1316)	100.0 (18)	AI, AII, E	Dairy	Castro-Hermida et al., 2009
The Netherlands	21.3 (183)	NR (1)	A	Dairy	Huetink et al., 2001
The United Kingdom	32.9 (283)	NR	A, E, C, D	Dairy	Minetti et al., 2014
	54.9 (556)	100 (31)	A, E	Dairy	Geurden et al., 2012

**NR: not reported*

G. duodenalis infection was more associated with younger cattle with the highest prevalence observed in one to six months old calves (Maddox-Hyttel et al., 2006; Cardona et al., 2011; Bartley et al., 2019). In Denmark, the highest *G. duodenalis* prevalence was observed in calves under one month old – 60.7% (229/377) (Maddox-Hyttel et al., 2006). Similarly, the high prevalence of 55.8% was observed in two-month-old calves in Germany (Geurden et al., 2012). Lower prevalence of the pathogen in calves under one-month-old were observed in Spain – 18.3% (11/78), The Netherlands – 16.7% (8/48), The United Kingdom – 31.8% (7/22), and Scotland – 39.4% (13/33) (Quílez et al., 1996; Huetink et al., 2001; Castro-Hermida et al., 2009; Minetti et al., 2014; Bartley et al., 2019). High prevalence of *Giardia* in Europe was also observed in cattle under six months of age with the prevalence ranging from 14.1% (10/71) in the Netherlands to 22.8% (21/92), 38.0% (46/121), and 43.6% (34/78) in Scotland, Spain and the United Kingdom, respectively (Quílez et al., 1996; Huetink et al., 2001; Minetti et al., 2014; Bartley et al., 2019). In Denmark and the United Kingdom, the prevalence in cattle over one year old was higher – between 20.2% (51/255) and 23.0% (32/139), respectively, in comparison with other age groups (Maddox-Hyttel et al., 2006; Minetti et al., 2014).

Although there are limited number of studies done on factors affecting the *G. duodenalis* prevalence at the cattle farms, some giardiasis-associated factors have been reported, such as cattle management, flooring type, maternity pen cleaning, calves separation, and feeding practices of young calves (Jäger et al., 2005; Hamnes et al., 2006; Maddox-Hyttel et al., 2006; Geurden et al., 2012).

It has been found that the flooring type could increase the prevalence of *G. duodenalis* in cattle herds. A significantly higher prevalence of *G. duodenalis* was observed in beef calves housed in deep litter housing with run-out compared to those in deep litter housing without run-out, winter run-out, or slatted floor management systems (Jäger et al., 2005). Differences in management practices have also been found to influence *G. duodenalis* prevalence in cattle and *G. duodenalis* was more likely to infect beef cattle (45.0%; n = 333) than dairy cattle (22.0%; n = 499) in study in Belgium (Geurden et al., 2008). However, zoonotic assemblage A was more commonly found in dairy cattle herds than in beef cattle (Geurden et al., 2008).

Regular cleaning of maternity pens at least four times per year, instead of cleaning them only after calving, reduced the risk of *G. duodenalis* infection in calves (Geurden et al., 2012). Similarly, the use of high-pressure cleaning combined with an empty period between introducing new calves was observed to decrease the shedding of cysts in calf pens (Maddox-Hyttel et al., 2006). Separating calves from the dam or limiting contact was another measure to minimize the risk of infection with *G. duodenalis* (Geurden et al., 2012). Additionally, feeding calves individually rather than in groups significantly reduced the prevalence of *G. duodenalis*, due to limited contact with other cattle and using personalized feeding equipment (Hamnes et al., 2006).

1.4.2. *Giardia duodenalis* clinical signs in cattle

In cattle, *G. duodenalis* causes giardiasis, and the disease is characterized by enteric symptoms such as diarrhea (O'Handley et al., 1999). The prepatent period ranges from seven to eight days in calves, and the infectious dose can be as low as 10 cysts (O'Handley & Olson, 2006; Dixon, 2021). The severity of the clinical signs varies with age being more pronounced in calves under 30 days old, but diarrhea may be observed in calves up to six months of age (O'Handley et al., 1999). Because of the chronic course of the disease, intermittent diarrhea and cyst shedding can also be observed frequently which complicates the treatment and diagnosis of the infection (O'Handley et al., 1999). Giardiasis can lead to malabsorption due to the reduction of the jejunum brush border surface area, which decreases the absorption of nutrients, electrolytes, and fluids (O'Handley et al., 1999; Ralston et al., 2003; Geurden et al., 2010). The reduction of the jejunum brush border surface can result in increased intestinal motility and reduced activity of the intestinal enzymes – maltase, lactase, and disaccharidase, which can decrease long term weight gain (O'Handley et al., 1999; Ralston et al., 2003; Geurden et al., 2010). Even though there are several reports about clinical signs in cattle, numerous studies have demonstrated no statistical association between the presence of *G. duodenalis* and diarrhea but only emphasizes the chronic course of giardiasis in cattle (O'Handley & Olson, 2006; Quílez et al., 1996; Maddox-Hyttel et al., 2006; Geurden et al., 2012; Minetti et al., 2013). Infected calves can shed *Giardia* cysts for at least 100 days, and there have been reports of prolonged shedding for more than 25 weeks without significant decline in the shedding intensity, which leads to increased environmental contamination and the infection of naïve calves (O'Handley et al., 1999; Ralston et al., 2003). The immune response against giardiasis in cattle develops slowly with the amount of *G. duodenalis* specific serum antibodies increase only 11 weeks post-infection. No intestinal immune response was observed three weeks after the start of infection, which could be the reason for prolonged cyst shedding in infected animals (Grit et al., 2014).

It is important to note that the presence of *Giardia* cysts in feces does not necessarily indicate that *G. duodenalis* is the causative agent of diarrhea or other clinical signs, as other pathogens can cause diarrhea in calves within the same age group, especially *Cryptosporidium parvum*, Bovine Coronavirus, Rotavirus, *Eimeria* spp., *Salmonella* spp., *Escherichia coli*, and

Clostridium spp.; therefore it is important to test the calves for other pathogens to confirm the infection (Blanchard et al., 2012).

1.4.3. *Giardia duodenalis* treatment and prevention in cattle and cattle herds

G. duodenalis treatment and prevention are crucial steps to minimize the spread of the parasite in cattle herds. Currently, there is no licensed treatment for giardiasis in cattle in the European Union (Geurden et al., 2010). The off-label use of benzimidazoles, such as albendazole and fenbendazole, which are commonly used to treat nematode and trematode infections in cattle, has been shown to clinically improve calf health by increasing the microvillus surface area and intestinal enzyme activity, compared to the control group (O'Handley et al., 2001). In a controlled study, administration of albendazole orally for three days straight reduced the *Giardia* cyst shedding by at least 90.8%, but treatment with fenbendazole for three days straight reduced the *Giardia* cyst shedding by 100% in the first week; however, the shedding intensified after three weeks after the treatment (Xiao et al., 1996). Similar results observed by O'Handley et al., 2000 where the cyst shedding intensity also increased after treatment. Therefore, even direct treatment of giardiasis in cattle can reduce the number of *Giardia* cysts, which could lessen the environmental load and transmission to other cattle and possibly reduce the clinical symptoms; this type of approach might not be economically beneficial due to re-shedding (Xiao et al., 1996; O'Handley et al., 2000). Hence isolation of the infected calves, if possible, is recommended (Xiao et al., 1996; O'Handley et al., 2000; Uehlinger et al., 2007). Although there are also no vaccines available against giardiasis for cattle (Geurden et al., 2010), a vaccination protocol used in dogs has been applied for cattle, but it did not reduce cyst shedding in cattle (Uehlinger et al., 2007).

G. duodenalis control and prevention in cattle herds should be highly prioritized to improve animal health and reduce risks to public health and economic losses within the livestock industry (Valdez et al., 2019; Brainard et al., 2020). Giardiasis causes chronic, intermittent diarrhea in calves and pathological changes in the jejunum associated with malabsorption of important nutrients in calves that influence weight gain (O'Handley et al., 1999; Ralston et al., 2003; Geurden et al., 2010). A long-term study done in lambs resulted in decreased weight gain and carcass quality in only five weeks after the initial exposure to the parasite (Olson et al., 1995).

Lack of specific treatments for *Giardia* in cattle highlights the importance of improving the overall health of the calves by supportive treatment and housing of animals in dry, warm environments (Thomson et al., 2017; Shaw et al., 2020). Strict hygiene measures such as frequent removal of feces, implementing an empty period between introducing new calves to the herd and using proper disinfection, should be enough to minimize the spread of *Giardia* in cattle herds (Geurden & Olson, 2011). Prioritizing the care of infected calves is also crucial for reducing the *Giardia* cyst load in the environment. The *Giardia*-shedding calves should be isolated from the healthy in separate pens and distanced from recovering calves to minimize the spread cyst load within the herd premises (Harp & Goff, 1998). Additionally, personnel attending to the animals should follow biosecurity principles – change clothes, wear single-use gloves, and use suitable disinfectants for hands, boots, and equipment (Harp & Goff, 1998).

Managing giardiasis within cattle herds poses significant challenges due to several factors: the pathogen's thick-walled cysts, low infectious dose – minimum of 10 cysts, extended shedding periods, and susceptibility across a wide range of hosts (Dixon, 2021). *Giardia* cysts are robust in the environment and can survive in cattle feces and soil for up to 12 weeks at 4 °C (Olson et al., 1999). Cysts lose infectivity after a week at -4 °C, but in moist and cool conditions, cysts can survive for several months (Olson et al., 1999; Feng & Xiao, 2011). Chlorine is often

used to inactivate pathogens in water, such as *Giardia*; however, a prolonged exposure time, for at least 60 minutes, is needed to inactivate all cysts in water and wastewater with chlorine at 0.5 ppm (Adeyemo et al., 2019). An effective approach to eliminate cysts in a herd involves washing pens with hot water (65 °C) and thoroughly drying the area before introducing new calves (Harp & Goff, 1998). Formaldehyde, ammonia, and hydrogen peroxide can inactivate *Giardia* cysts, by decreasing the cyst numbers by >3.3 log units after five minutes exposure to disinfection agent (Erickson & Ortega, 2006; Geurden & Olson, 2011).

Given that infected cattle can shed millions of *Giardia* cysts, it is crucial to implement proper treatment of manure collected from infected cattle. This proactive step is essential due to the potential risk the contaminated manure poses as a source of contamination for groundwaters, surface waters, fields, and crops (Boyer et al., 2009; Feng & Xiao, 2011). Effective management and treatment of manure becomes imperative to prevent potential environmental contamination and safeguard water sources and agricultural areas (Feng & Xiao, 2011). The survival of pathogens is usually impacted by the manure form – liquid, slurry, or solid, and if the manure has higher moisture content, the pathogens can survive for longer periods (Manyi-Loh et al., 2016). Lagoons, especially if covered as a part of manure treatment, provide proper anaerobic processes and can produce high enough internal temperatures that help eliminate pathogens such as *Giardia* (Manyi-Loh et al., 2016). Implementing lagoons for manure processing holds the potential to diminish the foodborne and waterborne *Giardia* risks for humans, mitigate exposure to mechanical vectors like cats and rodents, and prevent manure/slurry run-offs into the surrounding environment around herds (Nicholson et al., 2004).

1.5. *Giardia duodenalis* in domestic dogs

1.5.1. Epidemiology of *Giardia duodenalis* in domestic dogs in Europe

G. duodenalis has been one of the most common parasites detected in domestic dogs in Europe, with *G. duodenalis* assemblages A, B, C, and D being the most represented (Mravcová et al., 2019). The highest prevalence of *G. duodenalis* in dogs has been reported from Belgium, Germany, Spain, and the Netherlands in Europe, with the zoonotic assemblages reported from multiple European countries (Table 1.4) (Claerebout et al., 2009; Rehbein et al., 2019; Joachim et al., 2023).

Table 1.4. ***Giardia duodenalis*** **prevalence and genetic diversity detected in domestic dogs in Europe**

Country	**Prevalence in domestic dogs, % (Total no. of examined dogs)**	***G. duodenalis*** **assemblages**	**Reference**
Austria	NR* (70)	A, B, C, D	Joachim et al., 2023
Belgium	43.9 (357)	A, C, D	Claerebout et al., 2009
Bosnia and Herzegovina	15.6 (212)	NR	Omeragić et al., 2021
Czech Republic	NR (54)	C, D	Lecová et al., 2020
Germany	39.0 (31)	A, B, C, D	Rehbein et al., 2019

Country	Prevalence in domestic dogs, % (Total no. of examined dogs)	*G. duodenalis* assemblages	Reference
Germany	30.6 (376)	A, C, D	Sommer et al., 2018
	NR (60)	A, C, D	Leonhard et al., 2007
Greece	25.2 (879)	C, D	Kostopoulou et al., 2017
Italy	62.4 (14)	AI	Marangi et al., 2010
	41.0 (168)	AII, B, C	Agresti et al., 2022
The Netherlands	11.1–32.0 (1291)	NR	Uiterwijk et al., 2019
Poland	6.0 (217)	C, D	Piekara-Stępińska et al., 2021
	21.1 (128)	B, C, D	Piekarska et al., 2016
Portugal	33.8 (80)	C, D	Pereira et al., 2021
Romania	34.6 (614)	NR	Mircean et al., 2012
Spain	33.0 (194)	AII, BIII, BIV, C, D	Gil et al., 2017
	36.5 (348)	A, B, C, D	Adell-Aledón et al., 2018
The UK**	21.0 (870)	A, C, D	Upjohn et al., 2010

**NR: not reported; **The UK: the United Kingdom*

Several studies have reported the zoonotic assemblage A and B in dogs, especially the *G. duodenalis* sub-assemblage AI, AII, BIII, and BIV (Table 1.4) (Gil et al., 2017; Adell-Aledón et al., 2018; Joachim et al., 2023; Barasa et al., 2024). The dog-specific assemblages C and D were more commonly observed in Spain (Adell-Aledón et al., 2018), but zoonotic assemblages A and B in Germany (Pallant et al., 2015).

In domestic dogs, age has been proven to be a strong predicting factor regarding *G. duodenalis* infection, with the peak of shedding intensity around 12 months of age in a longitudinal study on *Giardia* effects during the first year of dogs' life (Hamnes et al., 2007). In the same study, the first peak of giardiasis was observed in three-month-old puppies with the prevalence of 8.7% (23/264), and then again in the 12-month-old dog group with the prevalence of 11.4% (18/158) (Hamnes et al., 2007). In Bosnia and Herzegovina, the *G. duodenalis* prevalence was higher (23.9%; 16/64) in dogs under six months old compared to 11.7% (17/111) in dogs older than six months (Omeragić et al., 2021). In Germany, similar results were observed, where the prevalence in dogs under six months old was 49% (47/96), compared to the 6–12 months old dogs (33.3%; 26/78) and 12–18 months old dogs (31.6%; 25/79), but the lowest prevalence was observed in dogs over two years old (12.5%; 8/64) (Sommer et al., 2018). In Spain, dogs between one and five years old exhibit a higher prevalence of 22% (7/32) compared to dogs under one year old (40%; 16/40) (Gil et al., 2017). In one study including seven European countries (Belgium, Germany, Spain, France, Italy, the Netherlands and the United Kingdom), higher odds of infection was observed in dogs under five months compared to 0.5-month to 2-year-old dogs, with significantly lower odds of infection in dogs older than five years (Epe et al., 2010). However, in a separate study in the United Kingdom, lover odds of infection was observed in dogs over one year old compared to dogs under 12 months old,

which supports previous reports about age being a predictive factor in dogs (Upjohn et al., 2010).

Besides age, there have been other factors which have been associated with increased risk of *G. duodenalis* in dogs, such as sex, breed and living conditions – single-animal households, dog shelters, dogs purchased from breeders (Epe et al., 2010).

Regarding living conditions, a higher *G. duodenalis* prevalence of 20.6% (n = 707) was observed in dogs that were the only animals in the household, compared to the households with more than two dogs (22.1%; n = 439) but the authors explained the reason was probably due to a small data sample rather than a real effect (Epe et al., 2010). Higher *G. duodenalis* infection rates were observed in dogs in shelters than dogs that came from breeders or had owners (Epe et al., 2010). Comparing results from seven countries (Belgium, Denmark, France, Italy, the Netherlands, the United Kingdom, and Spain), the significantly higher prevalence was in shelter dogs than dogs that came from a reputable breeder (Epe et al., 2010), which was supported by other studies done by Gil et al. (2017) in Spain, Mircean et al. (2012) in Romania, Sommer et al. (2018) in Germany, and Pereira et al. (2021) in Portugal. In one study, the opposite was observed – a higher prevalence was observed in dogs from a breeder (45.8%; 11/24) than in dogs from a shelter (40.4%; 88/218) (Adell-Aledón et al., 2018).

There has been contradictory information whether sex of the dogs has any effect on *G. duodenalis* clinical manifestation or cyst shedding intensity. In one study, female dogs shed *Giardia* more frequently (22.1%; n = 147) compared to male dogs (19.7%, n = 142) (Hamnes et al., 2007). In a large-scale study, higher *G. duodenalis* prevalence was observed in male dogs (25.8%; n = 1228) compared to female dogs (23.6%; n = 924) (Epe et al., 2010). No differences between the *G. duodenalis* prevalence and dogs' sex were observed in the UK (Upjohn et al., 2010). One study did show that neutered dogs had lower odds of infection compared to non-spayed dogs (Upjohn et al., 2010).

One study has compared differences between breed and mixed breed dogs, showing that Rottweilers had higher odds of infection than Staffordshire Bull Terriers (Upjohn et al., 2010). Another study observed that pure-bred dogs had higher prevalence and higher significance than mixed dogs to be infected with giardiasis (Fontanarrosa et al., 2006).

1.5.2. *Giardia duodenalis* clinical signs in domestic dogs

In domestic dogs, giardiasis is caused by four *G. duodenalis* assemblages – A, B, C, and D. The incubation period is from 2 to 15 days and can manifest as severe diarrhea, which can lead to severe dehydration, enteritis, abdominal pain, nausea, maldigestion, and malabsorption, with some dogs having foul-smelling diarrhea, steatorrhea, weight loss, and a delay in growth (Šmit et al., 2023). It has been reported that giardiasis in dogs can also alter the microbiome composition in the intestines, which can lead to dysbiosis-related diseases and therefore decreasing the overall health even more (Šlapeta et al., 2015; Certad et al., 2017). Association between diarrhea and *G. duodenalis* assemblages C and D was reported in dogs with clinical signs by Claerebout et al. (2009), while other studies failed to associate diarrhea with *G. duodenalis* assemblages or other pathogens (Uiterwijk et al., 2020; Scorza et al., 2021). It has been suggested that co-infections with other parasites, viruses, and bacteria might be a possible pre-disposing factor to develop the clinical signs associated with *Giardia*, but more research are needed (Berry et al., 2020; Kuzi et al., 2020). A strong association has been observed between *Giardia* and co-infection with Canine parvovirus and *Cryptosporidium* spp., which could delay not only the recovery, but also complicate the diagnostics of the pathogen (Kuzi et al., 2020). Changes in gut microbiota in puppies with chronic giardiasis have also been observed with an increase in facultative anaerobic, pro-inflammatory, mucus-degrading microbiota

species, as well as a decrease in *Lactobacillus johnsonii* (Boucard et al., 2021). IgA development in the mucosal surface of the intestines happened at 85 days of age indicating a delayed immune system response to *G. duodenalis*, which was also observed in cattle (Boucard et al., 2021).

Similar to cattle, diarrhea in dogs can be caused by other pathogens, such as *Clostridium perfringens, Salmonella* spp., pathogenic *Escherichia coli*, *Cystoisospora* spp., as well as other chronic diseases, such as idiopathic enteropathy. Thus, considering the chronic manifestation of giardiasis, these pathogens or chronic diseases should also be included in the differential diagnosis list (Volkmann et al., 2017).

1.5.3. *Giardia duodenalis* treatment and prevention in domestic dogs

For dogs, fenbendazole and metronidazole are registered to be used to treat giardiasis. It is important to note that the use of metronidazole should be limited to a second choice due to the risk of antimicrobial resistance and should only be used if fenbendazole is not efficient for the treatment of giardiasis (ESCCAP, 2025). A trial study comparing the efficacy of fenbendazole (50 mg/kg) and metronidazole (50 mg/kg) administered once a day for five days straight showed increased efficiency against giardiasis over time, with both treatments reducing the shedding to 97% of the pathogen at the end of the fifth day (Ciuca et al., 2021). It is recommended to prolong the treatment period if *Giardia* cysts are still observed in the diagnostic tests until giardiasis has been fully cleared (Ciuca et al., 2021). The treatment of giardiasis in dogs can be complicated by frequent reinfections, which can happen right after the treatment stops due to a lack of immunity development (ESCCAP, 2025).

Prevention is important to reduce the chance of reinfection, as there are no vaccines available against giardiasis in dogs. *G. duodenalis* cysts can survive for a long time in moist and cool environments, which include moist bedding or dog kennels. Therefore, it is important to keep the surroundings clean and, if possible, wash the materials in contact with the dog at least 65° C (Harp & Goff, 1998). Also, it is important to do routine testing of *Giardia* cysts, especially if intermittent diarrhea is observed, and, if necessary, repeat the testing multiple times with appropriate methods (Fiechter et al., 2012).

1.6. Epidemiology and clinical signs of *Giardia duodenalis* in wild red foxes and raccoon dogs in Europe

Compared to cattle and domestic dogs, there have been fewer reports regarding *G. duodenalis* in red foxes and raccoon dogs in Europe. Nevertheless, the prevalence of *G. duodenalis* in Europe in red foxes varied from 3.2% (9/123) in Bosnia and Herzegovina to 44.0% (46/104) in Sweden (Table 1.5), while the prevalence of *G. duodenalis* in raccoon dogs was reported only in Poland (Table 1.5) (Hodžić et al., 2014; Debenham et al., 2017).

Table 1.5. ***Giardia duodenalis*** **prevalence and genetic diversity detected in red foxes and raccoon dogs in Europe**

Species	**Country**	***G. duodenalis*** **prevalence, % (Total No. of examined animals)**	***G. duodenalis*** **assemblages**	**Reference**
Red fox	Bosnia and Herzegovina	3.2 (123)	NR*	Hodžić et al., 2014
	Croatia	4.5 (66)	AI	Beck et al., 2011
	Italy	7.0 (71)	NR	Papini et al., 2019
	Norway	4.8 (269)	A, B	Hamnes et al., 2007
	Romania	2.8 (273)	A, B	Onac et al., 2015
	Spain	8.1 (87)	NR	Mateo et al., 2017
	Sweden	44.0 (104)	B	Debenham et al., 2017
Raccoon dog	Poland	11.0 (18)	D	Solarczyk et al., 2016
	Romania	NR (1)	D	Adriana et al., 2016

**NR: not reported*

In Italy, the *G. duodenalis* prevalence in red foxes under one year old was 12% (3/25), compared to older foxes – 4.3% (2/46), and age was found to be a risk factor of infection (Papini et al., 2019). Age was also strongly associated with *G. duodenalis* in foxes from Romania where the prevalence in younger foxes was 12.3% (7/57), compared to adult foxes with the prevalence of 1.9% (3/160) (Onac et al., 2015). Increased risk of infection has been observed in male foxes, especially in juveniles in Sweden (Debenham et al., 2017). The positive foxes from Sweden shed 100 to 140,400 CPG, with all animals excreted the zoonotic *G. duodenalis* assemblage B (Debenham et al., 2017). Additionally, data from Spain show no co-infections between *Giardia* and other parasites (Mateo et al., 2017).

There were two reports on *G. duodenalis* in raccoon dogs in Europe—one from Romania and the other from farmed raccoon dogs in Poland (Adriana et al., 2016; Solarczyk et al., 2016). The farmed raccoon dogs from Poland shed 12,000-13,000 cysts per gram of feces (Solarczyk et al., 2016). There is a lack of studies on any risk factors associated with *G. duodenalis* infection in raccoon dogs.

Compared to cattle and domestic dogs, there is also lack of studies on the potential effects of *Giardia* on red foxes and raccoon dog health worldwide. Intermittent, chronic, and sometimes acute diarrhea, which was observed in domestic canids, could also affect wild canid species (Šmit et al., 2023). Wild canids can be carriers of zoonotic *G. duodenalis* assemblage A and B, especially red foxes (Hamnes et al., 2007; Beck et al., 2011; Onac et al., 2015; Debenham et al., 2017).

Regardless of the limited number of studies on giardiasis in red foxes and raccoon dogs, these animals can pose an environmental contamination risk with *G. duodenalis* cysts. Red foxes and raccoon dogs can be reservoirs of the parasite for domestic dogs and other wild canids by carrying the canine-specific *G. duodenalis* assemblage C and D. There are limited reports on zoonotic *G. duodenalis* assemblages in wild canids, but there has been evidence of the possible transfer of A and B assemblages to humans (Hamnes et al., 2007; Beck et al, 2011; Debenham et al., 2017).

1.7. *Giardia duodenalis* diagnostic methods in cattle, domestic dogs, red foxes and raccoon dogs

Several methods can be applied to detect *G. duodenalis* cysts in domestic and wild animals. These methods, which include microscopic, serological, or molecular examination, can also be used to detect *Giardia* in humans, with each providing different sensitivity and specificity.

Microscopical detection of *G. duodenalis* cysts in fecal samples involves several cost-effective and simple methods. Light microscopy is one of the easiest and simplest approaches to detect *G. duodenalis* cysts in feces (Koehler et al., 2014). For more cost-effective detection, different sample preparation techniques, such as using wet mount, flotation technique with saturated saline, glucose, or sucrose fluids, or formalin-ethyl acetate sedimentation techniques can be combined with different stain techniques to enhance the cyst detection (Alles et al., 1995; Quílez et al., 1996; Langkjaer et al., 2007; Rajurkar et al., 2012). These methods are less sensitive than more specific stains, such as immunofluorescence, or application of indirect detection serological methods, such as enzyme-linked immunosorbent assay (ELISA), or molecular methods such as RFLP (Alles et al., 1995; Rajurkar et al., 2012). Although *G. duodenalis* cysts can be seen under microscope without any additional staining (Figure 1.3), an expert examiner is needed to correctly identify *Giardia* cysts, due to poor visibility. To improve cyst visibility and increase the detection specificity, it is advised to apply additional stains, such as Lugol's iodine (Figure 1.4) or Trichome stain (Figure 1.5).

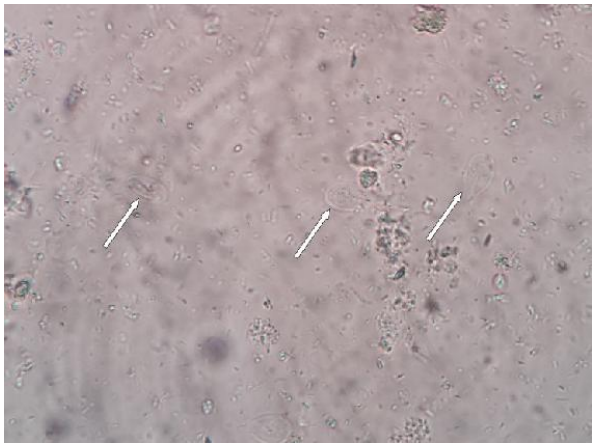

Figure 1.3. ***Giardia duodenalis* cyst (white arrows) under light microscopy without additional cyst staining (400x magnification)** (Kaya et al., 2018)

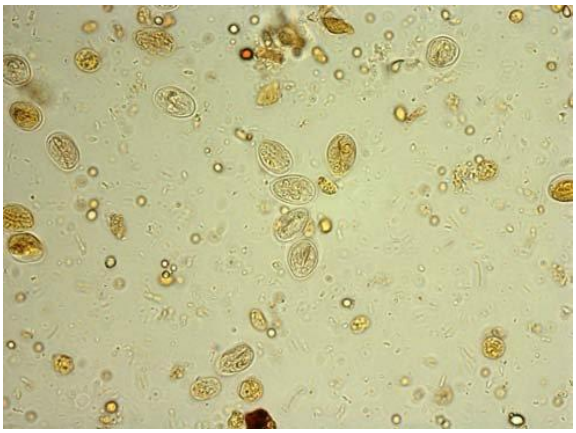

Figure 1.4. ***Giardia duodenalis* cysts under light microscopy, stained Lugol's iodine (400x magnification)** (Bayoumi et al., 2016)

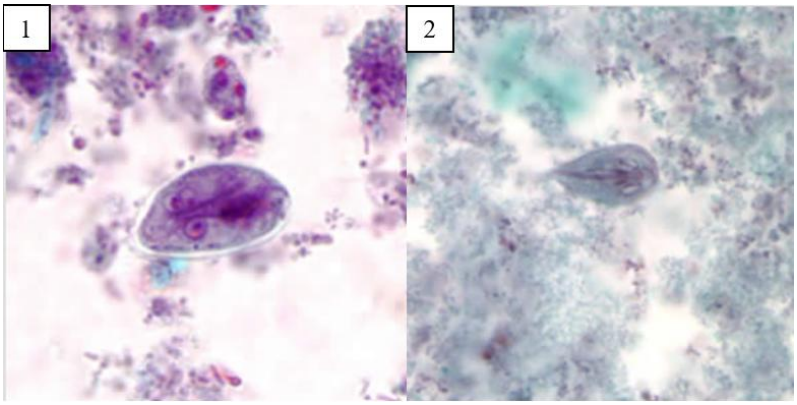

Figure 1.5. ***Giardia duodenalis*** **cyst (1) and trophozoite (2) under light microscopy, stained with trichome** (CDC, 2024)

Direct immunofluorescence (IMF) staining technique, which is an immunoassay diagnostic method, is expected to be a more sensitive and specific examination method. In IMF, the fluorescent-labeled anti-*Giardia* antibodies bind to the *Giardia* cyst wall antigens in both fecal or environmental samples and can be observed using a fluorescent microscope (Alles et al., 1995; Dixon et al., 1997; Rajurkar et al., 2012). Additionally, commercially available IMF staining kits can be modified to detect other pathogens, such as *Cryptosporidium* spp. making these staining kits efficient detection tools (Garcia et al., 1997). Although IMF can be used to stain fecal samples without any prior sample preparation, to enumerate the *Giardia* cysts per gram of feces, it is advised to use an appropriate fecal preparation technique, such as preparing one gram of sample with saturated sodium chloride solution, which is subjected to one flotation and multiple centrifugation steps (Kuczynska & Shelton, 1999; Maddox-Hyttel et al., 2006; Gulliksen et al., 2009; Geurden et al., 2012). IMF is regarded as the best method and golden standard for detecting and enumerating *G. duodenalis* cysts, as the fluorescent-labeled antibodies make the cysts more visible under the microscope (Figure 1.6).

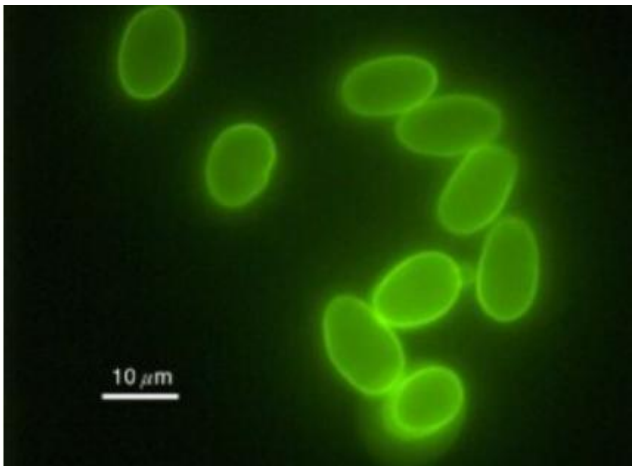

Figure 1.6. ***Giardia duodenalis*** **cysts stained with immunofluorescence (1000x magnification)** (Balderrama-Carmona et al., 2017)

Although the IMF has been regarded as the best method for detecting *Giardia* cysts, the implementation of the method can be costly, requiring not only trained personnel but also expensive equipment such as a thermostat and a fluorescent microscope (Uehlinger et al., 2017). However, with immunofluorescence, using different fluorogenic dyes, such as fluorescein diacetate and propidium iodide, and 4′,6-diamidino-2-phenylindole, it is possible to determine whether *G. duodenalis* cysts are viable (Thiriat et al., 1998).

Other quick methods to detect *Giardia* in feces are indirect detection methods such as ELISA, which targets the *Giardia*-specific cyst wall antigen, therefore offering easier application and is more cost-effective for rapid *Giardia* detection in both laboratory and field settings (Barbecho et al., 2018). It is important to note that with ELISA the cyst load in the feces cannot be determined, and the sensitivity and specificity of different commercially available ELISA kits can vary from 61% to 100% (Aziz et al., 2024). Although it is possible to detect IgG and IgA antibodies against *Giardia* in blood serum using ELISA, it is not a reliable diagnostic approach as serum antibodies appear approximately 8–11 weeks post-infection (O'Handley et al., 2003; Grit et al., 2014).

Molecular detection of *Giardia* cysts in feces is considered the most sensitive detection method, utilizing DNA isolation directly from feces. Improved detection results observed when additional cleaning of fecal debris and PCR inhibitors is done beforehand (Wilke & Robertson, 2009). The small subunit ribosomal RNA (*SSU rRNA*) gene is commonly targeted for *Giardia* species detection due to its high copy number and strong sequence conservation (Capewell et al., 2006). To detect *G. duodenalis* assemblages and sub-assemblages, genes such as glutamate dehydrogenase (*ghd*), *β-giardin* (*bg*), and triose-phosphate isomerase (*tpi*) are widely used (Cacciò & Ryan, 2008; Koehler et al., 2014). The *ghd* gene has moderate variability and can be targeted to distinguish between assemblages A–E and *G. duodenalis* assemblage AI and AII subtypes (Cacciò & Ryan, 2008; Capewell et al., 2021). The *ghd* gene is useful to identify assemblages A–H and can be used to distinguish between assemblage A sub-assemblages A1 and AII, but the *tpi* gene is used to target the sub-assemblages AI, AII, BIII, and BIV (Capewell et al., 2021). The *tpi* genes amplification process can be slow with low DNA yield, therefore it is suggested to combine the targeted genes, if possible (Capewell et al., 2021).

1.8. *Giardia duodenalis* from the "One Health" perspective – zoonotic transmission and giardiasis in humans

Since 2004, *G. duodenalis* has been included in the World Health Organization Neglected Disease Initiative and was ranked as the 8th most important foodborne parasite in Europe, while 10th in Eastern Europe (Savioli et al., 2006; Bouwknegt et al., 2018). *G. duodenalis* is the second most common protozoan that causes waterborne outbreaks in humans after *Cryptosporidium* spp. (Ma et al., 2022; Bourli et al., 2023). Although the true zoonotic impact of *G. duodenalis* is still not known, there are four proposed transmission cycles—livestock cycle, pet cycle, wildlife cycle, and human cycle—which are believed to sustain host-specific and zoonotic *G. duodenalis* assemblages A and B in mammalian hosts (Monis et al., 2009; Siwila, 2017).

While in animals, there are few studies describing the clinical manifestation of giardiasis, this disease has been extensively studied in humans with many chronic long-term effects were reported (Botero-Garcés et al., 2009; Akkaub & Buret, 2020).

Clinically, giardiasis causes either acute or chronic diarrhea, which can be accompanied by bloating, nausea, and abdominal pain, leading to lower cognitive functions, allergies, vitamin and mineral deficiencies (Botero-Garcés et al., 2009; Akkaub & Buret, 2020). Giardiasis has been associated with pancreatic cancer (Furukawa et al., 2011; Akkaub & Buret, 2020). In some cases, ocular pathologies, such as iridocyclitis, choroiditis, and retinal hemorrhages have been associated with *G. duodenalis* infection in humans (Corsi et al., 1998). Children have been reported to be more susceptible to ocular lesions, which are thought to be due to cell damage in the retina (Pettoello et al., 1990; Corsi et al., 1998). Inflammatory arthritis has been observed in some patients after two to four weeks after giardiasis and has been described in knee and ankle joints (Borman et al., 2001; Carlson & Finger, 2004). Myopathies due to hypokalemia have been described in immunocompetent and immunocompromised people (Cervelló et al.,

1993; Genovese et al., 1996). Chronic fatigue syndrome has been described in patients with giardia-caused enteritis – with more than 60% of patients reporting fatigue several months after the initial infection with at least 5% of the patients not being able to recover at all (Naess et al., 2012). Additional long-term effects, such as failure to thrive and stunted growth, have been observed in children when infected in the first years of their lives (Bergman et al., 2005; Botero-Garcés et al., 2009). Reports of stunted growth in children were more frequent from developing countries (Koruk et al., 2010). *G. duodenalis* disrupts iron and protein absorption, and, together with the socio-economic, socio-cultural and environmental factors, leads to growth failure, wasting and underweight, cognitive retardation and malabsorption (Simsek et al., 2004; Botero-Garcés et al., 2009; Koruk et al., 2010). There have also been reports of post-infectious irritable bowel syndrome, and secondary lactose intolerance, especially in patients with chronic giardiasis (Grazioli et al., 2006; Stark et al., 2007; Litleskare et al., 2015). There are still many uncertainties about the global disease burden of giardiasis in humans, even though there are more than 280 million cases worldwide with children being most affected, especially in developing countries (Dougherty & Bartelt, 2022). In these countries, giardiasis is a major cause of morbidity in children under five years of age (Lanata et al., 2013).

In Europe, giardiasis in humans has a seasonal pattern, with the infection peaks usually around September and November, but the least reported cases were from March and June (ECDC, 2022). Additionally, significantly higher percentage of reports was coming from males (56%) than females (44%) with the highest notification rate detected in the 0–4-year-old age group (ECDC, 2022).

Human giardiasis cases in Europe must be reported to the European Centre for Disease Prevention and Control (ECDC). From the ECDC reports, in 2018 there were a total of 15,546 reported human cases from 30 EU/EEA countries, excluding the UK, and in 2019, 2020, 2021 and 2022, the cases have steadily decreased, going to 10,894 in 2022 (ECDC, 2022). From 2018 to 2022, the highest report rate per 100,000 population has been from Belgium (9–20.8), followed by Bulgaria (7.2–16.3), and Sweden (5.7–12.4), with the lowest rates reported from Lithuania (0.2–0.6), Portugal (0.3–0.6), Greece (0.2–0.6) and Hungary (0.6–0.9) (Figure 1.7).

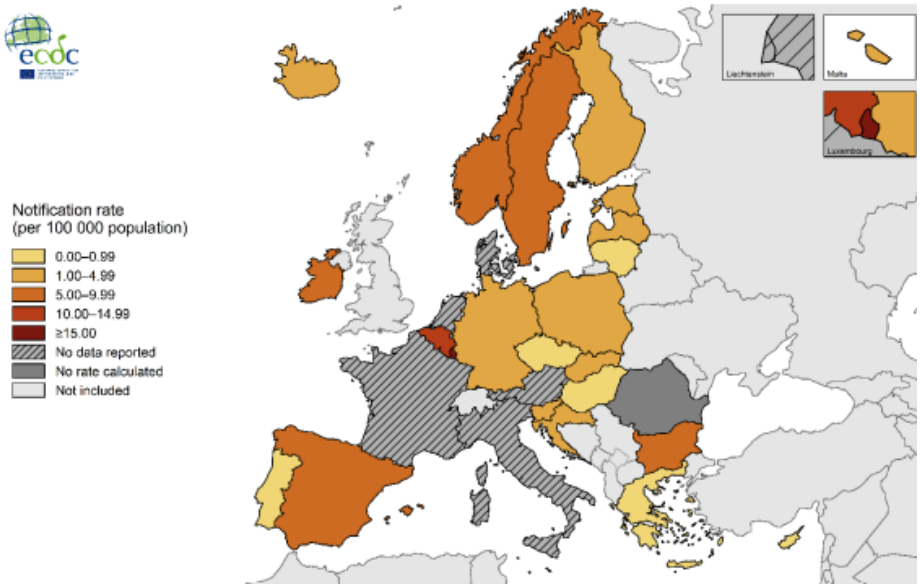

Figure 1.7. **Confirmed giardiasis cases in humans per 100,000 population by country, EU/EEA, 2022** (ECDC, 2022).

In Latvia, over a 20-year period, there have been a total of 1020 (average of 34 cases per year) giardiasis cases reported to the Centre of Disease Prevention and Control of Latvia with

the highest number of reports confirmed for 1–6- and 7–14-year-old children (Deksne et al., 2022). Deksne et al. (2022) tested an additional 584 unique patients from 22 days to 17-year-old with immunofluorescence and *Giardia* was detected in 7.2% (35) of the examined children, which shows that giardiasis in Latvia might be underdiagnosed and underreported in the human population (Deksne et al., 2022). To the best knowledge, no known outbreaks in humans have been reported due to the infection with *G. duodenalis* in Latvia.

1.9. *Giardia duodenalis* – foodborne transmission and food safety

Giardia can be spread via the foodborne transmission route. The viability of cysts is not affected by low temperatures, and cysts retained their infectivity for several weeks in the refrigerator (Ryan et al., 2019). The incubation period of giardiasis can be up to seven days, hence delay the identification of the infection source (Ryan et al., 2018; Ryan et al., 2019). *Giardia* cysts are covered by sticky surfaces; therefore, washing and rinsing with drinking water may not fully remove them from the surface or crevices of the produce (Robertson & Gjerde, 2000).

The main foodborne transmission routes for *G. duodenalis* are fresh fruits, vegetables and berries (Robertson & Gjerde, 2000; Dixon, 2021). Fresh fruits, berries, and vegetables can be contaminated by crop fertilization with manure, using contaminated manure for field irrigation, or washing the produce with contaminated water (Ryan et al., 2019). Infected workers, especially those who work with RTE products during packaging or preparation operations in large-scale industrialized factories may compromise the safety of products (Ryan et al., 2018). Additionally, a previously contaminated food-contact surface can alter the safety of a large proportion of batches, resulting in a wide distribution of contaminated products in a short time period (Ryan et al., 2018; Ryan et al., 2019).

Detection of *G. duodenalis* cysts in fresh produce is complicated due to low numbers of cysts retrieved from the surface of the produce. One official method to detect the parasite is available only for leafy greens and berries; it is expensive and uses immunomagnetic separation – International Organization of Standardization (ISO) approved Method No. 18744 "Microbiology of the food chain – Detection and enumeration of *Cryptosporidium* and *Giardia* in fresh leafy green vegetables and berry fruits" (Cook et al., 2006). A modified method has been described in which a smaller number of magnetic beads in the immunomagnetic separation step is successfully used to lower the costs (Utaaker et al., 2015).

Several studies have been conducted to study *Giardia* spp. prevalence in fresh leafy greens and berries. In Italy, 864 ready-to-eat (RTE) salads were examined for the presence of *Giardia* cysts utilizing immunofluorescence staining and molecular analysis. *G. duodenalis* assemblage A was found in four (0.6%) of the RTE packages (Caradonna et al., 2017). A later study by Barlaam et al. (2022) who examined 324 RTE salads and berries each revealed *Giardia* prevalence of 4.6% and the presence of *G. duodenalis* assemblages A, B, and E (Barlaam et al., 2022). In the Czech Republic, a total of 156 fresh berries were examined using molecular methods, revealing one (0.6%) sample positive for *Giardia* spp. with one to seven cysts per gram of strawberries (Dziedzinska et al., 2018). In Valencia, Spain, 129 leafy green vegetables from conventional and organic farms were examined using sedimentation technique, and real-time PCR for parasite detection; *Giardia* was found in six (23.0%) of the samples, with higher prevalence observed in the crops from ecological farms (27, 41.5%), compared to conventional farms (3, 4.7%) (Trelis et al., 2022). In Portugal, in a study by Faria et al. (2023), 100 RTE samples were examined for the presence of *Giardia* DNA, with 18 (18.0%) samples being positive for *G. duodenalis* assemblage A (Faria et al., 2023).

In European countries, there have not been any reported foodborne-related giardiasis outbreaks in humans; but in the USA, there have been at least 30 outbreaks from 1960 to 2016, mainly due to mixed green salads, unpasteurized milk, raw oysters, fruit, and home-canned salmon (Porter et al., 1990; Figgatt et al., 2017; Ryan et al., 2019).

1.10. *Giardia duodenalis* – water hygiene and waterborne outbreaks

Cyst size and relative cyst resistance to conventional water treatment (coagulation, sedimentation, filtration, and chlorine disinfection) improve survival of *Giardia* cysts during routine drinking and wastewater treatments (Betancourt & Rose, 2004; Baldursson & Karanis, 2011; Ryan et al., 2016). In raw water treatment, production of drinkable water requires several steps, including of removal of macroscopical debris, addition of chemical compounds (coagulants and flocculants) to combine particles into denser flocks so they sink to the bottom, filtration of water through several layers of granular medium with progressively smaller pore sizes to collect the residual matter, and lastly, disinfection of water either chemically (chlorine, chlorine dioxide, ozone) or with physically (ultraviolet rays) (Crittenden et al., 2012). However, these water treatments are not always successful at removing all *Giardia* cysts (Wallis et al., 1996; Castro-Hermida et al., 2014). In these water treatment plants, *G. duodenalis* assemblages AI, AII, and E were detected after treatment (Castro-Hermida et al., 2014).

Swimming in surface waters is another common way to become infected, especially if the surface waters are not protected from cattle and other animal waste, which is a significant risk of infection by *Giardia* (Putignani & Donato, 2010). Surface waters may be contaminated by fertilizing crops with contaminated slurry, manure, or pasturing livestock near water bodies (Smith et al., 2014a). River currents can also carry parasite cysts further from the initial source, complicating the outbreak investigation (Ruecker et al., 2007).

G. duodenalis is one of the main causes of human waterborne outbreaks (Adam et al., 2016). Since 2003, there have been five outbreaks caused by tap water contaminated by either leaking sewage in the water source or an inappropriate filtration system in Europe (Table 1.6).

Table 1.6. **List of *Giardia duodenalis*-related waterborne outbreaks in European countries since 2003**

***Giardia* species**	**Country, region**	**Year/-s**	**Source**	**Infected**	**Reference**
G. duodenalis	Norway, Trondheim	2003/ 2004	Water	12	Åberg et al., 2015
G. duodenalis assemblage A	Norway, Bergen	2004	Water (Leaking sewage pipes and insufficient water treatment)	2500	Robertson et al., 2004
G. duodenalis	Finland, Nokia	2007/ 2008	Tap water (Sewage contamination in the distribution network)	Unknown	Rimhanen-Finne et al., 2010
G. duodenalis	Belgium, Hemiksem	2010	Tap water (Contaminated with river water)	222	Braeye et al., 2010
G. duodenalis assemblage B	Italy, Bologna	2018/ 2019	Tap water	228	Resi et al., 2021

In the United States, *G. duodenali*s has been responsible for 242 outbreaks during 1971–2011 and 111 outbreaks between 2012–2017, most were related to various contaminated water sources (Adam et al., 2016; Conners et al., 2021).

Detection of *Giardia* cysts is complicated as large amounts of water need to be tested, and only one officially recognized method – ISO method No. 15553:2006 "Water quality— Isolation and identification of *Cryptosporidium* oocysts and *Giardia* cysts from water" for official *Giardia* cyst detection in water sources (ISO, 2006). Detecting *Giardia* in water is difficult because it requires a sample size of at least 100–1000 liters, expensive equipment, and reagents.

There are no regulations regarding *G. duodenalis* testing in drinking water in Latvia. Although the Cabinet of Ministers regulation Nr. 547 (2023) "Mandatory safety and quality requirements for drinking water, monitoring, and control procedures," states that drinking water needs to be free from parasites; but currently there are the only requirements for microbiological tests - for *Escherichia coli* and intestinal enterococci (CM nr 547, 2023).

1.11. *Giardia duodenalis* outbreaks in humans due to contact with cattle and canids

G. duodenalis cysts are hard to eliminate in cattle herds or other animal holdings due to their small cyst size (7–10 µm), large output from infected individuals, which can be up to 10^6 cysts per one gram of feces and *G. duodenalis* cyst resilience in the environment (Zambriski et al., 2013). Additionally, it is still not fully understood whether *G. duodenalis* is a true zoonosis as there are limited reports on human infection from animals, nevertheless, the potential *G. duodenalis* assemblage cross-species infection can be seen in Figure 1.8 (Cacciò et al., 2018).

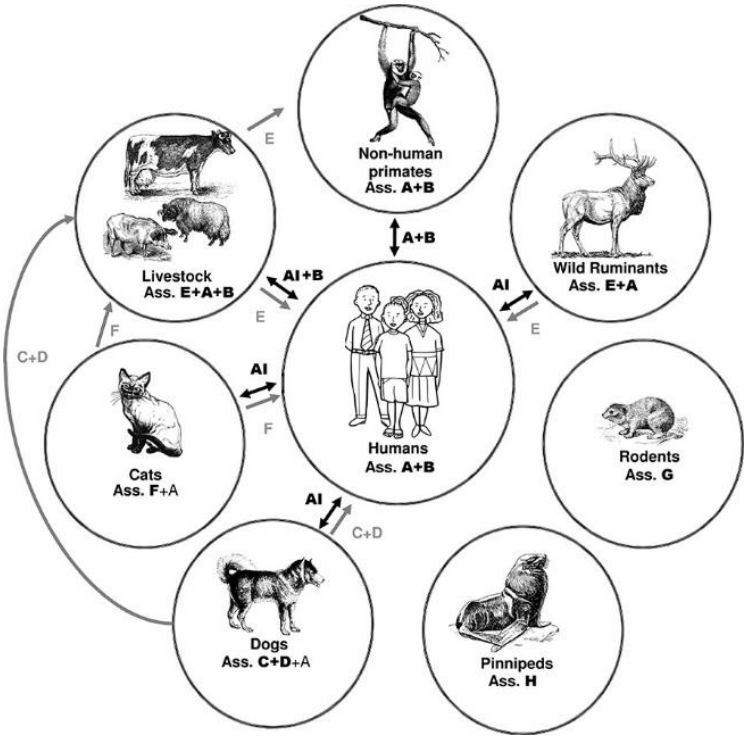

Figure 1.8. ***Giardia duodenalis* genetic diversity in various species and their zoonotic potential** (Cacciò et al., 2018)

Nevertheless, personal precautions, such as using single-use gloves and washing hands after contact with animals, should be taken, as even seemingly healthy animals can shed large

amounts of *G. duodenalis* cysts in the environment (Brook et al., 2008). There have been only a few sporadic and localized cases of the same *Giardia* genotype found in animals and humans who are living or working with them, usually in rural communities (Traub et al., 2004; Traub et al., 2009; Khan et al., 2011; Abdel-Moein & Saeed, 2016). Few sporadic cases of giardiasis have been recorded due to contact with cattle worldwide due to poor personal hygiene. *G. duodenalis* assemblage E was isolated from 25 children with diarrhea aged 1 to 12 from rural areas of Egypt and the same assemblage was found in calves from the same region, but it was not clear whether these children had direct contact with the sampled cattle herds (Abdel-Moein & Saeed, 2016). The presence of the same *G. duodenalis* sub-assemblage AI was identified in workers and calves from the same farm (Khan et al., 2011).

2. MATERIALS AND METHODS

The study was conducted in three different time periods from 2019 to 2025 and was elaborated at the Institute of Food and Environmental Hygiene of the Faculty of Veterinary Medicine and at the Institute of Food Safety, Animal Health and Environment "BIOR", the Department of Microbiology and Pathology, Parasitology and Microbial Genomic group.

The first study period focused on the prevalence, cyst load, and genetic diversity of *G. duodenalis* in cattle in Latvia. Additionally, information was gathered about herd management, and risk analysis was performed to identify the factors, which could affect the prevalence of *G. duodenalis* in cattle herds.

The second study period focused on the prevalence, cyst load, and genetic diversity of *G. duodenalis* in domestic dogs, as well as risk analysis was performed to identify factors that could increase the prevalence in domestic dogs.

The third study period focused on the prevalence, cyst load, and genetic diversity of *G. duodenalis* in red foxes and raccoon dogs.

Finally, data analysis was made to see which animal species could be the most significant source of *G. duodenalis* cysts for the general environment.

2.1. Study design for detection of *Giardia duodenalis* in cattle, domestic dogs, and wild canids

2.1.1. Sampling for detection of *Giardia duodenalis* in cattle in Latvia

From March 2019 to March 2020, cattle herds were visited to collect fecal samples for the detection of *G. duodenalis* and to gather information about herd management practices. A total of 973 individual cattle were sampled from four regions: Kurzeme (n = 283), Latgale (n = 91), Vidzeme (n = 244), and Zemgale (n = 355). A total of 853 female and 120 male cattle were examined. In total, samples were obtained from 32 herds, with the highest number of herds from Zemgale (n = 12), followed by Kurzeme (n = 9), Vidzeme (n = 9), and Latgale (n = 3) (Figure 2.1).

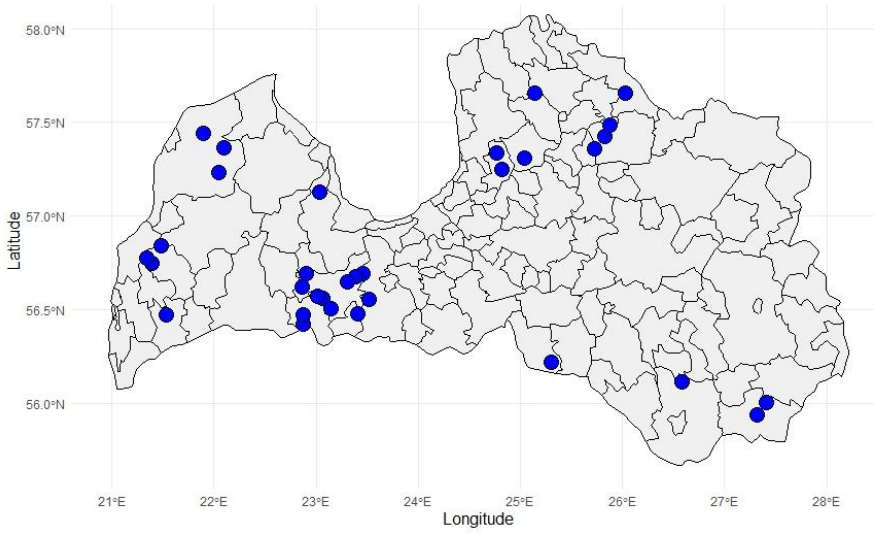

Figure 2.1. **Distribution of sampled cattle herds in Latvia**

Furthermore, fecal samples were collected from 13 cattle breeds with the majority of samples collected from Holstein Friesian (HM) (n = 699), followed by Holstein Red and White (HS) (n = 122); Latvian brown (LB) (n = 71), Limousin (LI) (n = 35), Danish Red (DS) (n =

19); mixed breed (XX) (n = 14) and 13 samples collected from six other breeds with number of collected samples varying from 1–4.

For each month when cattle were sampled, data on mean temperature, precipitation, and air humidity were acquired from Latvian Environment, Geology and Meteorology Center (LEGMC, www.klimats.meteo.lv) (Table 2.1).

Table 2.1. **Mean air temperature (°C), precipitation (mm), and air humidity (%) and total collected cattle fecal samples, during sampling period**

Month	Collected cattle fecal samples	Mean temperature (°C)	Mean precipitation (mm)	Mean air humidity (%)
March	153	2.2	49.2	81.0
July	70	16.2	87.3	78.0
August	27	17.0	51.8	77.0
September	290	12.4	82.1	79.0
October	152	8.5	81.4	87.0
November	117	4.0	73.2	89.0
December	164	2.7	56.1	90.0

The required sample size for the study was determined based on Latvia's cattle population size of 395,320 (Agricultural Data Centre Republic of Latvia, accessed on January 1st, 2020).

To calculate the minimal number of cattle needed for sampling, a 95% confidence interval, assuming a 50% infection rate within the population was used. At the end, a minimum of 384 cattle needed to be sampled. Even though the sampling approach was designed to be proportionally stratified across Latvian counties, only herds from which owners responded positively to the study were sampled and were not excluded from the study. Therefore, convenience sampling (haphazard or accidental sampling) was used (Etikan et al., 2015).

Potential herd owners or overseeing veterinarians were contacted for the sampling via telephone. A herd was visited once during the study period. The primary inclusion criteria were cattle herds with different management systems, such as organic, conventional, and untethered and tethered management types. Furthermore, the study aimed to represent a spectrum of herd sizes, encompassing large industrial operations to smaller family-owned cattle herds. If possible, fecal samples were gathered from both sexes and various cattle breeds. The exclusion criteria were if the owners did not agree to the study, or the agreement process for study was not finalized.

Up to 45 fecal samples were collected from each herd, distributed across the three age groups. The categorization of cattle age groups was established by considering the distinct management practices associated with different age brackets and the biology of *G. duodenalis*, without considering the physiological age of the cattle.

The categorization of age was done as follows:

- 0–3 months;
- 4–24 months;
- 24 months and above.

Calves up to 3 months of age experience a more pronounced clinical impact from *Giardia* and excrete a higher amount of *Giardia* cysts that could contaminate the environment (O'Handley et al., 2001; Trout et al., 2005; Mark-Carew et al., 2010). Additionally, calves in this age bracket often receive milk as an additional food source, reflecting their unique dietary needs and infection vulnerability (Rosenberger et al., 2017). Cattle between 4–24 months old are typically maintained on standard cattle feed and are grouped based on their similar dietary requirements and management practices (Curtis et al., 2018). Cattle older than 24 months, being

involved in calving and giving birth to new calves, are more likely to transmit infections to neonatal calves during birth or soon after due to the rise of excreted *Giardia* cysts around the perinatal period (Ralston et al., 2003; Mark-Carew et al., 2010).

The sampled cattle in each age group were chosen by convenience (Etikan et al., 2015). For example, the cattle were sampled, when they were about to defecate or had just freshly defecated, and the feces had no visual changes before collection (such as contamination with litter or hoof prints). If it was possible, when sampling the cattle from the 0–3-month-old group, random sampling technique was used to collect feces from calves with and without diarrhea. In herds where the predetermined sample quota could not be obtained from a particular age group, all available animals within that specific age group were sampled to ensure comprehensive coverage within each category.

Fecal samples were collected with a clean latex glove and put in a single-use plastic container; the latex glove was changed after every sample collection. Only the top part of freshly defecated feces was collected to minimize feces cross-contamination with other pathogens. An anonymous identification number was assigned for each cattle.

For each animal, a questionnaire was completed by the sample collector together with herd owner or veterinarian, recording the cattle's identification number, sex, breed, fecal consistency, and whether the cattle had been brought to the herd (Appendix 1). All sensitive data were made anonymous by assigning an individual laboratory number for each herd. Afterward, samples were put in a cooling box with cold elements, which ensured 4 °C until transported to the Institute "BIOR", Parasitology laboratory, on the same day of collection, where they were stored for up to two weeks at 4 °C until further testing.

2.1.2. Study design of *Giardia duodenalis* in domestic dogs in Latvia

The samples of feces from domestic dogs were collected from April 2020 to May 2022. In total, 373 dogs were tested for *G. duodenalis*, including 183 female and 190 male dogs. Of these, 328 were privately owned and 45 were shelter dogs. Fecal samples were collected from 64 dog breeds, which were categorized as "breed" dogs (218) and no-breed dogs (155). Regionally, samples were collected from Latgale (22), Kurzeme (57), Zemgale (88), and Vidzeme (206) (Figure 2.2).

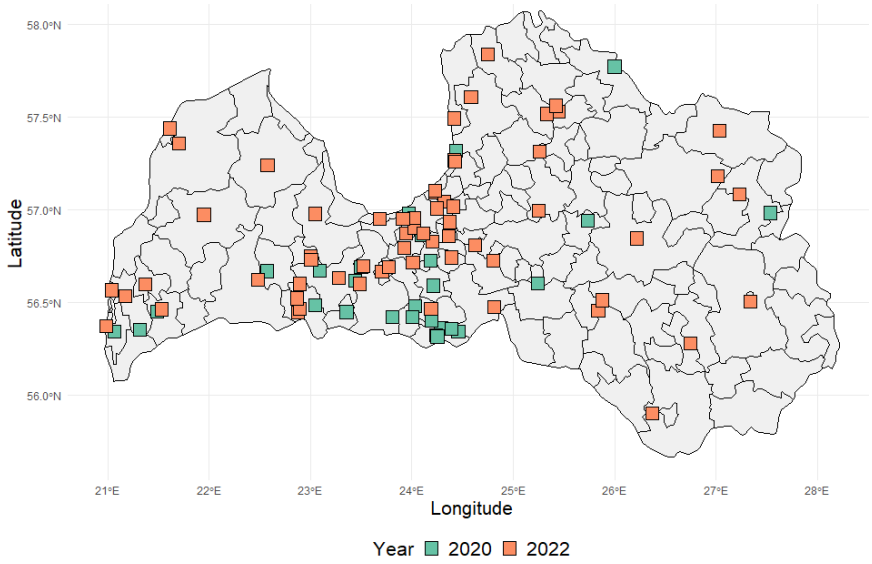

Figure 2.2. **Distribution of sampled domestic dogs in Latvia**

For each month when domestic dogs were sampled, data on mean temperature, precipitation, and air humidity were acquired from LEGMC (www.klimats.meteo.lv) (Table 2.2).

Table 2.2. **Mean air temperature (°C), precipitation (mm), and air humidity (%) and total collected dog fecal samples, during sampling period**

Month	Collected dog fecal samples	Mean temperature (°C)	Mean precipitation (mm)	Mean air humidity (%)
January	21	3.1	36.2	89.0
February	14	2.2	60.1	85.0
March	3	2.9	50.0	74.0
April	24	5.6	18.0	69.0
June	7	9.5	47.2	70.0
July	37	18.1	91.1	75.0
August	8	16.4	77.5	78.0
September	33	17.2	45.9	79.0
October	65	14.4	49.8	82.0
November	138	9.8	72.7	87.0
December	10	5.5	53.3	89.0

The required number of domestic dogs for this study was calculated based on a 95% confidence interval and assuming a 40% infection rate within the domestic dog population (Bouzid et al., 2015). Data from the Agricultural Data Centre Republic of Latvia was retrieved to calculate the necessary sample size for domestic dogs, which in 2020 was 132,750. The minimal necessary sample size was calculated to be 323 (Agricultural Data Centre Republic of Latvia, accessed on January 1st, 2020). A haphazard sampling technique was used to collect the samples – advertisements on social media, such as Facebook, and news outlets, such as BezTabu, www.lsm.lv, and the Institute "BIOR" homepage (www.bior.lv), were posted to reach a wider target audience. Individual invitations to participate in the study were also sent to veterinary clinics and dog shelters.

Descriptions of the study in Latvian with a detailed explanation of the feces collection process were provided to the owners, veterinarians, and dog shelter experts in both study periods. Participants were instructed to collect feces for three days straight, put them in a clean, waterproof container, and keep the collected samples at 4 °C until transported to the Institute "BIOR", Laboratory of Microbiology and Pathology, Parasitology group, until further testing, which was done within one week after the samples were delivered to the laboratory.

Age of the dogs was categorized in age groups by Harvey (2021):

- < 2 years old: puppies;
- 2 to 7 years old: adults;
- 8 to 11 years old: seniors;
- > 12 years old: geriatric.

At the laboratory, an individual identification number was added to provide anonymity. Fecal consistency was noted (liquid, soft, formed), and the samples were stored at 4 °C for two weeks until further testing.

2.1.3. Study design of *Giardia duodenalis* in red foxes and raccoon dogs in Latvia

From February 2020 to January 2023, a total of 219 red fox carcasses were collected in total, out of which 177 were from Latgale, 31 from Vidzeme, 7 from Zemgale, and 4 from Kurzeme, and tested for *G. duodenalis* (Figure 2.3).

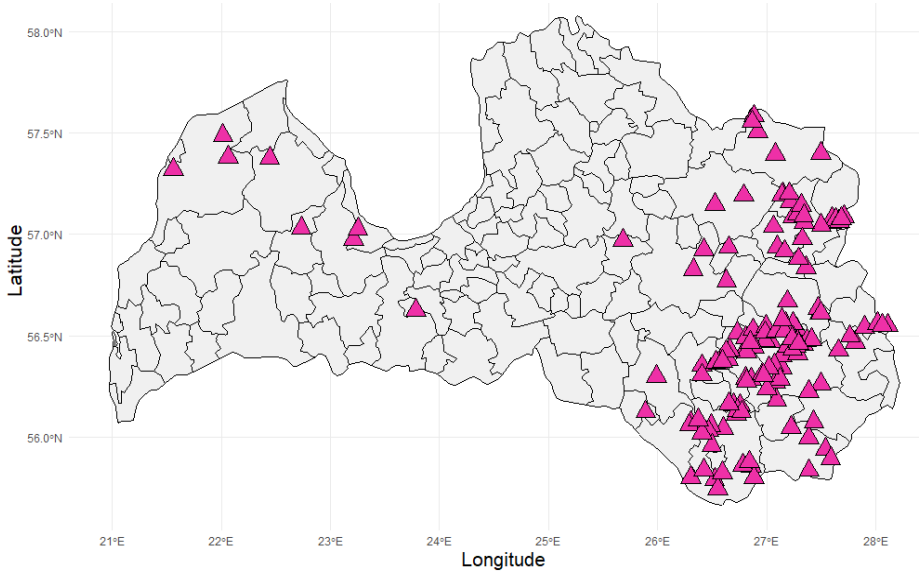

Figure 2.3. **Distribution of the hunted red foxes**

The red foxes were collected from 23 hunting parishes, which were then grouped in eight forestry districts of Latvia. The distribution of the sampled red foxes is summarized in Table 2.3.

Table 2.3. **Distribution of sampled red foxes by forestry districts of Latvia**

Region	Forestry district	Number of sampled red foxes
Latgale	Austrumlatgale	86
	Dienvidlatgale	91
Vidzeme	Centrālvidzeme	7
	Ziemeļaustrumu	18
	Ziemeļvidzeme	6
Zemgale	Zemgales	5
	Sēlija	2
Kurzeme	Ziemeļkurzemes	4

In the same period as the red foxes, a total of 78 raccoon dogs were collected and examined for *G. duodenalis*, out of these raccoon dogs, 69 were from Latgale, 7 from Vidzeme, and 2 from Zemgale (Figure 2.4).

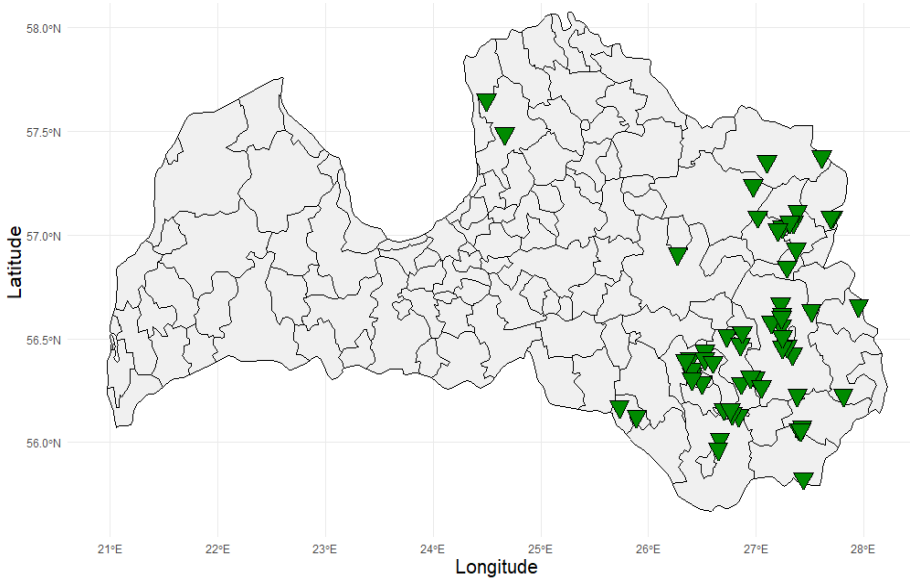

Figure 2.4. **Distribution of the hunted raccoon dogs**

The raccoon dogs were collected from 13 hunting parishes, which were grouped in six forestry districts of Latvia. The distribution of the sampled raccoon dogs is summarized in Table 2.4.

Table 2.4. **Distribution of sampled raccoon dogs by the hunting forestry district of Latvia**

Region	Forestry district	Number of sampled raccoon dogs
Latgale	Austrumlatgale	34
	Dienvidlatgale	35
Vidzeme	Centrālvidzeme	1
	Ziemeļaustrumu	3
	Ziemeļvidzeme	3
Zemgale	Sēlijas	2

For each month when red foxes and raccoon dogs were sampled, data on mean temperature, precipitation, and air humidity were acquired from LEGMC (www.klimats.meteo.lv) (Table 2.5).

Table 2.5. **Mean air temperature (°C), precipitation (mm), and air humidity (%) and total collected red fox and raccoon dog fecal samples, during sampling period**

Species	Month	Collected cattle fecal samples	Mean temperature (°C)	Mean precipitation (mm)	Mean air humidity (%)
Red foxes	January	19	-0.9	72.7	89.0
	October	66	9.2	54.9	87.0
	November	111	2.5	34.0	91.0
	December	23	-3.1	56.0	92.0
Raccoon dog	October	67	9.2	54.9	87.0
	November	11	2.5	34.0	91.0

Red foxes and raccoon dogs were shot by hunters via the Rabies vaccination and prevention program organized by the Food and Veterinary Service of Latvia (Zemkopības ministrija & Pārtikas un veterinārais dienests, 2021). After shooting the animal, the hunter determined the age of the animal (if possible) and sent the carcass to the Institute "BIOR". In the Institute "BIOR", Pathology group, the intestinal tract was removed, put in a waterproof container, and delivered to the Parasitology group, where it was put in the freezer (-30 °C) for up to a month. Afterwards, the intestinal tract was defrosted and feces were collected from the rectum, put in a waterproof container, labelled with an identification number, and stored at 4 °C for up to a week until further testing.

2.2. Microscopical analysis of fecal samples

2.2.1. Fecal samples preparation

To process the samples, a saturated sodium chloride (NaCl) method, as described by Kuczynska and Shelton with modifications from Maddox-Hyttel et al. (2006), was used (Kuczynska & Shelton, 1999; Maddox-Hyttel et al., 2006). The identification number was always written down on a new preparation tube throughout the preparation process.

To prepare the sample, one gram of the individual feces was weighed into a clean, single-use 15 ml centrifuge tube (SARSTEDT, Nümbrecht, Germany).

The sample purification and sedimentation process was as follows:

1. Four milliliters of distilled water was added to the fecal sample and mixed thoroughly for 30 seconds, or until the sample was thoroughly mixed/ using a vortex (Vortex V-1 plus, Biosan, Latvia). Then a 4 ml of saturated NaCl with a density of 1.18 was added and mixed thoroughly for 30 seconds, the 15 ml tube was centrifuged using Hermle Z446K (HERMLE Labortechnik GmbH, Germany) for one minute at 1540 x times gravity (g).
2. After, 8 ml of the supernatant was poured into a clean, single-use 50 ml centrifuge tube (SARSTEDT, Nümbrecht, Germany), to which distilled water was added until the 45 ml mark, and centrifuged for 10 minutes at 1540 x g.
3. The top layer was discarded in one go till the 15 ml mark, the sediment was vortexed until it was fully dissolved (approximately 30 seconds), and the distilled water was added till the 45 ml mark, and centrifuged again for 10 minutes at 1540 x g.
4. The top layer was discarded until the 15 ml mark in one go, and distilled water was added until the 45 ml mark (sediment was not vortexed), and was centrifuged again for 10 minutes at 1540 x g.
5. The top layer was discarded until the 5 ml mark, and the final top layer was removed with a 100-1000 µl single-channel micropipette (Transferpette® S, Brand, Germany) and a clean pipette tip until the 2 ml mark, and sediment were carefully mixed with the tip.
6. The sediment was transferred to a sterile 2 ml Eppendorf-type tube, and the tube was stored at 4 °C until further testing for up to one month.

2.2.2. *Giardia duodenalis* cyst staining with the immunofluorescence technique and microscopy

Giardia cysts were detected with the immunofluorescence technique using A100FLR-20X AquaGlo™ G/C Direct Reagent kit (Waterborne, INC, New Orleans, USA) according to the manufacturer's instructions.

Because the immunofluorescence stain is light sensitive, a light and waterproof moisture chamber was prepared using a slide box lined with moist tissue paper. For the preparation of

the fluorescein isothiocyanate (FITC) labeled monoclonal antibody solution (mAbs), 9.5 µl of B100-20 Dilution Buffer solution and 0.5 µl A100FLR-20X: AquaGlo G/C were mixed together in a clean Eppendorf type 2 ml centrifuge tube. This mixed solution was for a single sample. Therefore, the total amount of solution mixture was calculated based on the samples stained per staining session. A phosphate-buffered saline (PBS) solution was prepared by dissolving one PBS tablet (PanReac, AppliChem GmbH, Darmstadt, Germany) in 100 ml of deionized water and stirring until fully dissolved. For quality control, a positive control of 10 µl provided in the reagent kit was included in each batch and added to the first well of the first Teflon slide (ImmunoCell, Mechelen, Germany). For this detection kit, no negative control is needed.

The staining process with the immunofluorescence technique was as follows:

1. Positive control was added to the first slide well.
2. The purified 2 ml sample was thoroughly mixed using a vortex (approximately 15-30 seconds).
3. 10 µl of the purified material was applied to one Teflon slide well using a 0.5-10 µl single-channel micropipette (Transferpette® S, Brand, Germany) and sterile pipette tip.
4. The slide was dried at 37 °C in the thermostat until it was fully dry (approximately 15 minutes).
5. To fixate the slide, fully dried slides were put in a container with methanol (LiChrosolv, Merck KGaA, Germany) solution for 5 minutes and fully air-dried (approximately 5 minutes).
6. For staining with FITC-labelled anti-*Cryptosporidium*/*Giardia* mAbs solution, 10 µl of the mAbs solution was carefully applied and distributed on the slide well so that the pipette does not touch the well.
7. The slide was put upwards in the light-proof moisture chamber and put in the thermostat at 37 °C for 45 minutes.
8. The slide was washed with 150 µl of PBS solution to wash off the mAb. It was poured directly on the well with the sample using a 100-1000 µl single-channel micropipette and appropriate pipette tip. The PBS solution was poured not to touch the other wells. The wells were washed for five seconds and dried at 37 °C until fully dried (approximately 15 minutes).
9. For cover slide mounting, 4.5 µl of M101 No-Fade Mounting medium was added in the middle of each well, and a cover slide (40 x 50 mm) was put on the Teflon slide.
10. Fully ready slides were placed in a dry, light-proof chamber for up to a week until further examination.

All immunofluorescence-stained slides were analyzed using a fluorescence microscope (Nikon ECLIPSE Ti-E, USA) with a fluorescein isothiocyanate (FITC) filter using 200x magnification. The positive control was examined first for every batch. All brightly green-stained cysts (Figure 2.5) with typical morphology and size were counted in all wells.

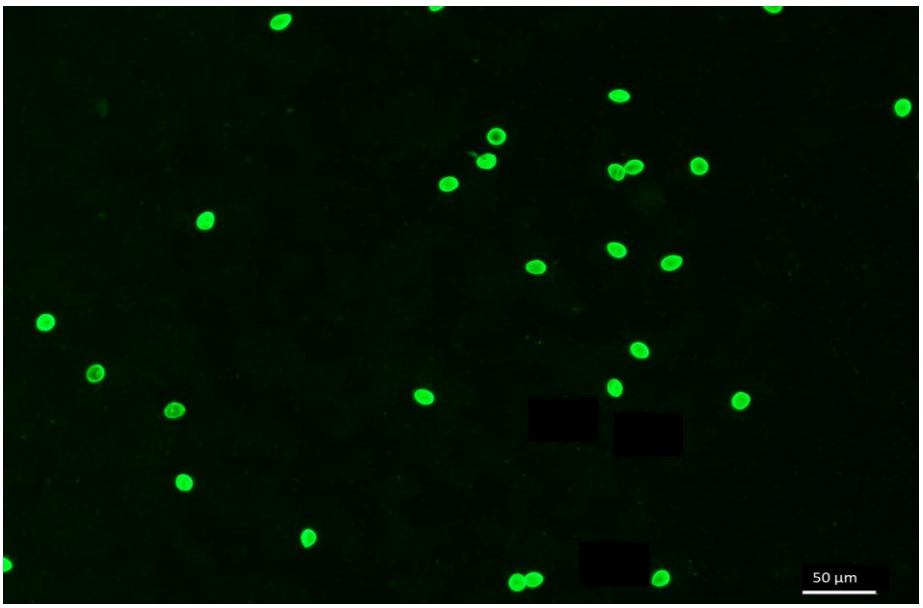

Figure 2.5. **Bright green *Giardia duodenalis* cysts with typical morphology stained with immunofluorescence stain. 200x magnification** (author: M. Mateusa)

G. duodenalis cysts are typically oval and 7.0–10.0 µm in size. The immunofluorescence staining kit that was used also detects *Cryptosporidium* spp. oocysts. To measure the cysts or differentiate between the larger *C. andersoni* oocysts and *Giardia* cysts, the Nis-Elements AR 4.00.00 program was used. Compared to *Giardia* cysts, *C. andersoni* oocysts are 7.4 ± 5.5 µm in size, but *Giardia* cysts are 7–10 µm in diameter (Figure 2.6) (Lindsay et al., 2000; Adam, 2001).

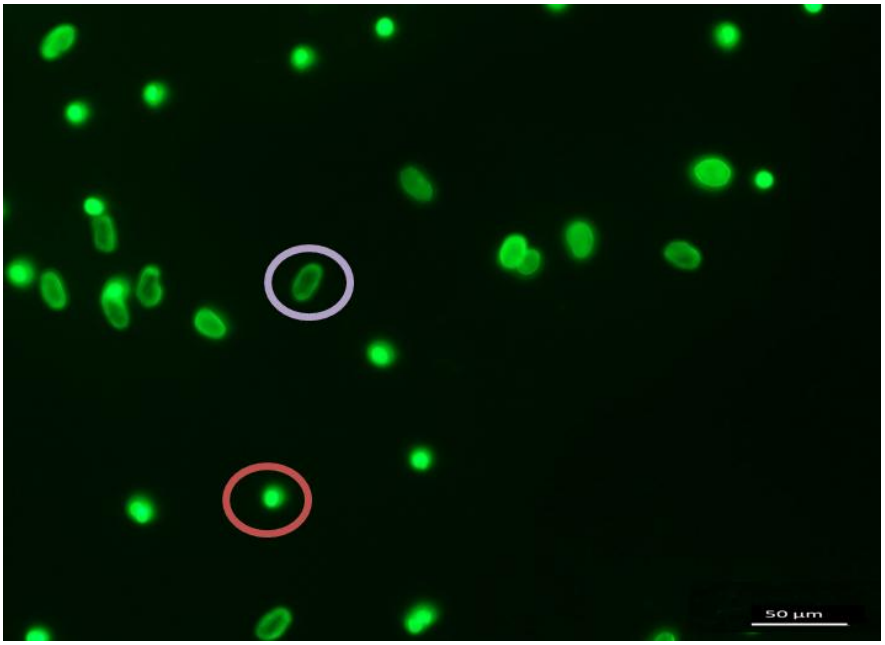

Figure 2.6. ***Cryptosporidium* spp. oocysts and *Giardia duodenalis* cysts comparison with the immunofluorescence method. Violet circle – *G. duodenalis* cyst, red circle – *Cryptosporidium* spp. oocyst. 400x magnification** (author: M. Mateusa)

2.3. *Giardia duodenalis* molecular identification

2.3.1. *Giardia duodenalis* DNA extraction from positive samples from positive cattle, domestic dogs, red foxes, and raccoon dogs

All microscopically positive samples were subjected to the molecular identification of *G. duodenalis*. In total, 145 IFM-positive fecal samples (82 cattle, 40 dogs, 23 red foxes, and raccoon dogs) were analyzed.

Genomic DNA was extracted from the 2 ml purified samples using the DNeasy® PowerSoil® Pro Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions (Qiagen, 2023), and the genomic DNA was isolated as follows:

1. Samples were centrifuged for 1 minute at 15,000 x g, and the top layer was discarded using a micropipette.
2. Approximately 250 µl of the sample was added to the provided lysis tubes with beads (PowerBead Pro tube), which contains buffer that protects nucleic acids from degradation, and 800 µl of the cell lysing CD1 solution was added.
3. The tube was put in homogenizer (Precellys 24, Bertin Technologies, France) with settings 6800-2x30-030, to ensure *Giardia* cell lysis.
4. The tube was centrifuged at 15,000 x g for one minute and supernatant was added to a clean 2 ml Microcentrifuge tube provided in the extraction kit, to which 200 µl of the inhibitor removal (such as inorganic and organic matter) CD2 solution was added, vortexed for five seconds, and centrifuged at 15,000 x g for one minute at room temperature.
5. 700 µl of supernatant was transferred to a clean 2 ml Microcentrifuge tube, to which 600 µl DNA-binding solution CD3 was added, vortexed for five seconds.
6. After, 650 µl of the lysate was added to the MB Spin Column, which contains silica membrane which selectively binds the DNA and centrifuged at 15,000 x g for one minute. The flow-through was discarded, and this step was repeated until all lysate had passed through the MB Spin Column.
7. The MB Spin Column was placed into a clean 2ml Collection tube, and 500 µl of washing buffer Solution EA was added in the middle of the tube, to remove proteins and other non-aqueous contaminants from the filter membrane of MB Spin column, and it was centrifuged at 15,000 x g for one minute.
8. The flow-through was discarded, the MB Spin Column was added back, and 500 µl of the ethanol-based wash solution CD5 was added, to further clean the DNA, by removing residual contaminants. The tube was centrifuged at 15,000 x g for one minute. An additional centrifuge step of 16,000 x g for two minutes was done to ensure that all solution CD5 had been removed from the MB Spin Column tube.
9. MB Spin Column tube was added to a 1.5 ml Elution Tube, and 100 µl of the elution Solution CD6 was added at the center of the filter membrane, to release the DNA from the MB Spin Column filter membrane and centrifuged at 15,000 x g for one minute. The MB Spin Column was discarded and isolated DNA was frozen at -20 °C until further testing.

2.3.2. PCR application for *Giardia duodenalis* detection

The identification of *G. duodenalis* was conducted using two consecutive nested PCR amplifications according to a method described by the European Reference Laboratory of Parasites by targeting the *beta-giardin* (*bg*) gene (EURLP, MI-09-rev-2). The manufacturer's instructions for the Taq PCR Master Mix kit (QIAGEN, Hilden, Germany) were followed. *Giardia* genomic DNA and nuclease-free water were used for quality control as positive and

negative controls, respectively. Oligonucleotide sequences used are summarized in Table 2.6 (Lalle et al., 2005; EURLP, MI-09-rev-2).

Table 2.6. **Oligonucleotide mixture names with their representative codes and sequences used for the identification of *G. duodenalis*** (Lalle et al., 2005)

Oligonucleotide mixture name	Code	Oligonucleotide sequence
SetA	BGFor71	5'-CCCGACGACCTCACCCGCAGTCG-3'
SetA	BGRev794	5'-GCCGCCCTGGATCTTCGAGACGA-3'
SetB	BGinfFor	5'-GAACGAACGAGATCGAGGTCCG-3'
SetB	BGintRev	5'-CTCGACGAGCTTCGTGTT-3'

A total of 10 µl of the DNA was processed for the initial PCR amplification. The necessary master mix was prepared for a single sample (Table 2.7) and multiplied by the samples processed that day, plus two – one for negative and one for positive control.

Table 2.7. **Master mix for the initial PCR amplification for *Giardia* genus detection**

Reagent	Volume, µl
ddH2O (QIAGEN, Hilden, Germany)	31.5
10x PCR buffer (QIAGEN, Hilden, Germany)	5
Taq DNA polymerase (QIAGEN, Hilden, Germany)	0.5
dNTP mix (QIAGEN, Hilden, Germany)	2
SetA (BGFor71; BGRev794)	1

The sample preparation for the initial PCR amplification was done as follows**:**

1. Master mix (Table 2.7) was prepared in a 1.5 ml Eppendorf-type tube, vortexed, and 40 µl of the mix was transferred to a 0.2 ml PCR tube on an ice block.
2. To each tube, 10 µl of DNA was added, vortexed, and centrifuged for five seconds.
3. Amplification was done in ProFlex PCR system (Thermo Fisher Scientific, USA) with the necessary amplification cycles (Table 2.8).

Table 2.8. **PCR conditions for *Giardia* genus detection**

PCR conditions	Time	Temperature, °C
Pre-denaturation	3 minutes	94
Amplification (35 cycles)	30 seconds	94
	30 seconds	55
	60 seconds	72
Final extension	10 minutes	72

4. After the amplification, tubes were centrifuged for five seconds and kept at 4 °C until the further nested PCR step.

2.3.3. Nested PCR for *Giardia duodenalis* detection

For nested PCR, a new master mix with SetB oligonucleotide mix was prepared for each sample (Table 2.9).

Table 2.9. **PCR master mix for nested amplification of *Giardia duodenalis***

Reagent	Volume, µl
ddH2O (QIAGEN, Hilden, Germany)	36.5
10x PCR buffer (QIAGEN, Hilden, Germany)	5
Taq DNA polymerase (QIAGEN, Hilden, Germany)	0.5
dNTP mix (QIAGEN, Hilden, Germany)	2
SetB (BGinfFor; BGintRev)	1

The DNA sample preparation procedure for nested PCR was as follows:

1. The necessary master mix (Table 2.9) was prepared in a 1.5 ml Eppendorf-type tube, mixed, and 45 µl of the mix was transferred to a 0.2 ml PCR tube on an ice block.
2. In each tube, 5 µl of the obtained PCR product was added, vortexed, and centrifuged for five seconds.
3. Amplification was done in ProFlex PCR system (Thermo Fisher Scientific, USA) with three amplification cycles (Table 2.10).

Table 2.10. **PCR conditions for *Giardia duodenalis* detection**

Amplification cycles	Time	Temperature, °C
Pre-denaturation	3 minutes	94
Amplification (35 cycles)	30 seconds	94
	30 seconds	53
	60 seconds	72
Final extension	7 minutes	72

4. At the end of the nested PCR reaction, tubes were centrifuged for five seconds and vortexed.
5. Amplifications of 511 base pairs (bp) were visualized with capillary gel electrophoresis (QIAxel Advances, QIAGEN, Hilden, Germany).
6. Nested PCR products with 511 bp were subjected to Restriction Length Fragment Polymorphism (RLFP) analysis.

2.3.4. Restriction Length Fragment Polymorphism analysis to detect *Giardia duodenalis* assemblages

For RLFP, restriction endonuclease HaeIII (New England Biolabs, USA) was used to recognize the 511 bp PCR products and detect *G. duodenalis* assemblages. The oligonucleotide sequences that are recognized by the HaeIII are 5'…GC▼CC…3' and 3'…CC▲GG…5'. For RFLP, a mix with restriction endonuclease was prepared (Table 2.11), to which 10 µl DNA obtained by nested PCR was added. A total of 81 nested-PCR-positive samples (62 cattle, 16 dog, 2 red fox and 1 raccoon dog) were subjected to the RLFP analysis.

Table 2.11. **Enzymatic digestion mix for Restriction Length Fragment Polymorphism analysis of *Giardia duodenalis* assemblages**

Reagent	Volume, µl
ddH2O	7
10x rCutSmart™ Buffer (New England Biolabs, USA)	2

Reagent	Volume, µl
HaeIII (New England Biolabs, USA)	1

After the enzyme mix was mixed with the DNA, it was put in PCR cycler (ProFlex PCR system, Thermo Fisher Scientific, USA) for 3 hours at 37 °C. Afterward, the obtained PCR-digested fragments were run on capillary electrophoresis (QIAxel Advances, QIAGEN, Hilden, Germany) to visualize the results of the 511 bp digestion to determine the *G. duodenalis* assemblage (Table 2.12).

Table 2.12. ***Giardia duodenalis*** **assemblages based on the size (in base pairs) of the beta-giardin fragments after HaeIII endonuclease digestion** (Lalle et al., 2005)

Assemblage	Digestion Fragments (*bp*)
A	201, 150, 110, 50
B	150, 117, 110, 84, 26, 24
C	194, 150, 102, 50, 15
D	200, 194, 117
E	186, 150, 110, 26, 24, 15
F	186, 150, 110, 50, 15
G	194, 165, 102, 50

2.4. Questionnaires

2.4.1. Questionnaire about management information at cattle herds

An overall questionnaire **(**Appendix 2) was designed to acquire information about cattle herds, herd management practices, calf management, and the surrounding herd area. Before the sample collection, a written consent was obtained from owners, herd managers, and overseeing veterinarians to collect fecal samples and the associated data.

Interviews were conducted in Latvian, in person, on the day of the sampling, by interviewing either the herd owner, herd manager, or overseeing veterinarian. The questionnaire was either filled with the help of the researcher, or the physical questionnaire was given to the managing person on-site to fill out, while the sample collection was done and collected afterward. After completing the questionnaire, an individual identification number was assigned for each interviewed herd to ensure anonymity.

The questionnaire was organized into five main sections:

1. General herd information: details about the herd (herd size, location, management type).
2. Calf management: information on calving location, separation from the dam, colostrum intake, calf grouping, and calf diarrhea occurrences.
3. Walking areas and pastures: walking area, and pasture seasons.
4. Feed and herd management: pasture manure management, equipment cleaning, deworming, rodent control, personnel hygiene, and biosecurity.
5. Herd surroundings: the presence of wild and domestic animals and distances from nearby farms and water bodies.

2.4.2. Questionnaire about management information on domestic dogs and wild canids

A questionnaire in Latvian with an explanation (Appendix 3) and a consent form was provided for each dog (Appendix 4).

The questionnaire was organized in six main sections:

1. General information (age, breed, sex, living habitat).
2. Daily activities and their frequency (walks in the city, forest, meadow, park).
3. Information about diarrhea.
4. Information about deworming (medication used, frequency).
5. Feed (raw, commercial, game meat, home-cooked).
6. Contact with other animals (including livestock).

Together with the red fox and raccoon dog carcasses, a filled-out sample submission form containing the information about the animals' age (if recorded) and the hunting parish was submitted. Age was determined by the hunters based on the animals' dental wear.

2.5. Data analysis

2.5.1. Descriptive statistics

The data were analyzed using OpenEpi (Dean et al., 2015) and RStudio with R version 4.4.2. (https://www.r-project.org/). The Mid-p Exact method in OpenEpi was used to calculate 95% confidence intervals (CI) for the prevalence and proportions of *G. duodenalis* positive animals, assuming binomial distribution. To calculate statistical significance, the two-tailed Fisher's exact test was applied and $p < 0.05$ was considered statistically significant.

For the study population, means and median were calculated for the age of the animals, as well as for the number of sampled cattle per herd.

An animal was classified as infected with *G. duodenalis* if at least one cyst was detected in the analyzed samples. For cattle, a herd was considered positive if at least one cattle shed *G. duodenalis* cysts.

Prevalence (2.1.) was calculated as follows:

$$\left(\frac{A}{B}\right) * 100 = X, \qquad (2.1.)$$

where A – positive samples;
B – total number of analyzed samples;
X – prevalence, %.

The total cysts per gram (2.2.) were calculated according to Maddox-Hyttel et al., (2016) as follows:

$$C * 200 = x \qquad (2.2.)$$

where C – total count of cysts in 10 µl;
x = CPG.

The means and medians of CPG were calculated for age groups, herd sizes, and sexes for cattle. For domestic dogs, means and medians were calculated for the CPG for age groups, breeds, and sex. For red foxes and raccoon dogs the CPG were analyzed for ages.

To test for associations between categorical variables such as age groups, sexes, herd sizes, breeds, and *G. duodenalis* infection status, a Chi-square test (χ^2) was used. Differences in CPG between groups were analyzed using the Kruskal-Wallis H test, as CPG values were non-normally distributed. Normality of CPG in each group was assessed using the Shapiro-Wilk test with $p < 0.05$ indicating deviation from normal distribution. If Kruskal-Wallis H test indicated significant differences between compared groups, pairwise comparison was done using the Wilcoxon ran-sum test or Dunn's test with Bonferroni correction, to adjust for multiple testing. Pearson's correlation coefficient was calculated to assess the relationship between environmental factors and *G. duodenalis* prevalence.

For cattle, herds were categorized into four size groups (<150, 151–250, 251–500, and >500) after the data collection to allow a meaningful statistical comparison while maintaining a balanced distribution of herds across the groups.

For domestic dogs, age was categorized into four age groups: puppies (up to 2 years), adults (2 to 7 years old), seniors (8 to 11 years old), and geriatric dogs (above 12 years old), after the data collection, following the classification by Harvey (2021).

For red foxes and raccoon dogs, age was categorized into defined age ranges. Red foxes were grouped in five age categories: 1–1.5 years, 2–2.5 years, 3–3.5 years, 4, and 5 years, but raccoon dogs in four groups – 1–1.5 years, 2–2.5 years, 3–3.5 years, and 4.5 years old.

2.5.2. Mapping of sampled animals and *Giardia duodenalis* prevalence, with the proximity from surface waters

For the geographic distribution of sampled and positive animal visualization, R and Rstudio version 4.2.2. (https://www.r-project.org/) with "sf", "ggplot2" and "rnaturalearth" packages were used. For each sampled animal, geographic coordinates (latitude and longitude) were available. Separate maps were generated for each species group to display positive and negative animals and *G. duodenalis* assemblage distribution.

To map out the assemblage A-positive animal proximity to waterbodies (<500m, 500-1000m, and 1000-1500m), cartographic outputs were produced using the Latvia TM projected coordinate reference system (EPSG:3059) (Latvian Geospatial Information Agency, 2022). Surface water features (rivers, lakes, ponds) were obtained from the Humanitarian OpenStreetMap Team (HOTOSM) dataset for Latvia (HOTOSM, 2023).

2.5.3. Risk and protective factor analysis in cattle, domestic dogs, red foxes, and raccoon dogs

For cattle, potential risk and protective factors associated with *G. duodenalis*, were analyzed using generalized linear mixed models (GLMMs), fitted using maximum likelihood (Laplace approximation) using R and RStudio version 4.2.2. (https://www.r-project.org/). The "lme4" package (Bates et al., 2014) was used to fit the models by applying the "glmer" function with a binomial family, including herd identification numbers (FarmID) as a random variable to account for the clustering within the herds. All factors were assessed at the herd level, except for cattle age, sex, breed, and presence of diarrhea (present/not present), which were assessed individually. Age was expected to be an important effect-modifying factor, therefore was included in all models. Only cattle younger than 1500 days were included in the GLMM analysis, as the number of *Giardia*-positive cases among older cattle was too low, and models including them failed to converge or yielded unstable estimates. In addition to the GLMMs, a GLM with a binomial family was used to assess regional differences in *G. duodenalis* prevalence in cattle, including age as an effect-modifying factor.

To analyze factors associated with *G. duodenalis* in domestic dogs, red foxes, and raccoon dogs, generalized linear modeling (GLM) was performed using a binomial family. Forward and backward stepwise selection was applied, based on the lowest Akaike information criterion (AIC) values.

For both animal groups, variables that showed significance ($0.05 \leq p < 0.1$) in the initial models were retained for the final GLMM and GLM analysis. The final models were optimized through stepwise variable elimination, ensuring that this process did not increase the Akaike information criterion (AIC). The multicollinearity was checked using the "vif" function from the "car" package and included factors that appeared significant in the single-factor analyses and also made biological sense. Model fit was evaluated using Tjur's coefficient of discrimination (Tjur's R^2), calculated with the "performance" package.

2.5.4. Calculation of adjusted *Giardia duodenalis* cyst shedding rates in cattle and canids

To estimate the potential environmental contamination from each animal species with *G. duodenalis*, adjusted daily cyst-shedding values were calculated. This was done by multiplying the median CPG by the average fecal mass per defecation (in grams) and the average number of defecations per day. Median CPG values were used from *G. duodenalis*-positive animals to reduce the influence of extreme outliers and better reflect central tendency of cyst excretion. The average weight of feces and defecation frequency for each species is shown in Table 2.13.

Table 2.13. **Average fecal mass per defecation and average defecations per day in cattle, domestic dogs and red foxes**

Animal species	Average fecal weight (g) per defecation	Average defecations per day	Reference
Cattle	1900	14	Aland et al., 2002
Domestic dogs	150	2	Wright et al., 2009
Red foxes	120	6.4	Ferreras et al., 2019

Because no specific data was available for raccoon dogs, it was assumed to be the same as red foxes, due to ecological similarities. The fecal output and defecation frequency were assumed to be consistent across age groups and breeds within each species to emphasize the overall environmental contamination rather than individual-level variation.

3. RESULTS

3.1. Prevalence, cyst load, genetic diversity, animal-level and herd-level factors potentially associated with *Giardia duodenalis* in cattle in Latvia

At least one herd was positive for *G. duodenalis* from each region of Latvia. The most positive herds were from Kurzeme (9/9; 100%, 95% CI: 65.5–100), followed by Zemgale (11/12; 91.7%, 62.5–100), Latgale (2/3; 66.7%, 95% CI: 20.2–94.4), and Vidzeme (5/8; 62.5%; 30.4–86.5). The locations of the positive cattle herds are shown in Figure 3.1.

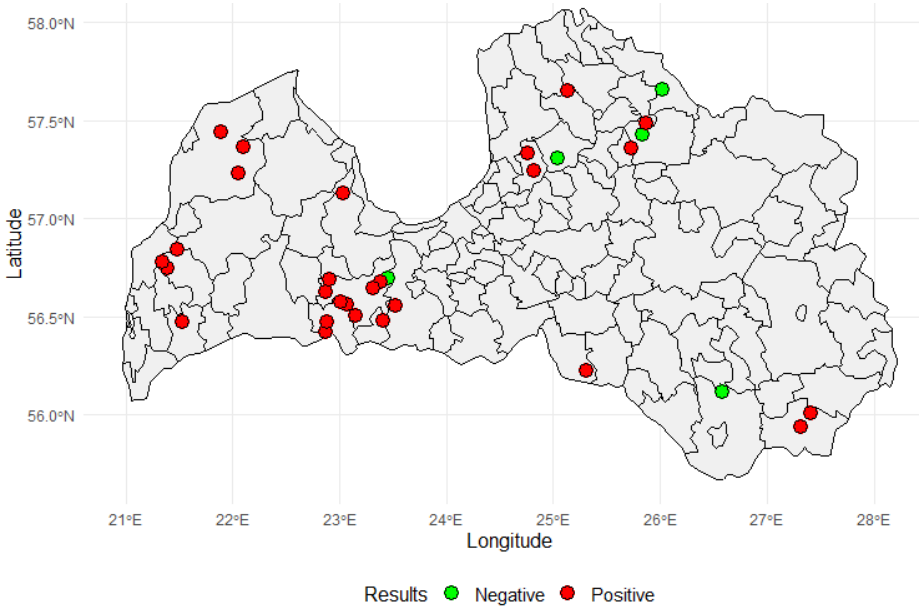

Figure 3.1. **Locations of the *Giardia duodenalis* positive and negative cattle herds**

G. duodenalis prevalence in herds reached 84.4% (95% CI: 67.8–93.6) with the 100% prevalence observed in herds with 251–500 and above 501 cattle per herd (95% CI: 59.6–100.0; 62.8–100.0, respectively), followed by 90.0% (95% CI: 53.1–100.0) in herds with less than 150 cattle and the lowest prevalence of 42.9% (95% CI: 15.7–75.0) was observed in herds with 151–250 cattle. In herds with 151-250 cattle, the highest mean CPG was observed (Table 3.1).

Table 3.1. **Number of *Giardia duodenalis* positive cattle herds and cyst load in cattle herds of different size**

Cattle herd size	Number of sampled herds	Positive herds	Mean CPG*	Min–Max CPG
<150	10	9[a]	2923[b]	200–24,200
151–250	7	3	16,689	200–56,600
251–500	7	7	6867	200–62,600
>501	8	8	5102	200–22,000
Total	32	27	6121	200–62,600

*CPG: Cysts per gram; [a] no statistical significance was observed between *Giardia*-positive herds and herd size ($p > 0.05$); [b] no statistical significance was observed between herd size and shed CPG ($p > 0.05$).

No statistical significance was observed between *Giardia*-positive cattle and herd size or cyst load ($p = 0.1$, $p = 0.1$, respectively) (Table 3.1).

The overall *G. duodenalis* prevalence in cattle was 8.4% (95% CI: 6.8–10.3). Regionally, the highest number of *Giardia*-positive cattle were from Kurzeme (34/283; 95% CI: 3.3–4.5), followed by Latgale (10/91; 95% CI: 5.1–8.2), Zemgale (28/355; 95% CI: 2.0–3.3), and Vidzeme (10/244; 95% CI: 2.4–3.3) (Figure 3.2).

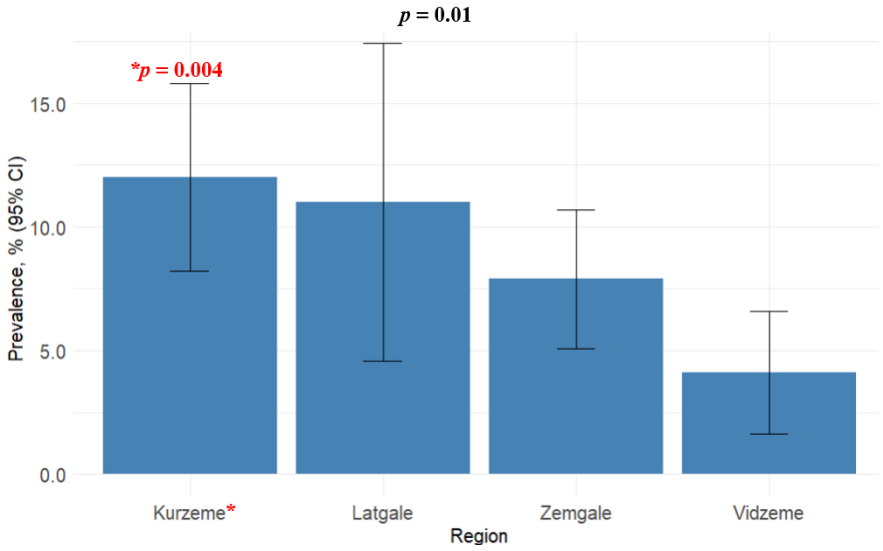

Figure 3.2. ***Giardia duodenalis* prevalence in individual cattle in regions of Latvia**

A significant difference was observed in *G. duodenalis* prevalence across regions ($\chi^2 = 12.1$, $p = 0.01$), and the highest prevalence was observed in Kurzeme ($p = 0.004$), compared to other regions.

The age of the sampled cattle ranged from one day to 4433 days (approximately 12 years), with a mean of 720 days and a median of 263 days. The number of cattle sampled per herd ranged from 10 to 45 (mean 30.4; median 34). The complete information about the gathered cattle is provided in Appendices 5 and 6.

The highest *G. duodenalis* prevalence was observed in the 0–3-month-old cattle group, followed by the 4–24-month-old and above 24-month-old cattle. The highest mean CPG and the highest proportion of diarrhea in the *Giardia*-positive cattle were also observed in the 0–3-month-old cattle group (Table 3.2).

Table 3.2. ***Giardia duodenalis* prevalence, diarrhea in cyst-shedding cattle, and cyst load per different cattle age groups**

Age groups	Total cattle analyzed	Positive cattle	Prevalence (95% CI)*	Positive cattle with diarrhea (95% CI)	Mean CPG** (Min–Max)
0–3 months	324	53	16.4[a] (12.7–20.8)	32.1 (21.1–45.5)	8109[b] (200–62,600)
4–24 months	281	19	6.8 (4.3–10.4)	15.8 (4.7–38.4)	1284 (200–9600)
>24 months	368	10	2.3 (1.4–5.0)	0.0	1780 (200–15,800)

*CI: confidence interval; **CPG: cysts per gram; [a]significant association was observed between age groups and *G. duodenalis* prevalence ($p < 0.05$); [b]no significant association was observed between age groups and CPG ($p > 0.05$).

A significant association was observed between age groups and *Giardia* prevalence χ^2 = 43.2, $p < 0.05$ (Table 3.2). No significant association was observed between cattle age groups and shed *G. duodenalis* CPG ($p > 0.05$) (Table 3.2).

The mean *G. duodenalis* CPG excreted among the cattle in Latvia was 5756 (min: 200; max: 62,600; median: 600). The youngest *Giardia*-positive calf was six days old with 200 CPG, but the oldest cattle was 7.2 years old with 15,800 CPG. The highest *Giardia* CPG shedding frequency with the broadest CPG range was also observed in cattle under three months old (Figure 3.3).

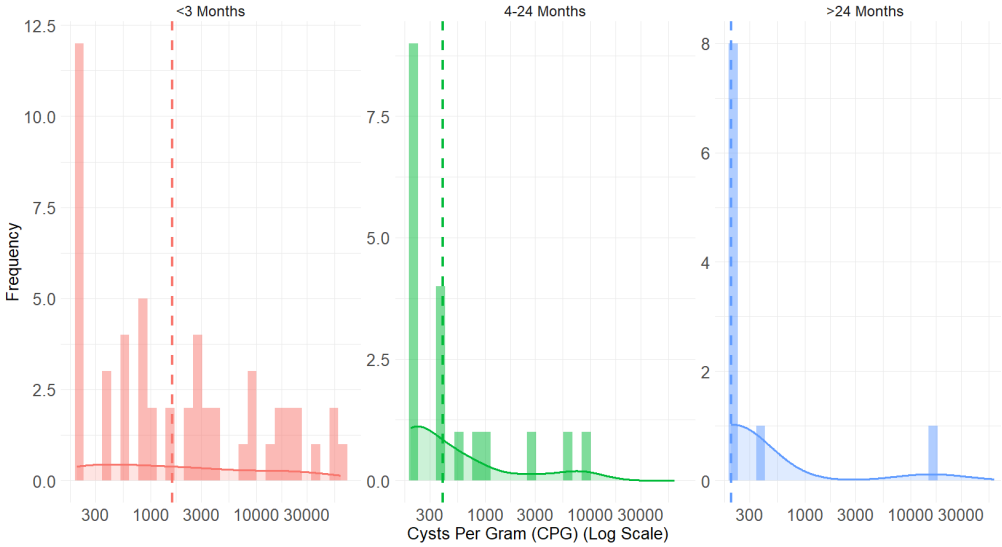

Figure 3.3. **Age-specific frequency distribution of *Giardia duodenalis* cysts in positive cattle.** The dashed line indicates the median CPG

The amount of *G. duodenalis* cysts varied significantly across cattle age groups (p = 0.0005 with the largest differences observed between calves under 3 months old and the other two groups: 4–24 months and >24 months old (Figure 3.4).

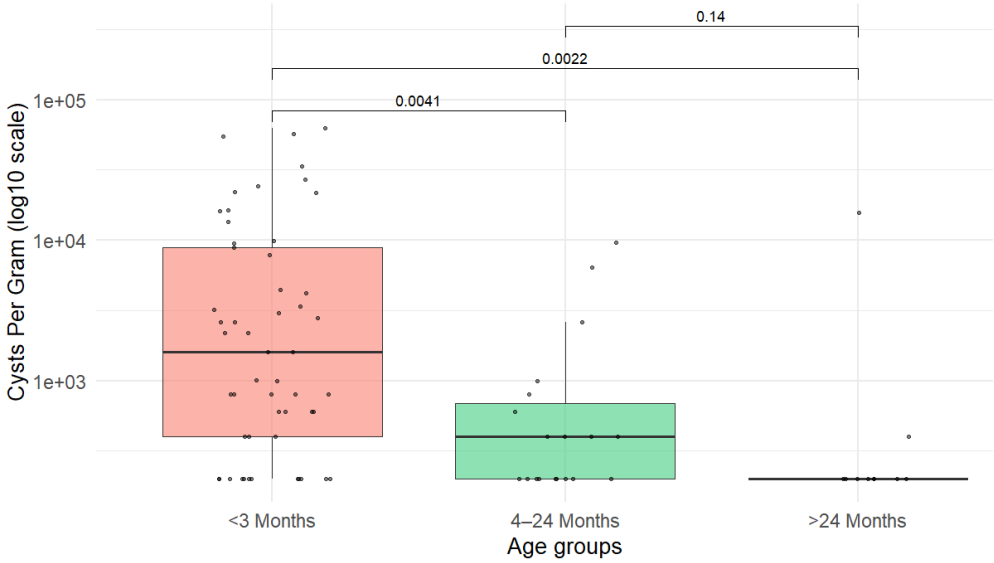

Figure 3.4. **Comparison of *Giardia duodenalis* cyst load across age groups.** Pairwise Wilcoxon test applied. Black, horizontal line within each box represents the median CPG per group

Among individual cattle, the highest *G. duodenalis* prevalence and highest proportion of *Giardia*-positive animals with diarrhea were observed in herds of 251–500 cattle, followed by cattle from less than 150 animals per herd. The highest mean and median CPG were detected in cattle from herds of 151–250 animals, followed by cattle from herds with 251–500 animals, while the lowest CPG was observed in cattle from herds with fewer than 150 animals (Table 3.3).

Table 3.3. ***Giardia duodenalis*** **prevalence, diarrhea in cyst-shedding cattle, and cyst load at cattle herds of different size**

Herd size, number of cattle	Total cattle analyzed	Positive cattle	Prevalence (95% CI)*	Positive cattle with diarrhea (95% CI)	Mean CPG** (Min–Max)	Median CPG
<150	259	26	10.0[a] (6.9–14.3)	23.1 (10.7–42.4)	2938[b] (200–24,200)	600
151–250	207	10	4.8 (2.5–8.8)	10.0 (0.0–42.6)	14,640 (200–56,600)	11,700
251–500	219	23	10.5 (7.0–15.3)	47.8 (29.2–67.0)	6191 (200–62,600)	1000
>500	288	23	7.9 (5.3–11.7)	4.5 (1.2–27.9)	4643 (200–55,000)	400

*CI: confidence interval; **CPG: cysts per gram; [a] difference in *G. duodenalis* prevalence and herd size was not significant ($p > 0.05$); [b] differences in *G. duodenalis* shedding between cattle from different herd sizes were not significant ($p > 0.05$).

Even though the *G. duodenalis* prevalence varied between herd sizes, there was no statistical significance between the herd size and prevalence ($\chi^2 = 5.7$, $p = 0.1$), and between herd size and cyst load ($p = 0.2$) (Table 3.3).

Between sexes, male cattle had 1.4 times higher *G. duodenalis* prevalence, compared to female cattle. In male cattle, higher proportion of diarrhea in *G. duodenalis*-positive cattle was also observed, compared to female cattle. Higher median and mean CPG were also observed in male cattle than in female cattle (Table 3.4).

Table 3.4. **Prevalence of *Giardia duodenalis* and cyst load in cattle of different sexes**

Sex	Total no. of cattle analyzed	Prevalence (95% CI)*	Positive cattle with diarrhea (95% CI)	Mean CPG** (Min–Max)	Median CPG
Female	853	8.0[a] (6.3–9.9)	14.5 (8.1–24.3)	5279[b] (200–56,600)	600
Male	120	11.7 (6.7–11.8)	18.4 (9.7–31.6)	8071 (200–62,600)	2200

*CI: confidence interval; **CPG: cysts per gram; [a] differences between *G. duodenalis* prevalence between cattle sexes was not significant ($p > 0.05$); [b] differences in *G. duodenalis* shedding between cattle sexes were not significant ($p > 0.05$)

No statistical association was observed between the *G. duodenalis* prevalence and sex ($\chi^2 = 1.4$, $p = 2.3$), or cyst shedding intensity ($p = 0.07$) (Table 3.4).

When further dividing cattle age groups according to their sexes, the highest *G. duodenalis* prevalence was observed in 0–3-month-old male cattle, compared to female cattle

in the same age group (Table 3.5) and in in the 4–24-month-old cattle group. The highest mean CPG was observed in the 0–3-month-old male cattle, compared to the female cattle in the same age group, but in the 4–24-month-old cattle group, female cattle had higher mean CPG, than male cattle (Table 3.5).

Table 3.5. **Prevalence of *Giardia duodenalis* by sex, diarrhea in cyst-shedding cattle, and cyst load in different cattle age groups**

Sex	**Female**			**Male**		
Age group	**Total no. analyzed/ Prevalence (95% CI)***	**Positive cattle with diarrhea (95% CI)**	**Mean CPG** (Min–Max)**	**Total no. analyzed/ Prevalence (95% CI)**	**Positive cattle with diarrhea (95% CI)**	**Mean CPG (Min–Max)**
0–3 months	219/18.3[a] (13.7–23.5)	12.7 (6.3–23.4)	7930[c] (200–56,600)	105/12.4[a] (7.5–20.2)	18.4 (9.7–31.6)	8661[c] (200–62,600)
4–24 months	264/7.3[b] (4.3–10.6)	23.1 (7.5–50.9)	1333 (200–9600)	12/8.3[b] (0.0–37.5)	0.0	400 (400–400)
>24 months	370/2.7 (1.4–5.0)	0.0	1780 (200–15,800)	0/0.0	0.0	NA***

*CI: confidence interval; CPG: cysts per gram; ***NA: not available; [a] no statistical differences were observed between *G. duodenalis* prevalence in the 0–3-month-old cattle and sexes ($p > 0.05$); [b] no statistical differences were observed between *G. duodenalis* prevalence in the 4–24-month-old cattle and sexes ($p > 0.05$); [c] no statistical significance was observed between CPG and sex in the 0–3-month-old cattle ($p > 0.05$).

Although the highest prevalence and the mean cyst load was observed in male cattle from the 0–3-month-old group, no statistical significances were observed ($p = 0.2$). Similar to the 0–3-month-old group, there was no significant difference in the prevalence of the pathogen in the 4–24-month-old group for both sexes ($p = 0.6$) (Table 3.5).

The highest prevalence of *G. duodenalis* was observed in DS cattle breeds, followed by XX, LB, HS, and HM, but all examined Limousine cattle breeds were negative (Table 3.6). The highest mean CPG was observed in the HS cattle breed group, followed by DS and HM (Table 3.6).

Table 3.6. ***Giardia duodenalis* prevalence and cyst load in cattle breeds**

Breed	**Total no. of samples**	**Positive cattle**	**Prevalence, %**	**95% CI***	**Mean CPG****	**Min–Max CPG**
DS	19	3	18.8[a]	5.2–37.6	7400[b]	2600–16,400
XX	14	2	14.3	4.0–39.9	400	200–600
LB	71	10	14.1	7.8–24.0	3420	200–21,600
HS	122	14	11.5	7.0–18.3	11,728	200–56,600
Other	13	1	7.7	1.4–33.3	200	200–200

Breed	Total no. of samples	Positive cattle	Prevalence, %	95% CI*	Mean CPG**	Min–Max CPG
HM	699	52	7.4	5.7-9.6	4815	200–62,600
LI	35	0	0	NA***	NA	NA

*CI: confidence interval; CPG: cysts per gram; HM: Holstein Friesian; HS: Holstein Red and White; LB: Latvian brown; LI: Limousin; DS: Danish Red; XX: mixed breed ***NA: not available; [a] no statistical differences were observed between *G. duodenalis* prevalence and cattle breeds ($p > 0.05$); [b] no statistical significance was observed between CPG and cattle breed ($p > 0.05$).

No statistical significance was observed between cattle breeds and *G. duodenalis* prevalence ($p = 0.3$); there was also no statistical significance observed between breeds and *G. duodenalis* cyst shedding ($p = 0.3$) (Table 3.6).

The monthly prevalence of *G. duodenalis* in cattle was from 0% in August to 13.1% in March 13.1% (Figure 3.5).

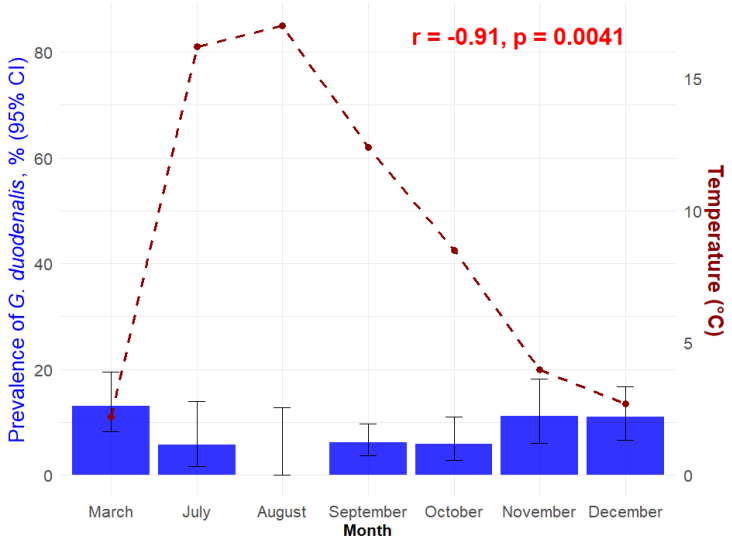

Figure 3.5. **Monthly prevalence of *Giardia duodenalis* in cattle and average monthly temperatures in 2019–2020**

A strong negative association was observed between average monthly temperature and *G. duodenalis* prevalence (Pearson's r = -0.9, $p = 0.004$; Figure 3.5). No significant association was found between monthly precipitation (r = -0.2, $p = 0.7$) or humidity (r = 0.6, $p = 0.16$).

To determine the *G. duodenalis* assemblages, all microscopically positive cattle samples were submitted for analysis, and of the 82 fecal samples that initially tested positive for *G. duodenalis*, the DNA amplification was successful in 62 samples (75.6%, 95% CI: 65.2–83.7).

Altogether, three *G. duodenalis* assemblages were identified. *G. duodenalis* assemblage E was found in 20 herds (74.1%, 95% CI: 55.1–87.1), mix of *G. duodenalis* assemblages A and E in 4 herds (14.8%, 95% CI: 5.3–33.1), but *G. duodenalis* assemblage A in 3 herds (12.0%, 95% CI: 3.3–30.8). In Kurzeme, *G. duodenalis* assemblage E was the most frequently detected assemblage in cattle herds, followed by *G. duodenalis* assemblage A and the mixed *G. duodenalis* assemblages A/E detected in one herd each (Table 3.7). In Latgale, both assemblage E and mixed assemblage A /E were detected, each in one herd. In Vidzeme, *G. duodenalis* assemblage E was also predominant among cattle herds, while assemblage A was detected in a single herd. In Zemgale, assemblage E was also the most detected *G. duodenalis* assemblage

among cattle herds, followed by the mixed assemblage A/E, with one herd testing positive for *G. duodenalis* assemblage A. The highest cyst load was observed in herds where assemblage E was detected, particularly in Kurzeme and Zemgale (Table 3.7).

Table 3.7. ***Giardia duodenalis*** **assemblage distribution in cattle among regions of Latvia**

Region	***G. duodenalis* assemblage**	**Number of herds with assemblage**	**Total examined herds per region/positive herds**	**Prevalence (95% CI)***	**Mean CPG** (Min–Max CPG)**
Kurzeme	A	1	9/9	11.1[a] (2.0–43.5)	600 (200–800)
	A/E	1	9/9	11.1 (2.0–43.5)	667 (200–2600)
	E	7	9/9	77.8 (45.3–93.7)	7771 (200–56,600)
Latgale	A/E	1	3/2	33.3 (6.1-–79.2)	8657 (200–5000)
	E	1	3/2	33.3 (6.1–79.2)	1333 (200–3000)
Vidzeme	A	1	8/5	12.5 (2.2–47.1)	200 (200–200)
	E	4	8/5	50.0 (21.5–78.5)	1500 (200–9600)
Zemgale	A	1	12/11	8.3 (1.5–35.4)	4900 (200–9600)
	A/E	2	12/11	16.7 (4.7–44.8)	5233 (200–33,400)
	E	8	12/11	66.7 (39.1–86.2)	9756 (200-62,600)

*CI: confidence interval; CPG: cysts per gram; [a] no statistical differences were observed between *G. duodenalis* prevalence and cattle breeds ($p > 0.05$).

There was no significant association between regions and *G. duodenalis* assemblage ($\chi^2 = 3.4$; $p = 0.8$) (Table 3.7). The distribution of the *G. duodenalis* assemblages is shown in Figure 3.6.

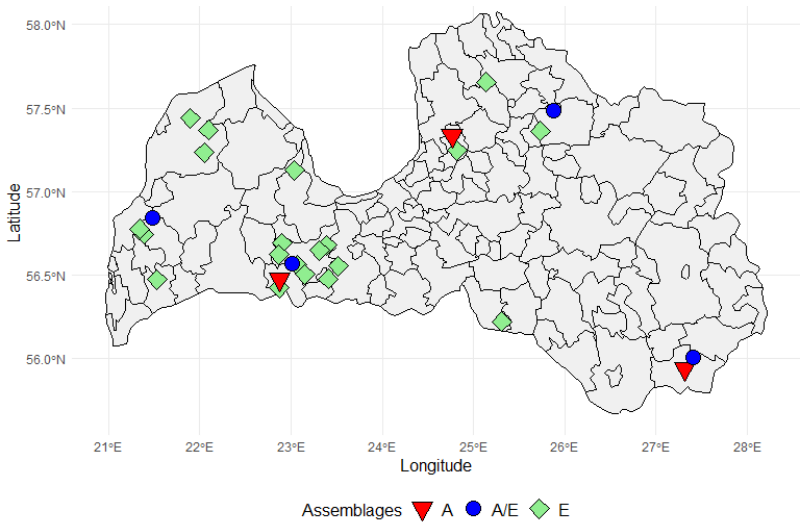

Figure 3.6. ***Giardia duodenalis*** **assemblage distribution across cattle herds in Latvia**

The zoonotic *G. duodenalis* assemblage A was detected in seven cattle (11.3%, 95% CI: 5.3–21.8), but assemblage E was detected in 55 cattle (88.7%, 95% CI: 78.2–94.). Assemblage A was identified in five cattle from the 4–24-month-old age group, and in two cattle from the >24-month age group, while assemblage E was prevalent across all age groups. Cattle from the 4–24-month-old age group, which were positive for assemblage A, shed the highest median number of cysts, compared to the cattle older than 24 months. Calves under three months old infected with *G. duodenalis* assemblage E excreted the highest number of cysts compared to the other two age groups. Calves from 0–3-month-old were positive for *G. duodenalis* assemblage E across the majority of the positive herds (Table 3.8).

Table 3.8. ***Giardia duodenalis*** **assemblages per age group, cyst load, and diarrhea in the cyst-shedding cattle**

Assemblage	Age group	No. positive cattle	Prevalence, 95% CI*	Median CPG** (Min–Max)	Diarrhea in positive cattle (95% CI)	No. of herds with assemblage detected
A	4–24 months	5	8.1 (3.5–17.5)	8000 (200–15,800)	0.0	4
	>24 months	2	3.2 (0.9–11.0)	600 (200–9600)	0.0	2
E	0–3 months	42	67.7 (55.5–78.0)	2400 (200–56,600)	28.6[a] (0.1–49.2)	23
	4–24 months	8	12.9 (6.7–23.4)	300 (200–6400)	12.5 (17.1–49.7)	7
	> 24 months	5	8.1 (3.5–17.5)	240 (200–400)	0.0	4

*CI: confidence interval; **CPG: cysts per gram; [a] no statistical differences were observed between *G. duodenalis* prevalence and cattle breeds ($p > 0.05$).

Although diarrhea was observed in calves, which were positive for assemblage E, differences were not significant ($p > 0.05$).

Potential individual and herd-level factors associated with *G. duodenalis* were analyzed by bivariable generalized linear mixed modeling, including age in days ("Age") as a fixed effect and herd identification number ("HerdID") as a random effects variable. In the initial single-factor models, seven variables were significantly associated (OR < 1; $p < 0.05$) with reduced odds of *G. duodenalis* infection (Table 3.9).

Table 3.9. **Initial fixed effects in general linear mixed models to determine potential protective factors associated with *Giardia duodenalis* in cattle in Latvia**

Model (AIC, Model Fit)	Variable	Odds Ratio (95% CI)	*z*-value	*p-value*
1 (527.4)	(intercept)	0.1 (0.0–0.1)	-16.5	< 0.01
	Age	0.4 (0.3–0.6)	-5.6	< 0.01
2. (527.1)	Age	0.4 (0.3–0.7)	-5.7	< 0.01

Model (AIC, Model Fit)	Variable	Odds Ratio (95% CI)	*z*-value	*p-value*
	Pasture season begins: April (ref)			
	Pasture season begins: May	0.4 (0.1–1.1)	-1.7	0.08
	No pasture	0.3 (0.1–0.9)	-2.2	0.03
3. (527.3)	(Intercept)	0.1 (0.07–0.2)	-9.6	< 0.01
	Age	0.4 (0.3–0.6)	-5.5	< 0.01
	Manure in closed space (ref)			
	Manure in open space	0.6 (0.3–1.0)	-2.0	0.04
	Manure kept in a pile	0.6 (0.3–1.2)	-1.4	0.16
4. (526.0)	(Intercept)	0.1 (0.07–0.14)	-12.2	< 0.01
	Age	0.4 (0.3–0.6)	-5.6	< 0.01
	Rodent control: Cat (ref)			
	Rodent control: No control	0.7 (0.2–3.2)	-0.4	0.66
	Rodent control: Poison	0.6 (0.3–0.9)	-2.4	0.02
5. (525.9)	(Intercept)	0.1 (0.06–0.14)	-11.8	< 0.01
	Age	0.4 (0.3–0.6)	-5.6	< 0.01
	Change of shoes for visitors: Yes	0.6 (0.4–1.0)	-1.9	0.05
6. (525.3)	(Intercept)	0.1 (0.07–0.16)	-10.3	< 0.01
	Age	0.4 (0.3–0.6)	-5.7	< 0.01
	Pet animals: Cat	0.6 (0.4–1.0)	-2.1	0.03

In addition to the protective factors, six variables were associated with increased odds ((OR) > 1; $p < 0.05$) with *G. duodenalis* infection in cattle (Table 3.10).

Table 3.10. **Initial fixed effects in general linear mixed models to determine potential risk factors associated with *Giardia duodenalis* in cattle in Latvia**

Model (AIC, Model Fit)	Variable	Odds Ratio (95% CI)	*z*-value	*p-value*
1 (524.8)	(Intercept)	0.04 (0.0–0.1)	-11.2	< 0.01
	Age	0.1 (0.07–0.4)	-3.9	< 0.01
	Can animal leave herd: No (ref)			
	Can animal leave herd: Yes	1.8 (1.0–3.2)	2.1	0.04
2. (524.9)	(Intercept)	0.06 (0.04–0.1)	-15.7	< 0.01
	Age	0.4 (0.3–0.6)	-5.5	< 0.01
	Calf isolation with diarrhea: No (ref)			
	Calf isolation with diarrhea: Yes	1.7 (1.0–2.7)	2.2	0.03
3. (524.4)	(Intercept)	0.05 (0.04–0.1)	-14.6	< 0.01
	Age	0.4 (0.3–0.6)	-5.7	< 0.01
	Walking area: No (ref)			

Model (AIC, Model Fit)	Variable	Odds Ratio (95% CI)	*z*-value	*p-value*
	Walking area: Yes	1.7 (1.1–2.7)	2.3	0.02
4. (524.8)	(Intercept)	0.06 (0.03–0.1)	-11.4	< 0.01
	Age	0.4 (0.3–0.6)	-5.6	< 0.01
	Drinking water in pasture: No (ref)			
	Drinking water in pasture: Yes	1.9 (1.1–3.6)	2.2	0.03
	No pasture	1.0 (0.6–1.9)	0.1	0.90
5. (526.8)	(Intercept)	0.06 (0.05–0.09)	-16.7	< 0.01
	Age	0.4 (0.3–0.6)	-5.6	< 0.01
	cattle can access surface water in pasture: No (ref)	0.4 (0.3–0.6)	-5.6	< 0.01
	cattle can access surface water in the pasture: Yes	1.6 (0.9–2.8)	1.7	0.09
6. (525.9)	(Intercept)	0.04 (0.01–0.1)	-6.2	< 0.01
	Age	0.4 (0.3–0.6)	-5.6	< 0.01
	Manure treatment: Fermentation (ref)			
	Manure treatment: Lagoon	2.7 (0.9–8.1)	1.8	0.07
	Manure treatment: No treatment	1.6 (0.6–4.7)	0.8	0.37

Calf isolation with diarrhea, which showed a y significant ($p < 0.05$) association with *G. duodenalis* infection (Table 3.10), was excluded from further analysis because it could be a consequence of *G. duodenalis* or other infectious disease rather than risk.

For the final model, all variables that were either statistically significant ($p < 0.05$) or showed a trend toward significance ($p \leq 0.1$) in the bivariable models were included in the multivariable analysis. A stepwise backwards selection approach was used, retaining only those variables whose exclusion would not increase the model's AIC. The final model of the final protective and risk factors is shown in Table 3.11.

Table 3.11. **Final in general linear mixed model for the potential for risk and protective factors associated with *Giardia duodenalis* in cattle in Latvia**

Model (AIC Model Fit)	Predictors	Odds Ratios (95% CI)	*z*-value	*p - value*
Final (521.2)	(Intercept)	0.2 (0.1–1.0)	-2.0	0.04
	Age	0.4 (0.3–0.6)	-5.7	< 0.001
	Can animals leave herd: Yes	2.2 (1.1–4.7)	2.2	0.03
	Pasture season beginning: May	0.2 (0.1–0.8)	-2.4	0.02
	No pastures	0.3 (0.1–0.9)	-2.2	0.03
	Manure kept in open pit	0.5 (0.3–0.9)	-2.3	0.02
	Manure kept in pile	0.3 (0.1–0.7)	-2.7	0.01

In the final model, age remained a strong protective factor and was significantly associated with a reduced likelihood of *G. duodenalis* infection. The final model showed no signs of multicollinearity (VIF < 1.2) and explained 13% (Tjur's R^2) of the probability of having *G. duodenalis* infection.

In summary, *Giardia duodenalis* prevalence in cattle in Latvia was 8.4% (82/973), shedding an average of 5756 CPG of feces. The highest prevalence of the pathogen of 16.4%

(53/324) was observed in the 0–3-month-old cattle age group, followed by 6.8% (19/281) in the 4–24-month-old and 2.7% (10/368) in the > 24-month-old age groups. Significantly higher *Giardia* cyst load was shed by cattle from the 0–3-month-old age group (p = 0.0005). The highest proportion of diarrhea was observed in the 0-3-month-old cattle age group; but no statistical significance was observed between diarrhea and the presence of *G. duodenalis* (p > 0.05). Herd prevalence was 84.4% (27/32) with the highest prevalence of 100% in herds with 251–500 (7/7) and more than 500 cattle (8/8), and 90.0% (9/10) in the herds with less than 150 cattle, but the lowest prevalence was observed in herds with 151–250 cattle (42.9%, 3/7). In individual cattle, *G. duodenalis* assemblage A was detected in 11.3% (7/62), but assemblage E was detected in 88.7% (55/62) of the positive cattle. *G. duodenalis* assemblage A was identified in five cattle from the 4–24-month-old age group (71.4%) and two cattle from the >24-month age group (28.6%). *G. duodenalis* assemblage E was detected in 76.4% (42/55) of the calves from 0–3 months old age group, followed by 14.5% (8/55) in the 4–24-month-old age group, and 9.1% (5/55) in the >24-month-old age group. One risk (ability to leave the herd premises) and five protective factors (age, pasture season beginning in May or no pastures; manure kept in open pit or pile) appeared to be associated with *G. duodenalis* infection in cattle in Latvia.

3.2. Prevalence, cyst load, genetic diversity, animal-level and housing factors potentially associated with *Giardia duodenalis* in domestic dogs in Latvia

Overall, *G. duodenalis* prevalence in domestic dogs was 10.7% (95% CI: 7.9–14.3), with the highest prevalence of 13.0% observed in Latgale (3/23; 95% CI: 4.5–32.1), followed by 12.1% in Vidzeme (25/206; 95% CI: 8.4–17.3), 8.8% in Kurzeme (5/57; 95% CI: 3.8–18.9), and 8.0% in Zemgale (7/87; 95% CI: 4.0–15.7) (Figure 3.7).

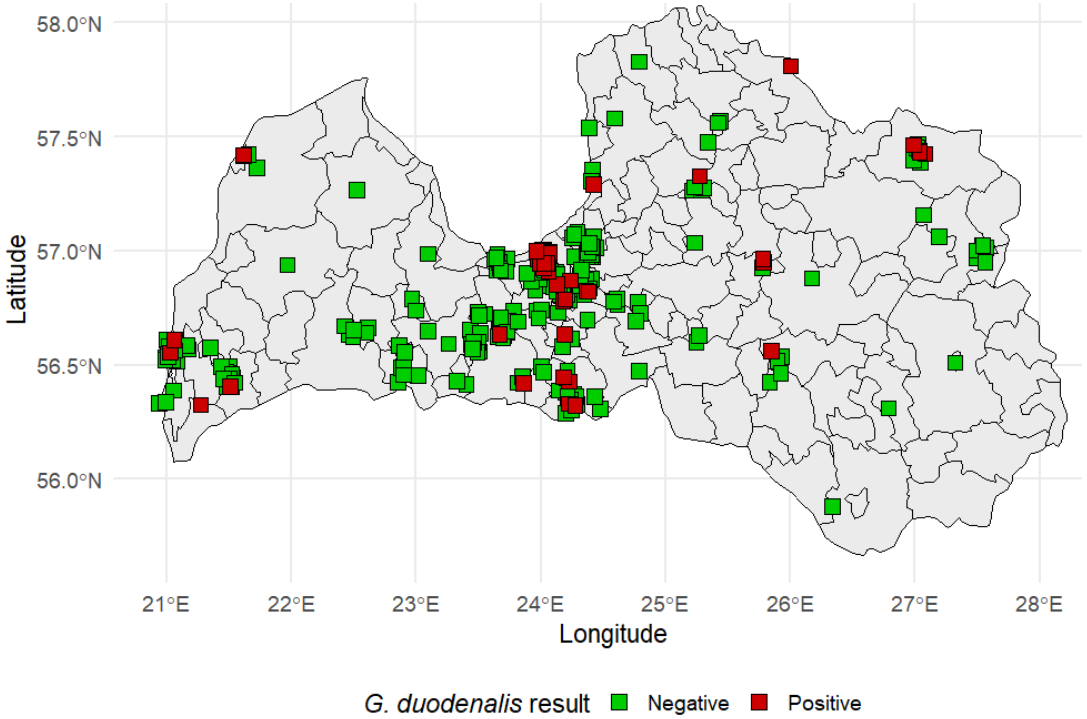

Figure 3.7. **Locations of *Giardia duodenalis*-positive and negative domestic dogs**

No statistical significance was observed between the prevalence of *G. duodenalis* in regions (p = 0.7). The data collected about domestic dogs is provided in Appendix 7.

The highest *G. duodenalis* prevalence was observed in the puppy age group, followed by senior dogs, and adult dogs, and the lowest prevalence was observed in the geriatric dog group, where only one dog tested positive for *G. duodenalis* (Table 3.12).

Table 3.12. **The overall prevalence and cyst load of *Giardia duodenalis* in domestic dogs of different ages**

Age group	No. of positive dogs	Total no. of dogs tested	Prevalence, % (95% CI)*
Puppies	12	65	18.5[a] (10.7–29.7)
Adults	18	193	9.3 (5.9–14.3)
Seniors	9	96	9.4 (4.8–17.1)
Geriatric	1	19	5.3 (0.1–26.5)
Total	40	373	10.7 (8.0–14.3)

*CI: confidence interval; [a] no statistical differences were observed between *G. duodenalis* prevalence and age group ($p > 0.05$).

There was no significant difference between the age groups and the *G. duodenalis* prevalence ($\chi^2 = 5.2$; $p = 0.2$; Table 3.12), and age groups and shed CPG ($p = 0.1$) (Figure 3.8).

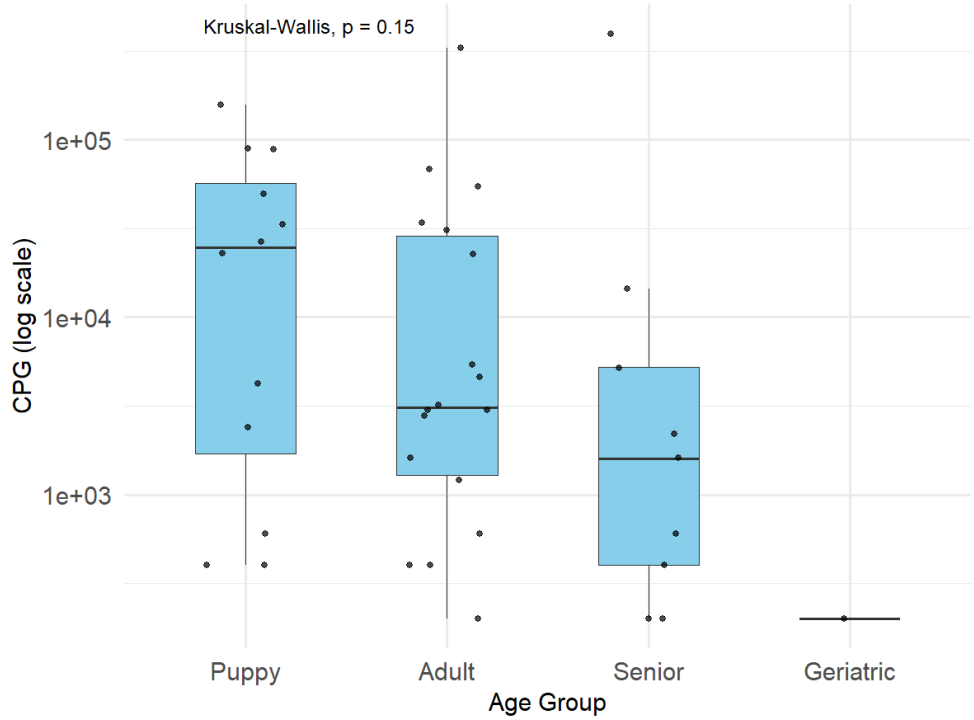

Figure 3.8. **Age-related variation in cyst load among *Giardia duodenalis*-positive domestic dogs**

Between purebred and mixed breed dogs, the highest prevalence was observed in mixed breed dogs, but the cyst load was similar (Table 3.13). In the purebred dog group, the highest *G. duodenalis* prevalence was observed in puppies, followed by adult and senior dogs, but in the mixed breed dogs, the highest *G. duodenalis* prevalence was also observed in the puppy group. The highest number of cyst load was observed in all age groups in the purebred dogs, compared to the mixed dog group (Table 3.13).

Table 3.13. ***Giardia duodenalis*** **prevalence and cyst load in purebred and mixed breed dogs**

Breed	Age group	Positive dogs	Total no. of dogs tested	Prevalence, % (95% CI)*	Mean CPG**	Min–Max CPG
Purebred	Puppies	10	49	20.4[b] (11.3–33.8)	38,200[e]	200–157,800
	Adults	9	116	7.5 (4.0–14.3)	17,133	400–54,600
	Seniors	3	46	6.5 (1.6–18.5)	4933	200–14,400
	Geriatric	0	7	0.0	NA***	NA
	Total	22	218	10.1[a] (6.7–14.9)	30,522[d]	200–157,800
Mixed	Puppies	2	16	12.5[c] (2.2–37.3)	46,900[f]	4200–89,600
	Adults	9	77	11.7 (6.1–21.0)	45,866	200–333,000
	Seniors	6	50	11.5 (5.0–23.3)	67,266	400–393,600
	Geriatric	1	12	8.3 (0.1–37.5)	200	NA
	Total	18	155	11.6[a] (7.4–17.7)	30,600[d]	200–36,600

*CI: confidence interval; **CPG: cysts per gram; ***NA: not available; [a] no statistical differences were observed between *G. duodenalis* prevalence and breed group ($p > 0.05$). [b]no statistical differences were observed between *G. duodenalis* prevalence in purebred dogs and age groups ($p > 0.05$); [c] no statistical differences were observed between *G. duodenalis* prevalence in mixed breed dogs and age groups ($p > 0.05$); [d,e,f] no statistical differences ($p > 0.05$) between CPG and purebred/mixed breeds, purebred and age groups, and mixed breeds and age groups, respectively.

There were no significant differences in the prevalence of *G. duodenalis* between the purebred and mixed breed dogs ($\chi^2 = 0.1$; $p = 0.7$), purebred dogs and respective age groups ($\chi^2 = 7.8$; $p = 0.08$), and mixed-breed dogs and age groups ($\chi^2 = 0.15$; $p = 0.9$). There were also no significant differences between shed CPG and purebred/mixed breed dogs ($p = 0.6$), purebred dogs and age groups ($p = 0.2$), and mixed breed dogs and age groups ($p = 0.9$) (Table 3.13).

Between sexes, higher prevalence and excreted higher number of *G. duodenalis* cysts was in male dogs compared to female dogs (Table 3.14). In female dog group, the highest prevalence was found in geriatric age group, followed by puppy age group, where the female puppies also excreted highest amounts of cysts (Table 3.14). In the male group, the highest *G. duodenalis* prevalence was observed in the puppy group, which was also 2.1 times higher than female puppies, while female puppies shed a higher number of *G. duodenalis* cysts, compared to male puppies (Table 3.14).

Table 3.14. ***Giardia duodenalis*** **prevalence and cyst load by sex and age groups in domestic dogs**

Sex	Age group	No. of positive dogs	Total no. of dogs tested	Prevalence, % (95% CI)*	Mean CPG**	Min–Max CPG
Female	Puppies	3	27	11.1[b] (3.0–28.9)	68,133[e]	26,800–89,600
	Adults	5	105	4.8 (1.8–11.1)	21,040	1200–68,200
	Seniors	3	43	7.0 (1.7–19.3)	5733	600–14,400
	Geriatric	1	8	12.5 (0.1–49.2)	NA***	NA
	Total	12	183	6.6[a] (3.7–11.2)	27,250[d]	200–89,600
Male	Puppies	9	38	23.7[c] (12.8–39.4)	30,155[f]	400–157,800

Sex	Age group	No. of positive dogs	Total no. of dogs tested	Prevalence, % (95% CI)*	Mean CPG**	Min–Max CPG
	Adults	13	88	14.8 (8.7–23.8)	35,523	200–330,000
	Seniors	6	53	11.3 (4.9–23.9)	66,866	200–393,600
	Geriatric	0	11	0.0	NA	NA
	Total	28	190	14.7[a] (10.3–20.5)	56,400	200–393,600

*CI: confidence interval; **CPG: cysts per gram; ***NA: not available; [a] statistical differences were observed between *G. duodenalis* prevalence and sex ($p < 0.05$). [b] no statistical differences were observed between *G. duodenalis* prevalence in purebred dogs and age groups ($p > 0.05$); [c] no statistical differences were observed between *G. duodenalis* prevalence in mixed breed dogs and age groups ($p > 0.05$). [d,e,f] no statistical differences ($p > 0.05$) between CPG and female/male dogs, female dogs and age groups, and male dogs and age groups, respectively.

There was statistical significance between male and female dogs (χ^2 =5.7, p = 0.01), but not between the shed CPG and sex (χ^2 = 0.6; p = 0.5). There were also no significant differences observed between female dogs and age groups (χ^2 = 4.8; p = 0.09), or male dogs and age groups (p = 0.5). No significant differences were observed between excreted *G. duodenalis* cysts in female dogs and age groups (p = 0.1), or male dogs and age groups (p = 0.5).

In shelter dogs, *G. duodenalis* prevalence was 6.7% (3/45; 95%CI: 2.3–17.9), while in owner dogs, it reached 11.3% (37/328; 95%CI: 8.1–15.2). There was no statistical significance between *G. duodenalis* prevalence in owner dogs and shelter dogs (p = 0.4).

Between months, the highest prevalence was observed in May (2/7; 28.6%; 95%CI: 3.7–71) while the lowest was in March (0/3; 0.0%) (Figure 3.9).

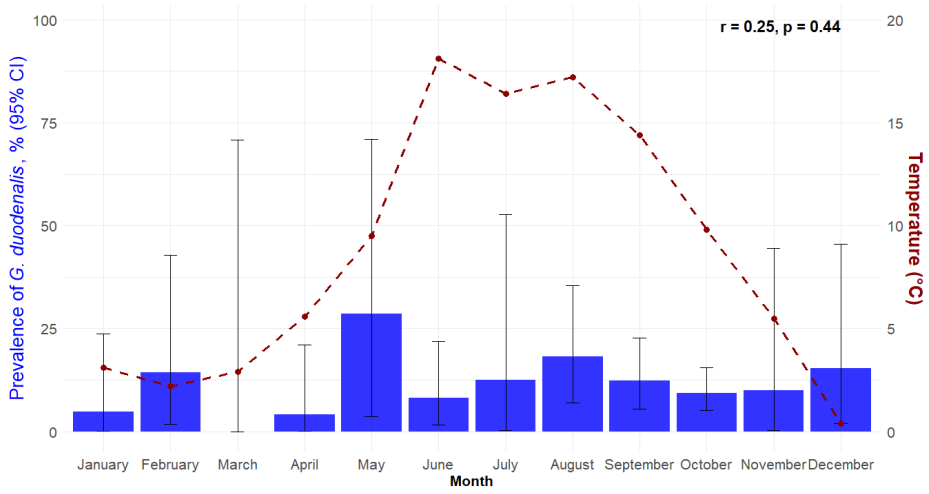

Figure 3.9. **Monthly prevalence of *G. duodenalis* in dogs and the average monthly temperatures in 2020–2021**

The average environmental temperature during the sample collection period had no significant effect on the prevalence of *G. duodenalis* (r = 0.2; p = 0.4). No significant association was found between monthly precipitation (r = 0.03, p = 0.9) or humidity (r = -0.09, p = 0.8).

Out of the 40 microscopically positive domestic dog samples, DNA amplification was successful from 16 samples 40.0% (95% CI: 26.3–55.4). *G. duodenalis* assemblage D was isolated the most (50.0%, 95% CI: 28.0–72.0), followed by assemblage C, 37.5% (95% CI:

18.4–61.5), and assemblage A, 12.5 % (95% CI: 2.2–37.3). The highest median CPG was in dogs with *G. duodenalis* assemblage D, followed by assemblage C and A (Table 3.15).

Table 3.15. ***Giardia duodenalis*** **assemblages in domestic dogs and median cyst load**

G. duodenalis **assemblage**	**No. of dogs positive**	**Mean CPG (Min–Max)**
A	2	37,000 (31,400–42,600)
C	6	102,040 (7200–285,600)
D	8	220,971 (14,000–393,600)

The potentially zoonotic assemblages A were observed in two female dogs. One was a nine-month-old puppy, but the other was a five-year-old adult dog. Dog-specific *G. duodenalis* assemblage C was observed in three male and three female dogs, with age varying from three months old to five years old, the mean of 3.4 years old. Assemblage D was observed in five female and three male dogs, with age ranging from 3 months old to 3 years old (mean of 3.4).

The locations of the *G. duodenalis* assemblages in Latvia are visualized in Figure 3.10.

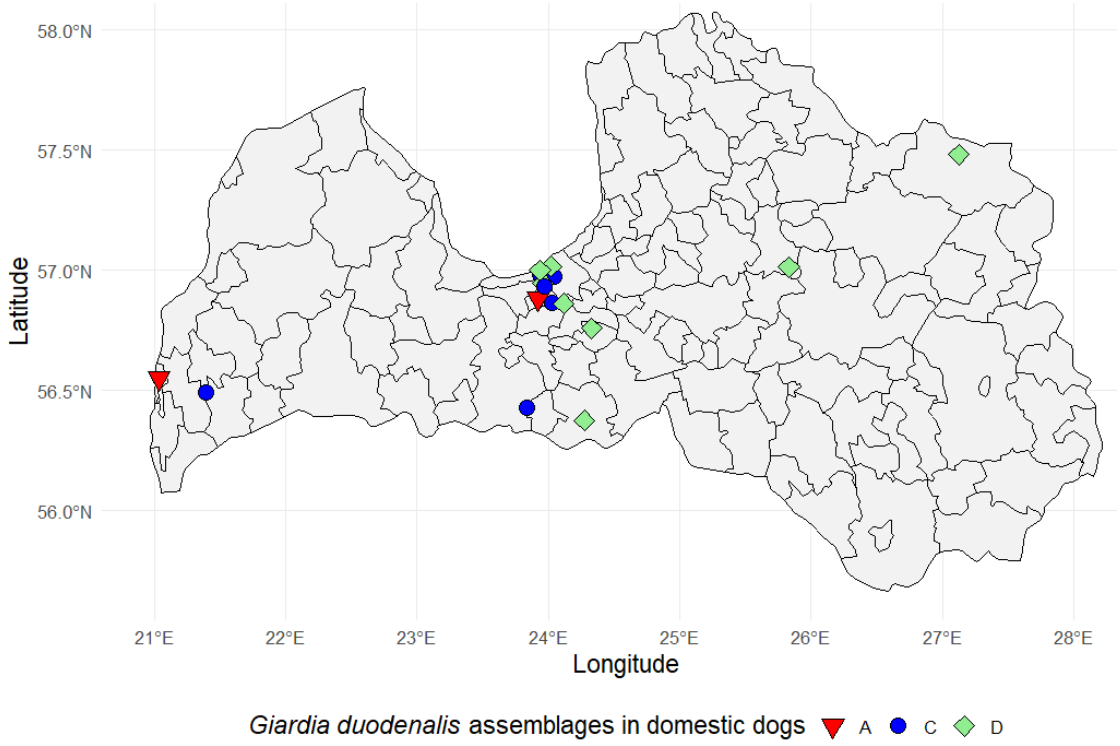

Figure 3.10. ***Giardia duodenalis*** **assemblage distribution in domestic dogs in Latvia**

Single-factor logistic regression analysis was performed first to assess the potential association between individual factors and *Giardia duodenalis* infection in domestic dogs. Age as a continuous variable did not show as statistically significant variable ($p = 0.15$), therefore age as a group was assessed in the initial logistic regression model. In total, four initial single-factor logistic regression models showed significance at $p < 0.1$, which were further considered for the multivariable analysis (Table 3.16).

Table 3.16. **Single-factor logistic regression models for predictors of *Giardia duodenalis* in domestic dogs in Latvia**

Model (AIC Model Fit)	Predictors	Odds Ratios (95% CI)	*z*-value	*p-value*
251.47	(intercept)	0.1 (0.05–0.1)	-8.9	< 0.001
	Sex: Male	2.5 (1.2–5.2)	2.5	0.01
257.42	(intercept)	0.1 (0.06–0.2)	-9.2	< 0.001
	Age group: Geriatric	0.5 (0.1–3.3)	-0.6	0.7
	Age group: Puppy	2.2 (1.0–4.9)	1.9	0.05
	Age group: Senior	1.0 (0.4–2.3)	0.01	0.98
251.71	(Intercept)	0.2 (0.1–0.4)	-4.7	< 0.001
	Activity outside city: with leash: Yes	0.4 (0.2–0.8)	-2.6	0.008
227.53	(Intercept)	0.07 (0.05–0.11)	-12.1	< 0.001
	Cryptosporidium spp.: Positive	9.9 (4.6–21.5)	5.8	< 0.001

A forward stepwise logistic regression was performed and the final model with AIC = 221.8 included three predictors – Sex ($p = 0.06$), Activity outside the city with a leash ($p = 0.02$) and *Cryptosporidium* spp. ($p < 0.001$). No multicollinearity was detected (VIF = 1 for all variables).

For the backward stepwise logistic regression model, 24 initial predictors were selected related to the domestic dog characteristics – host characteristics, activity, feeding and health status. After removing the non-significant variables (e.g. living place, activities in city, forest, meadow, access to farm animals, age in years, deworming frequency), the final model with AIC = 102.5 was obtained. The resulting model included fecal consistency, breed, access to slaughter by-products, deworming medication and *Cryptosporidium* spp. co-infection. Although the backwards model had lower AIC, which included borderline significant variables ($p < 0.1$), it increased the model's complexity.

The final model (Table 3.17) with the lowest AIC values was retrieved using the variables from the forward selection, based on the single, dependent variable models. The final model retrieved two risk and one protective factor affecting *G. duodenalis* in dogs in Latvia (Table 3.17).

Table 3.17. **Final logistic regression model for factors affecting *Giardia duodenalis* in domestic dogs in Latvia**

Model (AIC Model Fit)	Predictors	Odds Ratios (95% CI)	*z*-value	*p-value*
221.8	(intercept)	0.1 (0.05–0.1)	-12.1	< 0.001
	Sex: Male	2.5 (1.2–5.0)	2.5	0.01
	Activity outside city – with leash: Yes	0.4 (0.2–0.8)	-2.7	0.008
	Cryptosporidium spp.: Positive	10.0 (4.6–21.8)	5.8	< 0.001

The model explained about 15% of the variation in *Giardia duodenalis* infection based on Tjur's R^2, and no multicollinearity was detected (VIF = 1).

In summary, the prevalence of *G. duodenalis* in domestic dogs was 10.7% (40/373), with the highest prevalence in the puppy age group (under one year old) (18.5%, 12/65). Male dogs had a significantly higher prevalence than female dogs (p = 0.01), but no differences were observed between cyst shedding and sex (p = 0.05). In domestic dogs, the zoonotic assemblage A was detected in 10.5% of the dogs (2/19), and dog-specific assemblages C and D were detected in 31.6% (6/19) and 42.1% (8/19) of the dogs, respectively. Two risk factors – male dogs and co-infection with *Cryptosporidium* spp., and one protective factor – activity outside the city with a leash were associated with *G. duodenalis* infection in domestic dogs in Latvia.

3.3. Prevalence, cyst load, genetic diversity, and animal-level factors potentially associated with *Giardia duodenalis* in red foxes and raccoon dogs in Latvia

Overall, *G. duodenalis* prevalence in red foxes was 27.4% (60/219; 95% CI: 21.9–33.7), with the mean CPG of 3133 (min 300; max 47,600; median 700 CPG). The highest prevalence was observed in Latgale (52/183; 28.4%, 95% CI: 22.4–35.3), followed by Vidzeme (7/27; 25.9%, 95% CI: 13.4–44.7), Zemgale (1/5; 20.2%, 95% CI: 3.6–62.4), and no *G. duodenalis*-positive red foxes were observed in Kurzeme (0/4) (Figure 3.11).

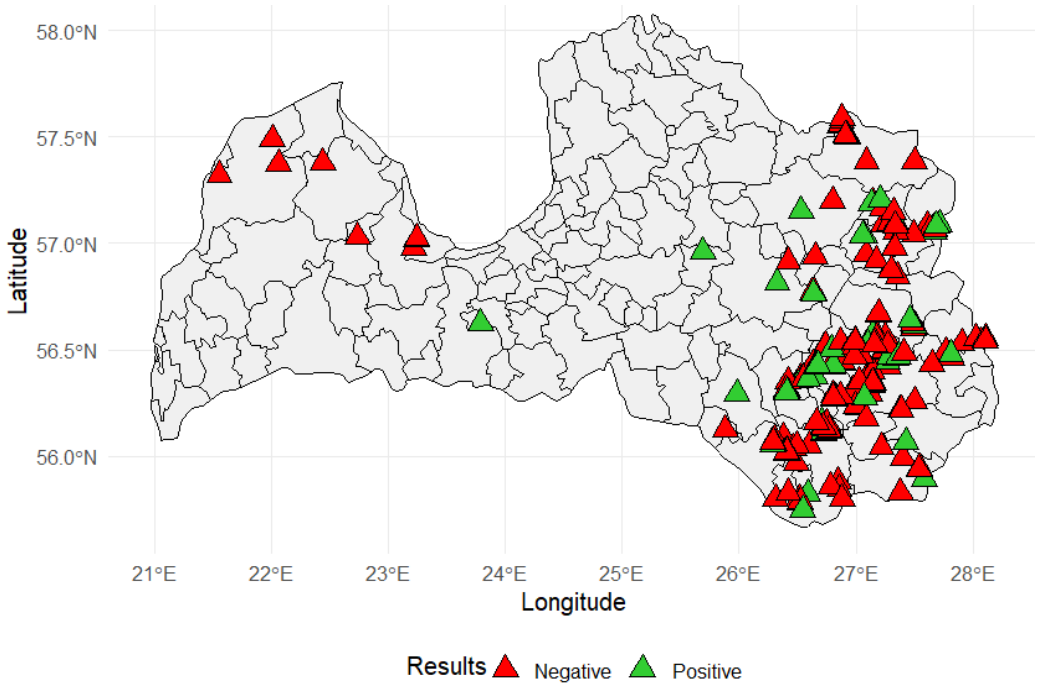

Figure 3.11. **Locations of *Giardia duodenalis*-positive and negative red foxes**

Out of the eight forestry districts, most of red foxes were examined from Dienvidlatgale and Austrumlatgale with the highest prevalence of *G. duodenalis* (50.0%, 95% CI: 9.5–90.5) observed in red foxes from Sēlija forestry and the lowest in red foxes from Ziemeļvidzeme forestry district (16.7%, 95% CI: 3.0–56.4) (Figure 3.12). *G. duodenalis* was not observed in red foxes from the Ziemeļkurzemes forestry district (0/4).

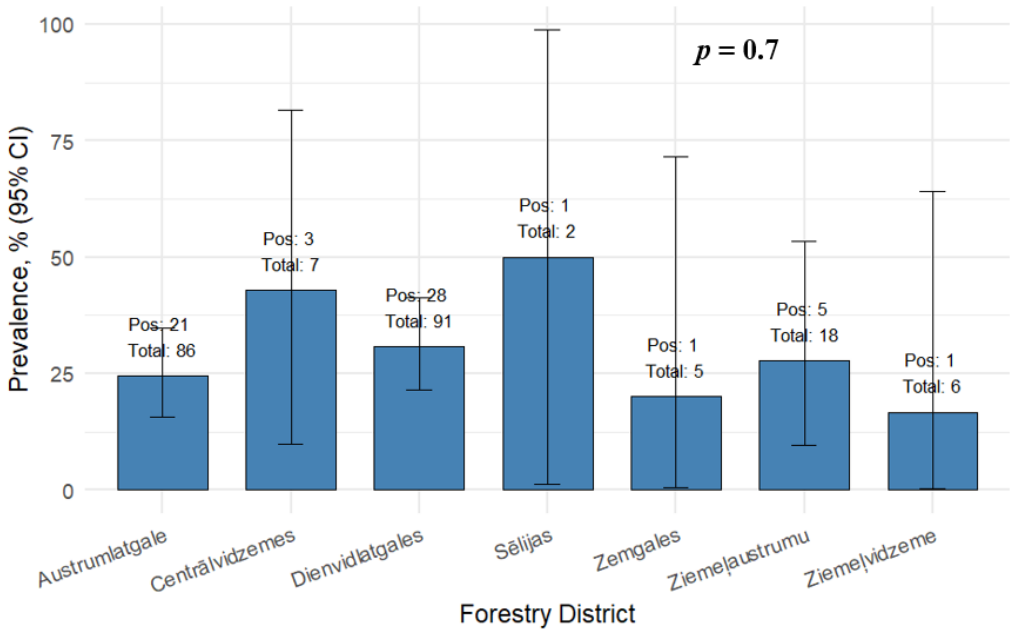

Figure 3.12. ***Giardia duodenalis*** **prevalence in different forestry districts in red foxes**

No statistical significance was observed between forestry districts and *G. duodenalis* prevalence in red foxes ($\chi^2 = 4.2$; $p = 0.7$).

Age in years was recorded for 169 red foxes with the average of 2.1 years (min: 1; max 5), and no age was recorded for 50 of the red foxes. The highest prevalence was recorded in the 5-year-old age group, followed by the 3–3.5 and 2–2.5-year age groups. The lowest *G. duodenalis* prevalence was observed in the 1–1.5-year-old red foxes (Table 3.18). Most of *G. duodenalis* cysts were excreted by the four-year-old red foxes, followed by the 2–2.5-year-old age group, and the least amount of *G. duodenalis* cysts were shed by the 1–1.5-year-old foxes (Table 3.18).

Table 3.18. ***Giardia duodenalis*** **prevalence according to the red foxes ages groups**

Age group, years	**No. of positive red foxes**	**Total no. of examined red foxes**	**Prevalence, % (95% CI)***	**Mean CPG** (Min–Max)**
1–1.5	8	47	17.2[a] (8.6–30.4)	875[b] (200–2600)
2–2.5	26	85	30.6 (21.8–41.1)	5408 (200–47,600)
3–3.5	10	30	33.3 (19.18–51.3)	920 (200–2800)
4	2	5	40.0 (11.68–77.1)	6400 (800–12,000)
5	1	2	50.0 (9.48–90.5)	2800
NR***	13	50	26.0 (15.88–39.7)	1200 (200–5400)

*CI: confidence interval; **CPG: cysts per gram; ***NR: not recorded; [a] no statistical difference was observed between *G. duodenalis* prevalence and age groups ($p > 0.05$); [b] no statistical difference was observed between shed CPG and age groups ($p > 0.05$).

Even though there were differences in the prevalence between age groups in red foxes, they were not significant ($\chi^2 = 4.5$, $p = 0.5$). No significant differences were observed between age groups and CPG ($p = 0.07$) (Table 3.18).

In raccoon dogs, the overall *G. duodenalis* prevalence was 30.8% (24/78, 95% CI: 21.6–41.7), with the average CPG of 14,008 (min 200; max 224,000; median 1200 CPG). *G. duodenalis*-positive raccoon dogs were found in two out of the three regions, with the highest

prevalence in Latgale (23/69; 33.3%, 95% CI: 23.4–45.1), followed by Vidzeme (1/7; 14.3%, 95% CI: 2.6–51.3). None of the examined raccoon dogs from Sēlija (0/2) forestry were positive for *G. duodenalis* (Figure 3.13).

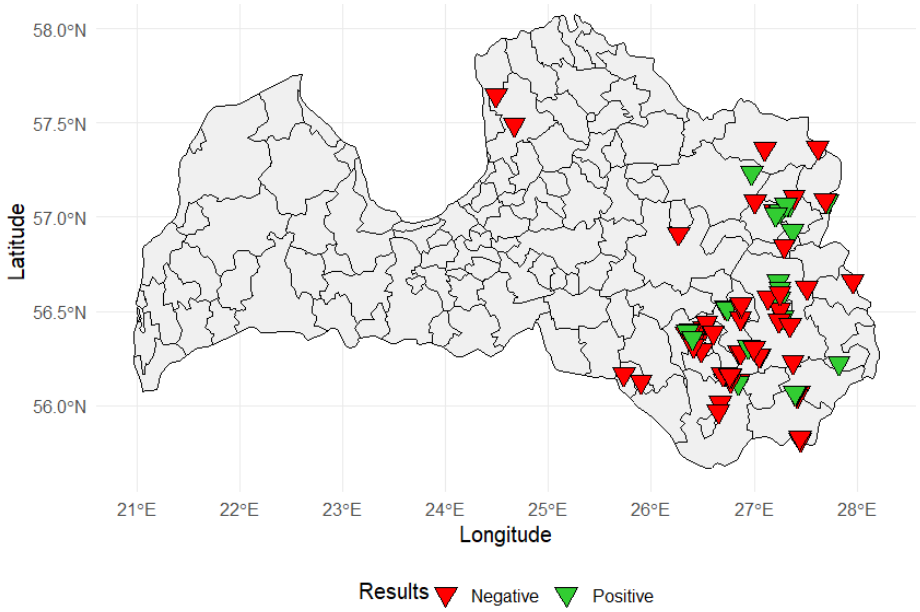

Figure 3.13. **Locations of *Giardia duodenalis*-positive and negative raccoon dogs**

G. duodenalis-positive raccoon dogs were found in 3 out of the six forestry districts. The highest *G. duodenalis* prevalence was observed in Austrumlatgale, but lowest in Dienvidlatgales forestries, but no positive raccoon dogs were observed in Centrālvidzeme (0/1), Sēlijas (0/2), and Ziemeļvidzemes (0/3) forestry districts (Figure 3.14).

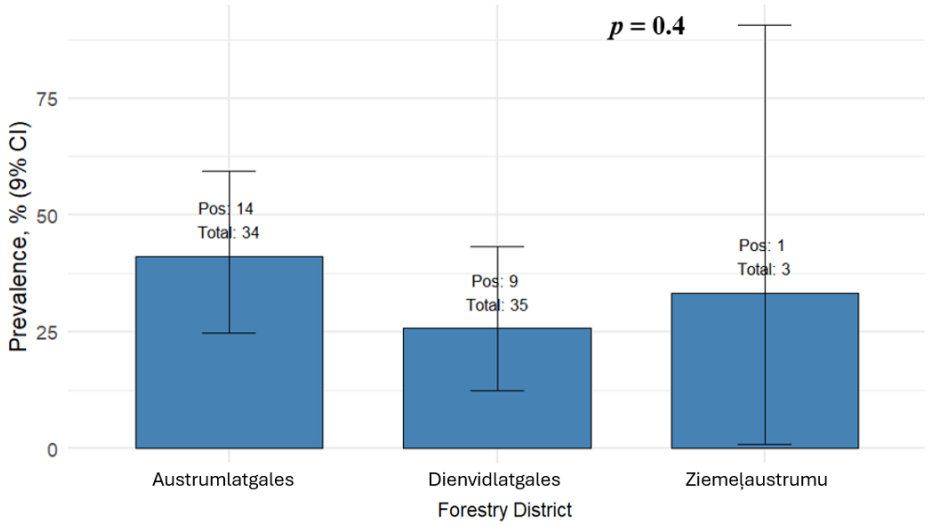

Figure 3.14. ***Giardia duodenalis* prevalence in raccoon dogs in different forestry districts of Latvia**

No significant differences were observed between forestry districts and *G. duodenalis* prevalence in raccoon dogs ($\chi^2 = 4.8$, $p = 0.4$).

Age in years was recorded for 55 raccoon dogs and the average age was 2 years (min 1; max 4.5), but no age was recorded for 23 of the raccoon dogs. From the raccoon dogs with recorded age, the highest prevalence was observed in the 1–1.5-year-old raccoon dogs, which

also shed the highest number of CPG, but the overall highest prevalence and excreted CPG was from the raccoon dogs with unknown age (Table 3.19).

Table 3.19. ***Giardia duodenalis*** **prevalence in raccoon dogs of different ages in Latvia**

Age group, years	No. of positive raccoon dogs	Total no. examined raccoon dogs	Prevalence, % (95% CI)*	Mean CPG** (Min–Max)
1–1.5	7	15	46.7[a] (24.88–69.9)	6171[b] (600–25,200
2–2.5	6	33	18.2 (8.28–34.8)	2733 (400–10,000)
3–3.5	1	6	16.7 (1.18–58.2)	600
4.5	0	1	0.0	NA****
NR***	10	23	43.5 (25.68–63.2)	27,600 (200–224,000)

*CI: confidence interval; **CPG: cysts per gram; ***NR: not recorded; ****NA: not available; [a] no statistical significance was observed between age groups and *G. duodenalis* prevalence ($p > 0.05$); [b] no statistical significance was observed between age groups and CPG ($p > 0.05$).

Despite differences in the *G. duodenalis* prevalence in raccoon dogs between age groups, no significant differences were observed ($p = 0.1$). No significant differences were also observed between raccoon dogs age groups and shed CPG ($p > 0.75$) (Table 3.19).

Out of the 23 microscopically positive red fox and raccoon dog samples, DNA from three animals were successfully amplificated (11.5%, 95% CI: 3.2–29.8). *G. duodenalis* assemblage D was detected in one, two-year-old red fox, which shed 37,000 cysts per gram of feces, and in one raccoon dog with 224,000 cysts (Figure 3.15). Age of the positive raccoon dog was not known. *G. duodenalis* assemblage C was shed by one, two-year-old fox, which shed 47,600 cysts per gram of feces (Figure 3.15).

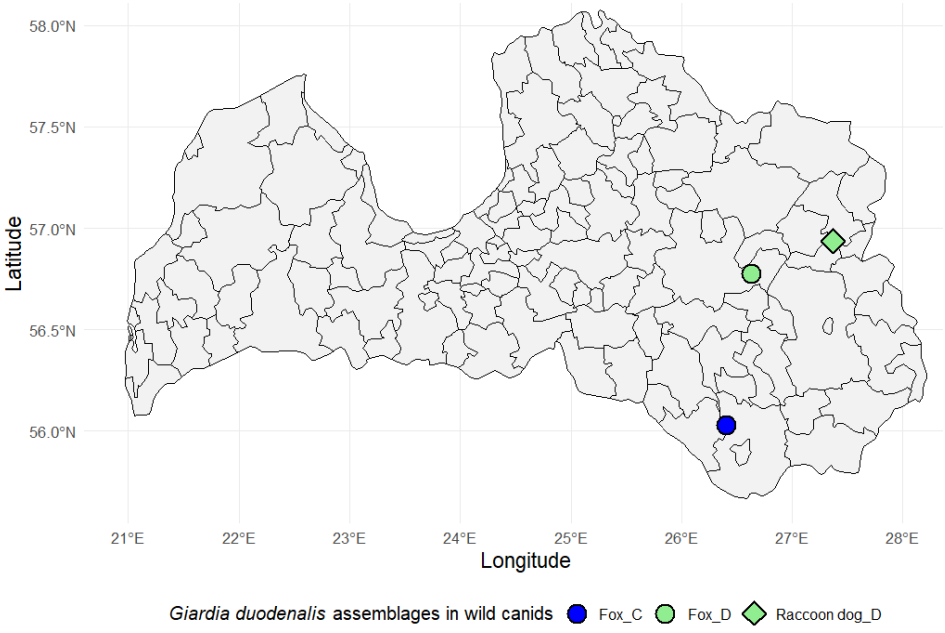

Figure 3.15. ***Giardia duodenalis*** **assemblage distribution in red foxes and raccoon dogs**

For the initial analysis, multiple univariable logistic regression models were run. Geographic region (hunting county or parish, and forestry district) did not show any effect on

Giardia infection in foxes. However, age in years ($p = 0.04$) and *Cryptosporidium* spp. co-infection ($p < 0.0001$) was statistically significant.

For the final model, foxes shedding *Cryptosporidium* spp. had significantly higher odds of also shedding *G. duodenalis* cysts. Co-infection with *Cryptosporidium* was also associated with age (Table 3.20).

Table 3.20. **Final logistic regression model for factors affecting *Giardia duodenalis* in red foxes in Latvia**

Model (AIC Model Fit)	**Predictors**	**Odds Ratios (95% CI)**	***z*-value**	***p-value***
165.7	(intercept)	0.03 (0.01–0.1)	-4.5	< 0.001
	Age: Years	2.3 (1.3–4.2)	2.7	0.007
	Cryptosporidium spp.: Positive	111.1 (11.5–1070.7)	4.0	< 0.001
	Cryptosporidium spp.: Positive + age (years)	0.3 (0.1–0.8)	-2.4	0.02

Final model showed no multicollinearity and explained 25.1% of the variation in *Giardia duodenalis* infection (Tjur's R^2).

Similarly to red foxes, same variables were added to the initial univariable analyses. Hunting county, parish or forestry district did not show any statistical significance ($p > 0.05$). Age showed a trend ($p = 0.07$) with younger raccoon dogs more likely to test positive. Also, the presence of *Cryptosporidium* spp. was strongly associated with *G. duodenalis* ($p < 0.001$).

The final logistic regression model of AIC = 75.7 showed strong *G. duodenalis* association with age and *Cryptosporidium* spp. (Table 3.21).

Table 3.21. **Final logistic regression model for factors affecting *Giardia duodenalis* in raccoon dogs in Latvia**

Model (AIC Model Fit)	**Predictors**	**Odds Ratios (95% CI)**	***z*-value**	***p-value***
165.7	(intercept)	0.2 (0.08–0.38)	-4.7	< 0.001
	Age: Years	0.1 (0.01–1.1)	-1.9	0.005
	Cryptosporidium spp.: Positive	16.0 (4.6–55.5)	4.5	< 0.001

No multicollinearity was observed and the model explained 29.7% of the variation (Tjur's R^2).

In summary, *G. duodenalis* prevalence in red foxes was 27.4% (60/219) with the highest prevalence observed in animals around five years old (50.0%, 1/2). No significant differences were observed between age and cyst shedding among the age groups in red foxes ($p = 0.07$). In raccoon dogs, *G. duodenalis* prevalence was 30.8% (24/78) with the highest prevalence observed in the 1–1.5-year-old age group (46.7%, 7/15), and no significant differences were identified between age and *G. duodenalis* cyst shedding ($p = 0.7$). In red foxes, assemblages C and D were detected in one animal each, but one raccoon dog was positive for assemblage D. In red foxes, increased prevalence of *G. duodenalis* was associated with older animals (OR 2.3,

$p = 0.007$) and co-infection with *Cryptosporidium* spp. (OR 111.1, $p < 0.001$). In raccoon dogs, increased *G. duodenalis* infection was associated with younger animals (OR 0.1, $p = 0.005$), as well as co-infection with *Cryptosporidium* spp. was also associated with *G. duodenalis* (OR 16.0, $p < 0.001$).

3.4. Environmental contamination potential of *Giardia duodenalis* from cattle and canids in Latvia with emphasis on the zoonotic assemblage A

To evaluate the environmental contamination potential of *G. duodenalis* in Latvia, the prevalence and the cyst load were assessed across all four animal species: cattle, domestic dogs, red foxes and raccoon dogs.

Raccoon dogs showed 3.7 times higher *G. duodenalis* prevalence compared to cattle and 2.9-times higher prevalence compared to domestic dogs. Red foxes, had a 3.3- and 2.9-times higher *G. duodenalis* prevalence, compared to cattle and domestic dogs, respectively (Figure 3.16).

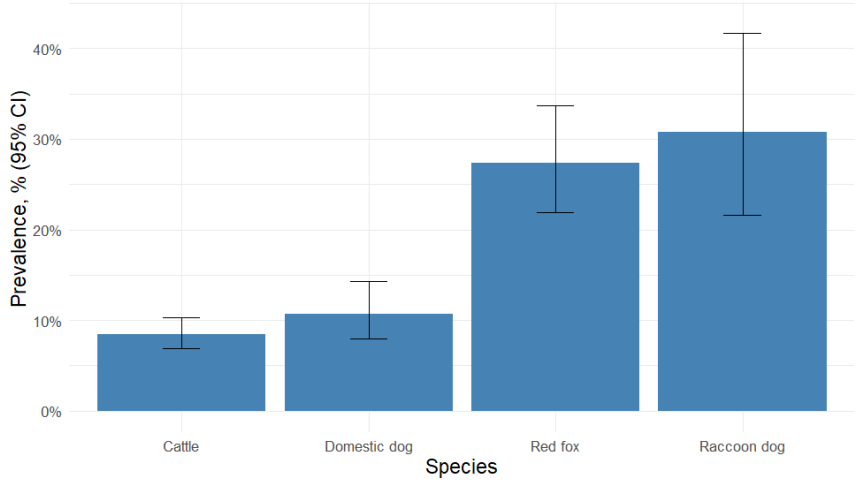

Figure 3.16. **Prevalence of *Giardia duodenalis* in cattle, domestic dogs, red foxes and raccoon dogs in Latvia**

Animal species was a significant factor that affected the prevalence ($\chi^2 = 83.8$; $p < 0.0001$). With generalized mixed modelling, including age as a potential cofounding variable, both raccoon dogs and red foxes had increased odds of being infected with *G. duodenalis* as shown in Table 3.22.

Table 3.22. **Logistic regression model assessing species and age-related odds of *Giardia duodenalis* infection**

Model (AIC Model Fit)	Predictors	Odds Ratios (95% CI)	*z*-value	*p-value*
1060.2	(intercept)	0.1 (0.1–0.2)	-14.8	< 0.001
	Species: Domestic dog	1.5 (1.0–2.2)	1.9	0.06
	Species: Red fox	3.5 (2.3–5.4)	6.0	< 0.001
1060.2	Species: Raccoon dog	3.1 (1.6–5.9)	3.4	< 0.001
	Age: years	0.9 (0.8–1.0)	-4.6	< 0.001

The *G. duodenalis* cyst load also significantly differed among the animal species ($p < 0.001$). Domestic dogs had the highest CPG values among all groups, followed by raccoon dogs (Figure 3.17).

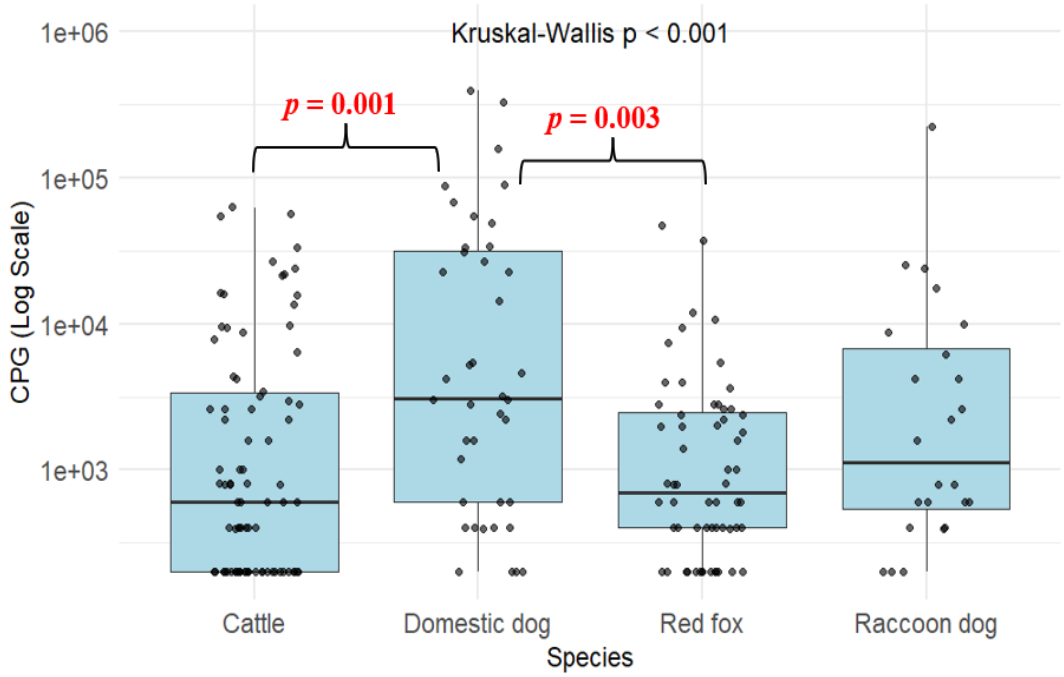

Figure 3.17. **Species-related *Giardia duodenalis* cyst load variations among cattle, domestic dogs, red foxes, and raccoon dogs in Latvia.** CPG not adjusted to the feces load per animal

Dunn's post hoc test with Bonferroni correction showed significantly higher CPG loads in domestic dogs compared to cattle ($p = 0.001$) and red foxes ($p = 0.003$). No significant differences were observed between other species ($p > 0.05$) (Figure 3.15).

After adjusting the CPG values for the average fecal/scat mass (grams per defecation) and the number of defecations per day, the potential adjusted daily cyst shedding per infected animal was calculated using the median CPG values to refine the environmental contamination potential (Table 3.23).

Table 3.23**. Median *Giardia duodenalis* cyst excretion rates per defecation event and per day, adjusted for fecal output and defecation frequency in positive cattle, domestic dogs, red foxes, and raccoon dogs in Latvia**

Species	Median CPG* in positive animals	Average weight of feces per defecation (g)	Average defecations per day	Cysts per defecation	Adjusted daily cyst-shedding per day
Cattle	600	1900[a]	14[a]	1,140,000	15,960,000[1]
Domestic dogs	3100	150[b]	2[b]	465,000	930,000[2]
Red foxes	700	120[c]	6.4[c]	84,000	537,600

Species	Median CPG* in positive animals	Average weight of feces per defecation (g)	Average defecations per day	Cysts per defecation	Adjusted daily cyst-shedding per day
Raccoon dogs	1200	120[d]	6.4[d]	144,000	921,600

*CPG: cysts per gram[a] Aland et al., 2002; [b] Wright et al., 2009; Ferreras et al., 2019. [d] Assumed to be the same as foxes, as no data is available, [1]statistical significance was observed between adjusted *G. duodenalis* cyst shedding in cattle and other species ($p < 0.05$); [2] statistical significance was observed between adjusted *G. duodenalis* cyst shedding between dogs and red foxes ($p < 0.05$).

The Kruskal-Wallis test showed a significant difference, when cyst shedding was adjusted ($p < 0.001$), and, the follow-up with Dunn's test with Bonferroni correction, showed that cattle had significantly higher *G. duodenalis* cyst output than domestic dogs, red foxes and raccoon dogs ($p < 0.05$). However, dogs excreted significantly higher cyst load than red foxes ($p < 0.05$) (Table 2.23).

A total of nine animals spread *G. duodenalis* assemblage A cysts in the environment. The majority of *G. duodenalis* assemblage A-positive cases were found in cattle (77.8%, 95% CI: 45.3–94.1), while 22.2% (95% CI: 6.3–54.7) of the dogs shed *G. duodenalis* assemblage A. In domestic dogs, the highest cyst count was excreted by the youngest animal, but in cattle, the highest cyst count was shed by older cattle. Infected animals were distributed across all regions and various housing systems, including biological and industrial farms, including three (42.7%, 95% CI: 15.7–75.0) herds with access to the pastures (Table 3.24).

Table 3.24. **Characteristics of Restriction Length Fragment Polymorphism confirmed *Giardia duodenalis* assemblage A positive animals**

Region	Residence	Animal species	Age, months	Ownership/ herd size	Housing and environment	CPG*
Kurzeme	City	Dog	2.4	Private owner	Apartment, activity in the city without a leash	33,400
Vidzeme	City	Dog	36	Private owner	Private house, activity in public parks, forests, meadows, with a leash	68,200
Vidzeme	Countryside	Cattle	4	>501	Industrial farming, access to pastures, no access to free water in pastures	600
Vidzeme	Countryside	Cattle	51	>501	Industrial farming, no access to pastures	200
Zemgale	Countryside	Cattle	4	251–500	Industrial farming, no access to pastures	9600
Zemgale	Countryside	Cattle	87	>501	Industrial farming, no access to pastures	15,800
Latgale	Countryside	Cattle	5	>501	Industrial farming, access to pastures, lake in the pastures	400

Region	Residence	Animal species	Age, months	Ownership/ herd size	Housing and environment	CPG*
Latgale	Countryside	Cattle	4	>501	Industrial farming, access to pastures, no access to free water in pastures	200
Kurzeme	Countryside	Cattle	6	251–500	Biological farming, access to pastures, no access to free water in pastures	2600

**CPG: cysts per gram*

To assess proximity to surface water sources, distances from *G. duodenalis* assemblage A-positive animals to the nearest river, pond or lake were calculated. Eight out of the nine *G. duodenalis* assemblage A- positive animals (six cattle, two dogs) were located within 1500 m of a surface waterbody, including three within 500 m (Figure 3.18).

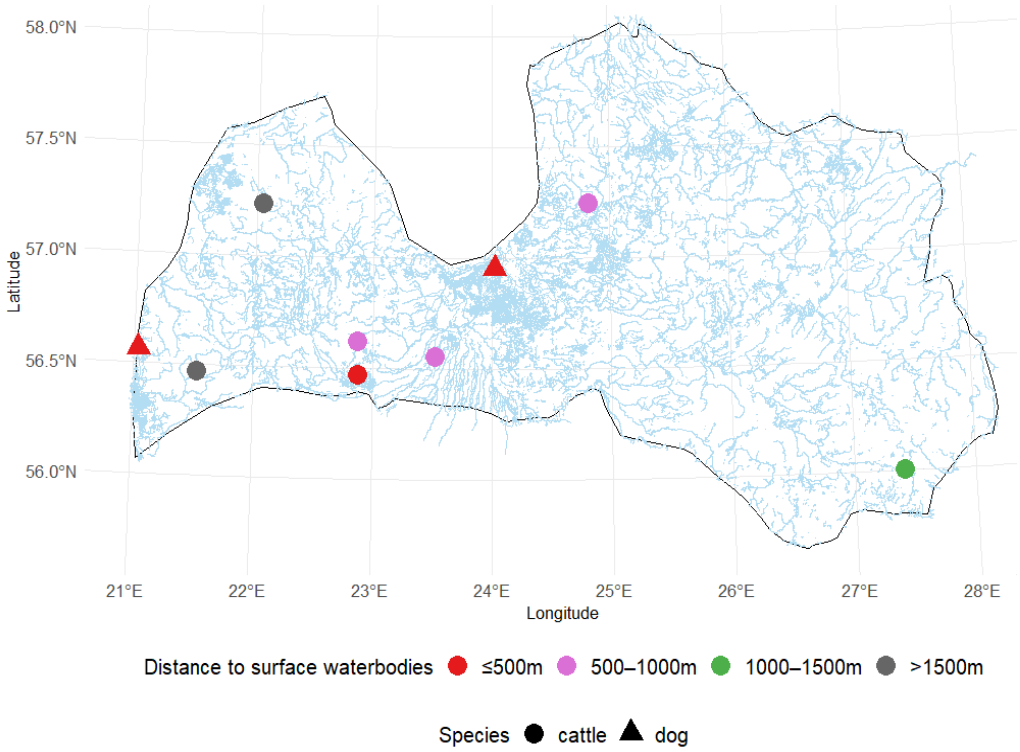

Figure 3.18. **Locations of *Giardia duodenalis* assemblage A-positive cattle and domestic dogs in relation to surface waterbodies**

Although the coordinates for domestic dogs were approximate, one dogs location was close to a named pond (Valhovas dīķis), while three cattle herds were within 2 km of major rivers – Bērze, Tērvete, and Engure (Table 3.25).

Table 3.25. **The distance between the nearest rivers and ponds to *Giardia duodenalis* assemblage A-positive domestic dogs and cattle in Latvia**

Species	CPG	Distance to river (m)	Nearest river	Distance to a lake or pond (m)	Nearest lake/pond
Dog	68,200	12.6	Unnamed stream	104.5	Unnamed lake or pond
Dog	33,400	96.5	Unnamed stream	368.7	Volhovas dīķis
Cattle	600	358.6	Unnamed stream	375.5	Unnamed lake or pond
Cattle	9600	574.1	Bērze	600	Unnamed lake or pond
Cattle	15,800	785.1	Tērvete	2346.1	Unnamed lake or pond
Cattle	400	880.3	Unnamed stream	1008.8	Unnamed lake or pond
Cattle	200	1369.3	Lūžupe	664.3	Unnamed lake or pond
Cattle	2600	2265	Engure	2252.5	Unnamed lake or pond
Cattle	200	2185.10	Unnamed stream	3315.9	Unnamed lake or pond

From the cattle questionnaires (Appendix 6), nine out of 32 (28.1%, 95% CI: 15.4–45.5) of herd owners reported open waterbodies in the pasture (such as river, ponds, lakes, ditches). Out of those, two farms reported lakes (33.3%, 95% CI: 9.2–70.4), and two other farms – rivers (33.3%, 95% CI: 9.2–70.4) available in the pastures. Out of these nine herds, six herd owners reported that the cattle could directly access the surface waterbodies in the pastures (66.7%, 95% CI: 35.1–88.3). In one farm, where one cow was positive for *G. duodenalis* assemblage A, the owner reported that the cattle in pastures could access the lake (16.7%, 95% CI: 1.1–58.2). Additionally, seven out of the nine farm owners (77.8%, 95% CI: 42.3–94.7), who reported the presence of surface waterbodies in the pastures, admitted that these waterbodies connected with another surface water via a river or a ditch.

All cattle herd owners used manure for field fertilization (100%), and eight of the herds stored manure in a pile next to the facilities (25.0%, 95% CI: 13.0–42.3), 16 stored manures in open pits (50.0%, 95% CI: 33.6–66.4), and eight stored in lagoons (25.0%, 95% CI: 13.0–42.3). Out of the 32 herds, 10 herd owners reported that they do not process manure or slurry before the use for field fertilization (31.2%, 95% CI: 17.8–48.7), with two herds using fermentation (20%, 95% CI: 4.6–52.1), and eight using lagoons (80%, 95% CI: 47.9–95.4). Regarding *G. duodenalis* assemblage A-positive cattle herds, in three out of the seven herds manure was used for field fertilization (42.9%, 95% CI: 15.7–75.0), and in five out of the seven herds, manure was s stored in open pits next to the farm facilities (71.4%, 95% CI: 35.2–92.4) (Table 3.24).

With GLMM, it was possible to determine that drinking water in the pastures (OR 1.9; $p < 0.03$), as well as cattle with access to open surface waters in the pastures (OR 1.6, $p < 0.1$), had an increased risk for cattle to become infected with *G. duodenalis* (Table 3.10).

In summary, comparing *G. duodenalis* prevalences among studied animals, both wild canids had significantly higher prevalence than domestic dogs and cattle ($p < 0.0001$), with red foxes having 3.5 times higher odds of infection than cattle. After adjusting the excreted *G. duodenalis* cyst load to the weight of feces produced by animals, cattle shed significantly higher

amounts of *G. duodenalis* cysts than other species ($p < 0.05$). While the higher prevalence of the zoonotic *G. duodenalis* assemblage A was in cattle (77.8%, 7/9), domestic dogs excreted a higher number of *G. duodenalis* assemblage A cysts in the environment (33,400–68,200). Three (42.7%) of the seven *G. duodenalis* assemblage A-positive cattle had access to pastures and three out of the seven *G. duodenalis* assemblage A-positive cattle herds were located within 2 km from a major river – Bērze, Tērvete, and Engure. From the questionnaires, 9 (28.1%) out of the 32 herd owners reported open waterbodies in the pastures (such as lakes and rivers). All interviewed herd owners used manure for field fertilization (32/32, 100.0%), out of which eight farms stored the manure in a pile next to the facilities (25.0%). Ten out of the 32 herds did not treat manure or slurry before field fertilization (31.2%) and three out of the seven owners of the cattle herds with zoonotic *G. duodenalis* assemblage-A positive cattle stored manure in open pits next to the farm facilities (71.4%).

4. DISCUSSION

4.1. Prevalence, cyst load, genetic diversity, animal-level and herd-level factors associated with *Giardia duodenalis* in cattle in Latvia

In this study, the overall prevalence of *G. duodenalis* in cattle was 8.4%, which was lower than previously reported in Europe, where the prevalence was up to 31.1% (Taghipour et al., 2022). In studies with application of immunofluorescence method for detection of *G. duodenalis* cysts, the higher overall prevalence rates in cattle were observed. For example, in Denmark, *G. duodenalis* prevalence was 43.6% (n = 1150), and in Greece – 41.3% (n = 254) (Maddox-Hyttel et al., 2006; Ligda et al., 2020). Studies utilizing other *G. duodenalis* diagnostic methods, such as enzyme-linked immunosorbent assay, retrieved varying prevalences between 32.2% (n = 503) in Italy, to 54.9% (n = 556) in the United Kingdom (Geurden et al., 2012). Immunofluorescence microscopy is considered one of the best methods for detection of *G. duodenalis* cysts in feces and other biological materials as it shares both high sensitivity and specificity (Gotfred-Rasmussen et al., 2016; Aziz et al., 2024). Hence the lower *G. duodenalis* prevalence in cattle in Latvia is likely due to differences in *G. duodenalis* prevalence existing between regions in Latvia. For example, the prevalence of *G. duodenalis* was higher in Kurzeme (12.0%), compared to Vidzeme (4.0%). Regional differences have been observed in Norway, where the prevalence in cattle varied from 44.5% (n = 461) in calves from Nord-Trøndelag region to 46.6% (n = 450) in Rogaland region, and to 55.6% (n = 475) in Oppland region, which was more likely due to collecting samples in different seasons rather than regional differences (Hamnes et al., 2006). In our study, we also observed differences in *G. duodenalis* prevalence between months, with the highest prevalence observed in winter months – November and December (11.0% each), while no positive cases were detected in August, and this was supported by a strong negative correlation between average monthly temperature and prevalence ($r = -0.9$; $p < 0.05$). This could be due to reduced environmental survivability in warmer months and is in align with previous research, which suggests that *G. duodenalis* cyst survivability is reduced in warmer months due to cyst exposure to the sun's UV-rays and increased temperature (Alum et al., 2013; Masina et al., 2019; Wang et al., 2023). It is important to acknowledge, however, that the differences in *G. duodenalis* prevalence could be due to the variable sample size collected each month. Nevertheless, the observed trend in seasonality shows insight into the potential influence of environmental conditions on *G. duodenalis* prevalence in cattle.

The highest *G. duodenalis* prevalence and highest number of cysts were shed by calves under three months old ($p < 0.05$) compared to cattle between 4–24 months old. The lowest prevalence of *G. duodenalis* was seen in adult cattle. Also in other studies, where *G. duodenalis* was mainly found in younger animals, they were more likely to excrete a higher number of *G. duodenalis* cysts (Huetink et al., 2001; Castro-Hermida et al., 2009; Minetti et al., 2014). In Denmark, *G. duodenalis* prevalence in under one-month-old calves was as high as 31.8% (7/22) in the United Kingdom, followed by 24.0% (229/377) in Denmark, and 20.9% (29/139) in Spain (Maddox-Hyttel et al., 2006; Castro-Hermida et al., 2009; Minetti et al., 2014). The youngest calf excreting *G. duodenalis* cysts was six days old in our study. Neonatal calves can get infected with giardiasis immediately after birth and can shed the first *G. duodenalis* cysts only two days after birth (Wade et al., 2000). Additionally, the cellular immune response against *G. duodenalis* starts at five weeks of age, and the immune system is effective enough to reduce the number of *G. duodenalis* cysts only by weeks 14 to 15 of the calf's development (Grit et al., 2014). In Germany, where 441 calves were tested, calves over one month of age shared higher *G. duodenalis* prevalence than neonatal calves, and the prevalence in four-week-old calves was 38.0%, compared to one- to two-week-old calves with the prevalence between 10.0% and

20.0% (Jäger et al., 2005). In another study, where a total of 333 beef calves were examined, the maximum *G. duodenalis* prevalence of 55.0% was observed in four- to ten-week-old beef calves, compared to the calves below four weeks old (34.0%) (Geurden et al., 2008). In the present study, both prevalence and cysts shed by the cattle above two years old were lower than calves which may indicate the role of older cattle in the *G. duodenalis* spread to the newborn calves and passive contamination of the farm surrounding area (Ralston et al., 2003).

G. duodenalis was detected in 84.4% of the examined herds, with the highest prevalences observed in herds with 251–500 and over 500 cattle per herd. Similarly, high herd-level prevalences have been reported in Spain (96.6%; n = 60), Germany (100.0%; n = 31), and the United Kingdom (100.0%; n = 31) (Castro-Hermida et al., 2007; Geurden et al., 2012). In our study, no significant differences ($p > 0.05$) were observed between herd size, *G. duodenalis* prevalence and cyst load. Similar results were also observed in dairy and beef calves and adult cattle, where no differences were observed between herd sizes (Uehlinger et al., 2011). Although convenience sampling was used in this study, the results still provide meaningful insight into *G. duodenalis* prevalence in cattle in Latvia (Tyrer & Heyman, 2016).

Male cattle had both a higher *G. duodenalis* prevalence and higher cyst load than female cattle. Sex predisposition in cattle regarding *G. duodenalis* has been studied before with contradictory results. Some studies show no differences in prevalence between male and female cattle (Oh et al., 2021; Onder et al., 2020). In the study done by Oh et al. (2021), comparing *G. duodenalis* prevalence between calves under 12 weeks of age, no significant differences were observed between male (5.7%; 23/402), and female (5.6%; 21/373) calves. Similar results were observed by Onder et al. (2020), where no significant differences were identified between male (30.2%; 90/298) and female (30.3%; 46/152) young calves and adult cattle. Nevertheless, significant differences were observed in three different studies. In one study, across all ages, male cattle had the significantly higher (35.3%; 36/102) prevalence compared to female cattle (25.6%; 108/422) ($p > 0.05$) (Heng et al., 2022). In other studies, differences between sexes were observed in cattle aged from 10 to 150 days, where all examined female cattle were negative (Baazizi et al., 2025), but in another, the *G. duodenalis* prevalence in male cattle was 20%, with the result being statistically significant (Heng et al., 2022). An earlier study has hinted at a potentially stronger immune response to pathogens in pre-pubertal female calves (Carroll et al., 2015), which might influence their reaction to *G. duodenalis* infection.

All successfully isolated *Giardia* cysts from cattle were confirmed as *G. duodenalis*. Two *G. duodenalis* assemblages were identified in our study – *G. duodenalis* assemblage E, and in four herds, mixed infection of *G. duodenalis* assemblages A and E was detected. In previous reports from several countries, the mixed infection of *G. duodenalis* assemblage A and E in herds was more common in France, Germany, Italy, and the UK (Geurden et a., 2012). The prevalence of mixed *G. duodenalis* assemblages A and E varied from 21.0% (3/14) in Italy to 44.0% (4/9) in the UK (Geurden et al., 2012). Additionally, in our study, across all age groups and herd sizes, *G. duodenalis* assemblage E was the predominant, which has been widely distributed across Europe (Hamnes et al., 2006; Langkjær et al., 2007; Geurden et al., 2012). *G. duodenalis* assemblage E is cattle-specific and can be present in all ages but more frequently in adult cattle (Castro-Hermida et al., 2011; Minetti et al., 2013). *G. duodenalis* assemblage E contributes to lower immunity in cattle, therefore prolongs the chronic course of the disease (Dreesen et al., 2012). Even though *G. duodenalis* assemblage E is considered cattle-specific, it was isolated from humans, mostly in rural areas (Abdel-Moein & Saeed, 2016; Fantinatti et al., 2016; Zahedi et al., 2017; Garcia et al., 2021). In Brazil, *G. duodenalis* assemblage E was found in three preschool children from a slum (Fantinatti et al., 2016). In Australia, six people were positive for *G. duodenalis* assemblage E, and all isolates were identical to a sheep-derived *G. duodenalis* assemblage E (Zahedi et al., 2017). In Egypt, *G. duodenalis* assemblage E was identified in 25 children feces after close contact with assemblage E-positive cattle (Abdel-

Moein & Saeed, 2016). These children were with or without gastrointestinal manifestations (Abdel-Moein & Saeed, 2016).

The zoonotic *G. duodenalis* assemblage A was detected mainly in cattle older than four months. Other studies reported that *G. duodenalis* was frequently observed in younger cattle (Trout et al., 2005; Trout et al., 2006; Geurden et al., 2012). In Scotland, Spain, and the UK, *G. duodenalis* assemblage A has been found in cattle of all ages (Castro-Hermida et al., 2009; Minetti et al., 2014; Bartley et al., 2019). There have been no associations between diarrhea or any other clinical signs and *G. duodenalis* assemblage A in cattle (Castro-Hermida et al., 2009; Minetti et al., 2014; Bartley et al., 2019). Similarly to *G. duodenalis* assemblage E, scarce reports on *G. duodenalis* assemblage A in humans infected from cattle were published. In New Zealand, human and cattle shared the same *G. duodenalis* assemblage A (Garcia et al., 2021). Because of *G. duodenalis* assemblage A has been more frequently observed in cattle in Europe, infected animals could be a potential source of human infections (Geurden et al., 2008; Geurden et al., 2012; Minetti et al., 2014; Bartley et al., 2019; Dixon et al., 2011). In the present study, DNA amplification was successful in 75.6% (62/82) of the positive cattle fecal samples, which could be due to the low number of *G. duodenalis* cysts in the positive samples. Low DNA concentration may be attributable to the low numbers of *G. duodenalis* cysts, as well as the presence of inhibitors, such as lipids or bile salts, could have influence the performance of the RFLP (Schrader et al., 2012). Nevertheless, this study shows that both *G. duodenalis* assemblages A and E are frequently observed in cattle in Latvia.

Multiple animal, management and environmental level protective and risk factors were found to be associated with *G. duodenalis* in cattle in Latvia. In the initial GLMM, seven factors were determined to be protective – age, no access to pastures, pasture season starting in May, manure storage in open pits, rodent control with poison, the presence of cats in the herd, and change of shoes for visitors. Out of these seven initial factors, in the final GLMM, five factors were statistically significant ($p < 0.05$) or showed a trend ($p \leq 0.1$) – age, pasture season starting in May, no access to pastures, and manure kept in an open pit or piles were shown as protective factors.

Age exhibited a protective effect against *G. duodenalis* in cattle with significantly reduced odds of infection in older cattle ($p < 0.05$). This result has been observed before, where a 22 to 150 times higher risk of shedding *G. duodenalis* cysts was observed in calves between 9–18 days and above 18 days old (Gow & Waldner, 2006). Maddox-Hytell et al. (2006) reported that *G. duodenalis* cyst excretion increased with calf age within the first month of life. Calves as young as four days old can excrete *G. duodenalis*, but calves from the ages of five to ten weeks old were more likely to shed *G. duodenalis* cysts intermittently (O'Handley et al., 1997; O'Handley et al., 1999; Ralston et al., 2022). Generally, calves were more prone to clinical manifestation of giardiasis with intermittent diarrhea and weight loss as the main clinical signs (O'Handley et al., 1999; Geurden et al., 2010). As discussed before, it has been assumed that the adaptive immunity during giardiasis is incomplete in calves; therefore, reinfections occur (Dreesen et al., 2012). In experimentally infected calves, *G. duodenalis* cysts were shed for up to 100 days with a slow introduction of cellular and humoral response, which explains the chronic progress of the disease (Grit et al., 2014). Decrease in *G. duodenalis* prevalence and in the cyst load in our study alongside with the cattle age emphasizes the heightened vulnerability of young calves to *G. duodenalis* infection.

Two seemingly contradictory protective factors appeared in the GLMM models – pasture season beginning in May and no pastures at all. Both appeared to reduce the odds of giardiasis, suggesting that timing and management of the surrounding pasture may be crucial. One possible explanation could be wet soil and lower nutritional quality of the pastures during the early spring months, which can lead to a compromised immune system due to a decrease in vitamin B12, folic acid, and iron intake, as well as a higher chance of contact with other endoparasites

(Wade et al., 2000; Lejune et al., 2010; McCarthy et al., 2022). Delaying pasture access to May might allow for drier environmental conditions and reduced contact with mud and stagnant water, which can lead to lower environmental cyst load (Wade et al., 2000). When cattle are only kept indoors, feeding is often constant and provides balanced nutrition, therefore increasing immunity, but with higher density, chances of direct transmission of *G. duodenalis* as well as accumulation of other pathogens in the herd facilities increases (Wade et al., 2000; Dixon, 2021). Both management systems can be protective under specific conditions, and the observed effects could reflect a balance between environmental exposure and nutritional or hygiene-related effects.

Keeping manure in an open pit or in piles minimized the effect of *G. duodenalis* in cattle in our study. Keeping manure in a pile or open pit which allows run-offs near the herd premises has no biological explanation as it should not decrease the disease prevalence at the visited herds. As *G. duodenalis* cysts are robust in environments, appropriate measurements need to be considered when managing manure (Millner et al., 2014; Vermeulen et al., 2017). Open-type manure piles often self-heat in the central core, but the sides of the piles cannot produce internal heat to reduce pathogen viability; therefore, it is important to manage proper manure treatment plans (Millner et al., 2014). The heat in the core needs to reach at least 60 °C to destroy most pathogens, including cysts (Spencer & Guan, 2004). If lagoons are used for manure storage, it reduces the risk of manure and slurry run-offs, contact with mechanical vectors as well as food and water-borne outbreaks risks for humans (Nicholson et al., 2004; Millner et al., 2014). All the visited herds used manure as field fertilization, even if manure was not held in a lagoon, which could further increase the risk of environmental pollution with *G. duodenali*s. During rainfall events, surface run-off may transfer cysts from contaminated soils, open manure pits, or manure-spread fields into nearby surface water bodies (Alhusen et al., 2011; Rochelle-Newall et al., 2019). During cool and wet periods, the *G. duodenalis* cyst concentrations in surface waters can increase threefold compared to dry conditions, and water contamination during rainfalls has been linked to higher human giardiasis cases (Alhusen et al., 2011; Rochelle-Newall et al., 2019). Therefore, careful attention should be paid to how and where manure is applied to reduce the environmental contamination in the cattle herds.

Rodent control using poison appeared as a protective factor against *G. duodenalis.* All seven *G. duodenalis* assemblages have been reported in rodents, with *G. duodenalis* assemblage A sub-assemblages AI and AII reported from 4.9% (3/61) to 87% (53/61) (Li et al., 2023). It seems that rodent control is an important factor that could prevent the spread of *G. duodenalis* in the herds, especially the zoonotic *G. duodenalis* assemblages (Daniels & Hutchings, 2001; Li et al., 2023). Also, cats as pets seemed to reduce the potential of *G. duodenalis* in the cattle herds, which could be used in a rodent control. This finding only emphasizes that rodent control needs to be a part of an effective herd management strategy to potentially reduce the transmission of pathogens. Nonrestricted access of pets to the herd also poses a biosecurity risk because they can transfer pathogens, including *G. duodenalis,* from the environment directly to the cattle or contaminate their feed or water (Wells et al., 2002; Sarrazin et al., 2014).

Finally, providing a change of clothes, especially shoes appeared to reduce the *G. duodenalis* infection in cattle herds in Latvia. Change of clothes is an important way to limit the introduction of new pathogens into the herd (Nöremark et al., 2016). *G. duodenalis* cysts are small, of 7–10 μm in diameter; therefore, it is important to implement proper biosecurity protocols to stop the introduction of new pathogens from other herds, especially with shoes (Adam, 2001; Rashid et al., 2016). A study done in Italy showed that shared personnel between herds, such as veterinarians and technicians, can increase the spread of diseases exponentially; but if proper cleaning protocols are in place, such as a change of shoes, the probability of disease transmission had significantly decreased (Rossi et al., 2017). A change of shoes for visiting

veterinarians and other third-party visitors is necessary to reduce the introduction of *G. duodenalis* and minimize the transmission risk to other herds.

As for risk factors, in the initial GLMM, six factors appeared to increase the risk of *G. duodenalis* in cattle in Latvia – ability for cattle to leave the herd premises, isolating calves with diarrhea, a walking area next to herd premises, drinking water in the pastures and access to surface water at the pastures. In the final GLMM, only one risk factor appeared to increase the risk of *G. duodenalis* in cattle – cattle can leave the herd premises (OR 2.2, $p < 0.05$).

Walking areas (paddock) around the farm and outdoor movement (between premises) seemed to increase the risk of *G. duodenalis.* While reducing animal density at the farm facilities together with environmental factors, such as exposure to sun rays, can lower the infectivity of *G. duodenalis* cysts, walking areas are rarely, if ever, cleaned or changed. Therefore, the environmental cyst load could be higher at walking paddocks compared with in-farm facilities, which are cleaned more frequently (Boyer & Kuczynska, 2010; Wang et al., 2023).

The isolation of calves with diarrhea as a risk factor was more likely to be due to calves already being infected with a *G. duodenalis* or other pathogens or non-infectious diarrhea. In this case, the observed association likely reflected the timing of the clinical signs rather than a causal effect of isolation, therefore was removed from further analysis. However, it is worth to note that diarrhea is one of the major causes of young calf death, and effective management protocols to isolate these animals is necessary (Cho & Yoon, 2014). While *G. duodenalis* rarely causes acute diarrhea, there are many other pathogens, which cause severe gastrointestinal diseases in calves, such as *E. coli*, *Salmonella* spp., *Clostridium* spp., *Eimeria* spp., *Cryptosporidium* spp., rotavirus, bovine coronavirus, and bovine viral diarrhea virus (Cho & Yoon, 2014). Additionally, poor overall calf management and inadequate colostrum feeding can cause non-infectious diarrhea (Al Mawly et al., 2015).

Two similar risk factors increased the odds of risk of infection for cattle – available drinking water in the pasture provided by the owner and access to surface water in the pasture. Those findings are important since water is one of the primary infection routes for *G. duodenalis*. Although it is important to provide clean drinking water in the pastures, the water containers may not be cleaned often enough or thoroughly and facilitate persistence of *G. duodenalis* cysts and other pathogens (Lewerin et al., 2019). In future studies, if drinking water is provided to the grazing cattle, it should be noted whether the water containers are cleaned and whether it is possible for cattle to contaminate these containers with feces.

Regarding open water sources in the pastures, this factor is in line with the protective factor – no access to pastures, suggesting that environmental exposure could play a central role in *G. duodenalis* transmission in cattle. Surface waters, such as ponds, ditches, streams, lakes, or rivers, can be contaminated by direct defecation in the water source and fecal run-off from the pastures (Castro-Hermida et al., 2009). High levels of *G. duodenalis* cysts have been found in a river near cattle herds, with cyst concentration varying from two to 400 cysts per liter of water (Castro-Hermida et al., 2009). Another study shows that within 500 m of cattle housing, low levels of *G. duodenalis* cysts were observed in the nearest surface water (Budu-Amoako et al., 2012). Several large outbreaks have been reported in humans due to swimming in rivers or streams, but none of them were linked to contamination due to cattle feces (Adams et al., 2016). Nevertheless, *G. duodenalis* cysts can survive in water for a long time, especially in cool conditions, and if high turbidity or organic matter is present in a flowing water source, it can physically shield *G. duodenalis* cysts from UV radiation (Wang et al., 2023). No model data is available for *Giardia*, but for *Cryptosporidium*, which also is a waterborne parasite and commonly observed in cattle in Latvia, the settling velocity is 0.1 m per day, that ensures slow settling and combined with river flow, the pathogen may not reach the bottom of the river as a sediment (Vermeulen et al., 2019).

4.2. Prevalence, cyst load, animal-level and housing factors potentially associated with *Giardia duodenalis* in domestic dogs in Latvia

The *G. duodenalis* prevalence in dogs in this study was 10.7%. The highest prevalence was observed in dogs from the Latgale (13.0%), while the lowest was 8.0% in dogs from Zemgale. Previous studies across Europe show that the mean *G. duodenalis* prevalence in dogs was higher varying from 28.5% (80/281) in Belgium to 14.6% (38/260) in the UK (Epe et al., 2010). A more recent study done in dogs in Spain shows *G. duodenalis* prevalence of 48.6% (n = 252) (Mateo et al., 2023). While not significant ($p > 0.05$), some regional differences between *G. duodenalis* prevalences were observed in Latvia. A reflection of environmental factors, such as the density of surface waterbodies, moisture retention in soil and general precipitation levels could increase the survival and transmission of *Giardia* cysts (Hadi et al., 2016). In contrast with our study, the regional differences in the prevalence of *G. duodenalis* were reported in dogs in Norway. Dogs from Eastern Norway had a higher prevalence, possibly because of the density of the dogs or possible climate differences between the regions (Hamnes et al., 2007).

An age-related pattern was observed in this study, with puppies showing both higher *G. duodenalis* prevalence and increased cyst load compared to other age groups. Similar findings were observed in a study done in dogs under one year old, where the prevalence of *G. duodenalis* was higher in 12–month-old dogs than in the 3–month-old dog group without significant differences (Hamnes et al., 2007). Other studies showed a similar pattern of younger dogs being infected without statistical significance (Lopez-Arias et al., 2019; Remesar et al., 2022). This could be due to more naïve immune systems of younger dogs and it may not protect properly against pathogens, waning of maternal antibodies and gut microbiota development (Chastant & Mila, 2019).

Male dogs were more frequently infected than female dogs ($p < 0.05$). While male dogs did simultaneously shed higher numbers of *G. duodenalis* cysts, there was a significant difference observed between sexes ($p < 0.05$). Differences in the prevalence of the pathogen between sexes have been reported previously. In a study by Fontanarrosa et al. (2006), male dogs were more infected than female dogs, but no statistical differences were noted. This was also in line with the study of Epe et al. (2010), which included dogs from seven European countries. In a longitudinal study with dogs under one year old, females had a higher *G. duodenalis* prevalence than male dogs (Hamnes et al., 2007). This could be due to the exploitative nature or increased levels of androgynous hormones in males, which could decrease immunity (Klein, 2000).

G. duodenalis prevalence (11.3%; 37/328) was higher in owner dogs than shelter dogs (6.7%; 3/45). This could be partially explained by the smaller sample size collected from shelter dogs. However, similar findings in shelter dogs were previously explained by kennel dogs continuous exposure to *Giardia* cysts (Adell-Aledón et al., 2018). Nevertheless, it is more commonly reported that shelter dogs share higher *G. duodenalis* prevalence than owner dogs due to lack of proper cleaning and sanitation, a higher stress environment, high animal density, and high turnover rates (Epe et al., 2010; Mircean et al., 2012; Turner et al., 2012; Gil et al., 2017). While shelter dog data offer valuable insights on *G. duodenalis* prevalence, their inclusion could influence the parasite prevalence estimates, as they might not fully represent the broader homeless dog population in Latvia.

In domestic dogs, zoonotic *G. duodenalis* assemblage A and two canine-specific *G. duodenalis* assemblages C and D were detected in this study. Although the RFLP had a low success rate (15.3%), these results are still substantial and show an insight into the assemblages that could be found in dogs in Latvia.

The canine-specific *G. duodenalis* assemblages D and C were the most isolated and higher number of cysts were shed by the dogs with both *G. duodenalis* assemblages. *G.*

duodenalis assemblages C and D were also the most observed assemblages in dogs in Europe. In Romania, *G. duodenalis* assemblages D and C were found in 70.0% (42/60) and 16.7% (10/60) of examined dogs, respectively (Adriana et al., 2016). In the same study in Romania, one dog was positive for mixed infection with *G. duodenalis* assemblages C and D, while another dog was shedding *G. duodenalis* assemblage E, which is cattle-specific (Adriana et al., 2016). In Poland, *G. duodenalis* assemblages C and D were also more prevalent in domestic dogs, but one dog was positive for the zoonotic *G. duodenalis* assemblage B (Piekarska et al., 2016). In a different study done in Poland, *G. duodenalis* assemblage A was detected in 1.7% of the examined dogs (Zygner et al., 2006).

G. duodenalis assemblage A was observed in two dogs – one in a nine-month-old puppy, and second – a five-year-old dog in the present study. *G. duodenalis* assemblage A has been reported in dogs in Poland, Germany, Belgium, the UK, Sweden, The Netherlands and Spain (Mravcová et al., 2019). Similarly to cattle, there are ongoing discussions about whether humans can get infected by dogs with the zoonotic *G. duodenalis* A and B assemblages. Some studies have reported associations between human giardiasis and *Giardia*-positive dogs which lived in the same households or had close interaction (Traub et al., 2004; Traub et al., 2009). Although observations of cross-infection between dogs and humans are rare, the presence of this assemblage can still pose a risk for human infection.

Multiple animal and husbandry-associated protective and risk factors were found to be associated with *G. duodenalis* in domestic dogs. Regarding protective factors, activity outside city with a leash appeared to minimize the risk of being infected with *G. duodenalis* in domestic dogs. As for risk factors, male dogs (sex), age (puppies) and co-infection with *Cryptosporidium* spp. were found to increase the probability of *G. duodenalis* infection.

One protective factor was observed – activity outside the city with a leash ($p < 0.05$), while other types of activity, including activity in the city with a leash and activity in nature with or without a leash, were not significant ($p > 0.05$). Using a leash outside urban areas can minimize the roaming of the dog, which further minimizes the contact with *G. duodenalis*-infected feces. Dogs on a leash are less frequently sniffing the ground that could minimize the contact with contaminated material or water as well it reduces the contact time with other dogs (Westgarth et al., 2010). Another study found that off-leash activities were positively associated with increased parasitism in dogs (Smith et al., 2014b). Additionally, in our study, the high prevalence of *G. duodenalis* was observed in red foxes (27.3%; 60/219) and raccoon dogs (30.8%; 24/78), and these species often are observed near urban and non-urban areas. It is important to educate the dog owners about the importance of using a leash while taking the dog for a walk, as *G. duodenalis* can be shed in large quantities by other infected animals (Dixon et al., 2021).

G. duodenalis prevalence was significantly higher in male had than in female dogs ($p < 0.05$). Male dogs shed higher number of *G. duodenalis* cysts, compared to females. In study by Tysnes et al., 2014 higher odds of infection with *G. duodenalis* were intact and neutered male dogs, especially in intact dogs, which could be explained by either differences in hormone distribution or differences in the dog's behavior. French et al., (2023) confirmed higher odds of giardiasis in intact dogs above 12 months old rather than in younger, neutered dogs ($p < 0.05$). Contrary results were observed in study by Mircean et al. (2012), where no differences in increased risk of infection were observed between sexes ($p > 0.05$), while Smith et al. (2014b) described higher risk in female dogs ($p < 0.05$). Considering these contradictory findings on the differences in the prevalence of *G. duodenalis* between sexes, it could be possible that additional questioning about the neuter status should be considered to truly understand whether the sex of the animal alone has an increased risk.

Age appeared as a risk factor associated with increased *G. duodenalis* prevalence in present study. Younger dogs were more likely to be infected with *G. duodenalis*, compared to

older dogs ($p < 0.05$). This has also been observed in a study done in the UK, where younger dogs also had an increased infection risk ($p < 0.05$) (Upjohn et al., 2010). In Italy, dogs under five years old were more likely to be infected with *G. duodenalis* (Papini et al., 2005). In a large-scale study, including over two million dogs, age groups of under five months old and from five months to two years old, had an increased risk of infection (Mohamed et al., 2013). Although it is still not clear whether there is a zoonotic route between dogs and humans, *G. duodenalis* is still one of the most common parasites in dogs, which cause chronic, intermittent diarrhea, and needs specific and long-term treatment (Traub et al., 2004; Traub et al., 2009; Epe et al., 2010; Tysnes et al., 2014). Due to the course of giardiasis in dogs, long-term shedding of *G. duodenalis* cysts is also possible, and animals contaminate the environment and disseminate the disease among other canid species (Tysnes et al., 2014).

Co-infection with *Cryptosporidium* spp. was another other risk factor that significantly increased odds of infection with *G. duodenalis* in domestic dogs in this study. The high prevalence of both parasites in dogs has been reported before (Hamnes et al., 2007; Smith et al., 2014b). *Cryptosporidium* spp. is a protozoan parasite that also tends to affect younger animals and can cause diarrhea (Thompson et al., 2005). A frequent identification of both parasites simultaneously shows the need to test dogs for both parasites – *G. duodenalis* and *Cryprosporidium* spp., especially if diarrhea or chronic, intermittent diarrhea is present (Overgaauw et al., 2009; Matos et al., 2015). From One Health perspective, dogs can be infected with not only the zoonotic *Cryptosporidium parvum*, which has caused multiple outbreaks in humans, but also with dog-specific *C. canis* and cattle-specific *C. andersoni* (Rosanowski et al., 2018). Sharing of zoonotic *G. duodenalis* assemblage A and B by dogs indicates that they could pose a risk of zoonotic transmission not only to humans but also to the environment and other animals (Simonato et al., 2017; Cacciò et al., 2018; Rosanowski et al., 2018).

4.3. Prevalence, cyst load, genetic diversity, and animal-level factors potentially associated with *Giardia duodenalis* in red foxes and raccoon dogs in Latvia

Prevalence of *G. duodenalis* in red foxes (27.4%; 60/219) was higher than domestic dogs (10.7%; 40/373). In our study, most of the red foxes were examined from the eastern part of Latvia, which is relatively sparsely populated therefore may serve as an indicator for the presence of pathogens in wildlife. Red foxes were hunted as part of the Rabies vaccination program at the eastern EU border. Only a few studies have been done on the *G. duodenalis* prevalence in red foxes in Europe, where prevalences varied from 44.0% (n = 104) in Sweden to 4.8% (n = 269) in Norway, 4.5% (n = 66) in Croatia, and 2.8% (n = 273) in Romania (Hamnes et al., 2007; Beck et al., 2011; Onac et al., 2015; Debenham et al., 2017).

We observed an increased *G. duodenalis* prevalence with the rise in foxes' age, with the lowest prevalence observed in the 1–1.5-year-old age group compared to older animals. This is unusual as *G. duodenalis* has been previously reported in Norway and Italy in juvenile red foxes (Hamnes et al., 2007; Papini & Verin, 2019). The age of the red foxes in this study was determined by the hunters. Therefore, age might be underestimated or overestimated. Although *G. duodenalis* does affect younger animals more, if the animal is under chronic stress or affected by an accompanying disease, such as mange mites, this may result in compromised immune system with chronic course of infection and long-term shedding of cysts (Soulsbury et al., 2007; Thompson et al., 2008).

A higher number of *G. duodenalis* cysts was shed by the 2–2.5-year-old red foxes while the lowest – by the younger red foxes. A similar observation was reported in red foxes in Sweden (Debenham et al., 2017), while other studies did not report the intensity of *G. duodenalis* cyst shedding (Hamnes et al., 2007; Mateo et al., 2017; Papini & Verin, 2019).

Because red foxes tend to roam not only in their natural environment and expand their habitat to the urban areas, but this behaviour also poses a risk of environmental contamination, as foxes can become long-term reservoir of *G. duodenalis* (Hamnes et al., 2007; Beck et al., 2011; Onac et al., 2015).

Among wild canids, the highest *G. duodenalis* prevalence (30.8%; 24/78) was in raccoon dogs. Similarly to red foxes, the raccoon dogs were also hunted in the eastern part of Latvia, which might not represent the true *G. duodenalis* prevalence in the country. Fewer studies have been done on the estimations of the prevalence of this parasite in raccoon dogs, compared to red foxes. The prevalence of *G. duodenalis* in farmed raccoon dogs from Poland was 11% (n = 18) (Solarczyk et al., 2016). Meanwhile, in another study done in Poland, *G. duodenalis* was not found in wild raccoon dogs (Osten-Sacken et al., 2017). In studies outside Europe, the prevalence of *G. duodenalis* in raccoon dogs was from 1.7% (4/233) to 7.2% (22/305) (Zhang et al., 2016; Liu et al., 2025).

In raccoon dogs, more positive animals were observed in the youngest age group. This finding was in agreement that younger animals are prone to giardiasis infection (Boucard et al., 2021). Although there is a lack of studies on the prevalence of *G. duodenalis* in raccoon dogs in Europe, this study provides a good insight into the potential situation of the wild canids in Latvia.

Two red foxes were positive for the canine-specific *G. duodenalis* assemblages D and C in present study. In several studies in Europe, zoonotic *G. duodenalis* assemblages A and B have been reported in Europe in red foxes as well. In Romania, two red foxes were positive for *G. duodenalis* assemblage A and B (Ocan et al., 2015). In Norway, five foxes were positive for *G. duodenalis* assemblage A with the *G. duodenalis* AI genotype detected in two foxes. Out of those foxes, one was positive for *G. duodenalis* assemblage A genotype which was previously recovered from a roe deer (Hamnes et al., 2007). Additionally, *G. duodenalis* assemblage B was further genotyped as BIII, which has been reported in a human sample in Norway (Hamnes et al., 2007). In Sweden, *G. duodenalis* assemblage B was detected in four foxes (Debenham et al., 2017). These studies from other European countries show that red foxes especially can carry zoonotic assemblages. Although the zoonotic *G. duodenalis* assemblage A or B were not observed in this study, their zoonotic transmission cannot be excluded in Latvia.

One raccoon dog was positive for canine-specific *G. duodenalis* assemblage D. There is a lack of studies on *G. duodenalis* assemblages in raccoon dogs in Europe and worldwide. One study from Romania reports *G. duodenalis* assemblage D from one raccoon dog (Adriana et al., 2016). In farmed raccoon dogs in Poland, two animals were positive for *G. duodenalis* assemblage D (Solarczyk et al., 2016). Outside of Europe, *G. duodenalis* assemblages C and D were reported from China (Zhang et al., 2016; Liu et al., 2025). Because there is a lack of studies done on raccoon dogs and reports about zoonotic assemblages found in these animals, we cannot determine whether these animals pose a zoonotic risk. Nevertheless, they carry canine-specific assemblages, so they could be a reservoir for infection in domestic dogs.

A low success rate of the RFLP was observed from samples of red foxes and raccoon dogs. Intestinal tracts from red foxes and raccoon dogs were frozen before the fecal sample was retrieved because of the complicated sample logistic to the laboratory, that could affect the *G. duodenalis* cyst structure and further molecular analysis (Wilke & Robertson, 2009).

In red foxes, in the final model, infection with *G. duodenalis* was significantly associated with age and co-infection with *Cryptosporidium* spp., especially in younger animals.

G. duodenalis infection was associated with older red foxes; but no significant association was observed between age groups ($p > 0.05$). While *G. duodenalis* has been previously reported in association with age in red foxes across Europe, there is a lack of studies using linear models to identify and qualify risk factors for *G. duodenalis* infection (Hamnes et al., 2007; Beck et al., 2011; Onac et al., 2015; Debenham et al., 2017; Papini & Verin, 2019). Nonetheless, this

finding is supported by the increased *G. duodenalis* prevalence and the increased number of *G. duodenalis* cysts shed by older red foxes.

G. duodenalis co-infection with *Cryptosporidium* spp. was also observed in red foxes in this study. *Cryptosporidium* spp. prevalence in red foxes has been reported to be from 2.2% (6/269) to 3.2% (4/123) in Norway and Bosnia Herzegovina, but no co-infections with *G. duodenalis* were detected (Hamnes et al., 2007; Hodžić et al., 2014). In contrast, we found a strong association between the two parasites, with co-infection significantly more likely to occur in younger foxes ($p < 0.05$). Red foxes can carry several *Cryptosporidium* species, including the zoonotic *C. parvum*, human-specific *C. hominis*, canine-specific *C. canis*, pig-specific *C. suis* and *C. ubiquitum*, which is often found in cattle and wild ruminants (Mateo et al., 2017; Berrera et al., 2020). The zoonotic *G. duodenalis* assemblages A and B have also been reported as co-infection with *Cryptosporidium* spp. (Hamnes et al., 2007; Ocan et al., 2015; Debenham et al., 2017). While zoonotic *G. duodenalis* assemblages were not reported in this study, red foxes still could contaminate the environment, especially facilitating transmission of the pathogen among wildlife, areas near livestock, and urban areas. There were around 32,000 red foxes in Latvia with roaming area of nomadic adults up to 25.9 km^2 in 2024 (Meia & Weber, 1995; Walton et al., 2018; Oficiālais statistikas portals, 2025). During the dispersal period, red foxes can move distances longer than 60 km (Meia & Weber, 1995; Walton et al., 2018). Combined with chronic *G. duodenalis* cyst shedding in canids, red foxes can contribute to heavy environmental contamination with both pathogens (Epe et al., 2010).

As for raccoon dogs, *G. duodenalis* co-infection with *Cryptosporidium* spp. was a risk, but age was a protective factor in the final model. Limited prevalence data for *Cryptosporidium* spp. in Europe had been reported as well. Two studies have been done in Poland, where the *Cryptosporidium* spp. prevalence was from 17.6% (11/51) to 24.1% (21/87), with *C. canis*, *C. suis*, and *C. erinacei* species detected in raccoon dogs (Osten-Sacken et al., 2017; Perec-Matysiak et al., 2023). Raccoon dogs can also carry the zoonotic *C. parvum* (Matsubayashi et al., 2004). Raccoon dogs were introduced in Latvia in 1940s for the fur industry and are considered a newly established invasive species (Paulauskas et al., 2016). With the introduction of invasive species in the native environment, a new reservoir or even host may become established for pathogens, and raccoon dogs can spread at least 25 zoonotic pathogens (Tedeschi et al., 2021). There were around 27,000 raccoon dogs in Latvia, and they do cover smaller areas compared to red foxes, ranging from 193 to 391 hectares. (Süld et al., 2017, Statistiskas portals, 2025). A smaller roaming area may indicate a more localized environmental transmission, that could lead to the shed of *G. duodenalis* cysts in more concentrated areas, which is favorable for further environmental transmission.

Compared to red foxes, younger raccoon dogs had a higher odds ratio of infection with *G. duodenalis* (OR 2.3; $p < 0.05$). These similarities were also observed in domestic dogs. In domestic dogs, giardiasis is more chronic, and cysts can be intermittently shed for several months (Hamnes et al., 2007), but there is a lack of studies on *G. duodenalis* in raccoon dogs, including association with age or clinical manifestation.

A notable observation was the consistent co-infection between *G. duodenalis* and *Cryptosporidium* app. across all three canid species but not in cattle. One possible explanation could be due to sampling timing for cattle – if possible, we tried not to focus on cattle who had signs of diarrhea. For dogs, the sampling was done by the owners, who could have had bias by sending samples of dogs with gastrointestinal symptoms. However, all red fox and raccoon dogs were tested, not considering any clinical signs; therefore, this could show that co-infection of both parasites is an important topic in canids. These results highlight the importance of including wild canids in parasite monitoring, as their movement patterns, possible long-term cyst shedding, and proximity to herds and urban areas might play a significant role in environmental contamination.

4.4. Environmental contamination potential of *Giardia duodenalis* from cattle and canids in Latvia with an emphasis on the zoonotic assemblage A

The species-level prevalence and cyst load of cattle, domestic dogs, red foxes, and raccoon dogs were compared to better understand which of the examined species across three different environments: rural, urban, and wildlife, reveal the highest environmental transmission potential of *G. duodenalis* in Latvia.

Significantly higher *G. duodenalis* prevalence ($p < 0.05$) was in wild canids than pets and livestock in our study. This result may reflect ecological, behavioral, and management-related differences between wild, domestic and productive animals. Unlike dogs and productive animals, endoparasitic diseases are not controlled in wild canids, allowing them to persist in wild animals (Laurenson et al., 2005). Both wild canids are also scavengers, which could increase exposure to contaminated sources (such as feces) and help sustain the *G. duodenalis* transmission cycle without being clinically affected (O'Bryan et al., 2018). Additionally, both red foxes and raccoon dogs tend to roam, and all herd owners reported seeing wild carnivores near their herds. Zoonotic *G. duodenalis* assemblage A and B have been reported in cattle and wild canids so they could introduce these pathogens into cattle herds (Hamnes et al., 2007; Ocan et al., 2015; Debenham et al., 2017). The lack of monitoring of *G. duodenalis* in wildlife and their contact with both natural water sources and forest products, like mushrooms and berries, which are commonly foraged in Latvia, make these animals high-risk *G. duodenalis* shedders (Geldreich, 1996; Grivins, 2021).

Initially, domestic dogs excreted significantly higher cyst load ($p < 0.05$) than cattle and wild canids, but after adjusting the cyst load to the produced average weight of feces per animal species, cattle shed significantly higher ($p < 0.05$) *G. duodenalis* cysts compared to the three canid species. Additionally, 22 out of the 32 interviewed cattle herd owners did not use any treatment before using manure for field fertilization. In 2016, cattle in Latvia created at least 3420 tons of manure, out of which, 6.8% was left at the pastures (Priekulis et al., 2018). Most of the manure that is used in field fertilization in Latvia originates from cattle (Köninger et al., 2021). In the study by Köninger et al. (2021), most of the manure was also not properly treated prior to use in fields in the European Union member states. Using untreated manure for field fertilization can lead to groundwater and surface water contamination (Köninger et al., 2021). *G. duodenalis* cysts can survive for up to several months, especially in cold and wet environments such as puddles (Wang et al., 2023). Regarding canid species, domestic dogs have direct contact with humans, noting their possible contribution to human infection with *G. duodenalis* (Dixon, 2021). Compared to wildlife, the is a higher density of dogs in urban and sub-urban environments, which increases the load of *G. duodenalis* cysts in the environment and dog contact (Papini et al., 2009). There are still discussions about whether dogs contribute to transmitting the zoonotic *G. duodenalis* assemblages A and B (Cai et al., 2021). Nevertheless, out findings reinforce the need for public hygiene interventions, responsible pet waste management, and proper manure treatment before using it for field fertilization.

G. duodenalis assemblage A was detected in cattle and domestic dogs, which had access to surface water sources, highlighting a potential zoonotic risk and water contamination in Latvia. Of the seven cattle herds in Latvia, where *G. duodenalis* assemblage A was detected, one herd was located 570 meters from the river Bērze and another 785 meters from the river Tērvete. Neither of the cattle from these herds had pastures, but the owners of the farms used manure to fertilize the fields. This may lead to the transmission of feces containing zoonotic *G. duodenalis* to both surface and underground water sources, hence the whole ecosystem may be affected (Oliver et al., 2005). Rainfall has been associated with increased vertical and horizontal transfer of *Giardia* due to increased run-off from the fields and the farm facilities if manure or slurry is not properly taken care of (Oliver et al., 2005). *Giardia* cysts do not attach to natural

soil particles, and, in cases of overflow, they can travel independently (Dai & Boll, 2003). To minimize water contamination, it is important to decrease the slurry run-offs from the facilities, properly decontaminate the manure to minimize the viability of *G. duodenalis* cysts before field fertilization, and, if possible, remove direct access to surface water sources in the pastures (Oliver et al., 2005). These findings highlight that agriculture and productive animals, particularly cattle farming, can serve as a significant source of environmental contamination of *G. duodenalis*, emphasizing the need for integrated control measures within the One Health approach. There is evidence that in Latvia, human giardiasis cases are underreported and underdiagnosed (Deksne et al., 2022). During a 20-year period, a total of 1020 cases have been officially reported (average 34 per year) to the Centre of Disease Prevention and Control of Latvia, with most cases being reported between one and six-year-old children (Deksne et al., 2022). Furthermore, data from the clinical diagnostic laboratory showed that *G. duodenalis* prevalence was 2.2% (n = 18,367) when testing feces for *Giardia* antigen, however, in a prospective study, where 584 patients were analyzed, *G. duodenalis* prevalence reached 7.2% (n = 42/584) when applying the immunofluorescence method (Deksne et al., 2022). These *G. duodenalis* findings in humans, especially in the prospective study, highlight that this parasite is present in the human population in Latvia, which reinforces the importance of monitoring animal and environmental sources of *Giardia* and supports the need for integrated prevention strategies under the One Health framework. To fully understand the environmental contamination risks posed by the zoonotic *G. duodenalis* assemblage A in Latvia, further research is needed on its prevalence in surface water bodies, wastewaters and the effectiveness of wastewater treatment plants in removing *Giardia* cysts from drinking water meant for human use.

CONCLUSIONS

1. The prevalence of *G. duodenalis* in individual cattle (8.4%) was low in Latvia. Significantly higher *G. duodenalis* prevalence was observed in 0–3-month-old calves (16.4%, $p < 0.05$) and in male cattle (11.7%), which also excreted the highest cyst load (8017 CPG).
2. Only cattle-specific *G. duodenalis* assemblage E was detected in 74.1% of the herds, with 55 cattle were positive (88.7%), while only the zoonotic *G. duodenalis* assemblage A was detected in three herds (12.0%) with seven (11.3%) cattle being positive, highlighting the need for molecular surveillance to mitigate the zoonotic risks.
3. Key protective factors against *G. duodenalis* in cattle were age (OR 0.4, $p < 0.01$), pasture and manure management, implementation of biosecurity protocols (OR 0.7; $p < 0.05$) and change of shoes for visitors (OR 0.6; $p = 0.05$), while the risk factor was cattle able to leave the farm (OR 2.2; $p < 0.05$), underlining the role of management factors in the spread of *G. duodenalis* at cattle farms.
4. *G. duodenalis* prevalence in domestic dogs was 10.7% with the highest prevalence observed in puppies (18.5%), and male dogs were exposed to a higher risk of infection compared to female dogs ($p = 0.01$).
5. Three *G. duodenalis* assemblages were detected in dogs: canine-specific assemblages C and D, and the zoonotic assemblage A indicating potential zoonotic risk to humans.
6. In dogs, co-infection with *Cryptosporidium* spp. was associated with an increased risk of infection with *G. duodenalis* (OR 10.0, $p < 0.01$); in contrast, on-leash activities outside the city areas were protective (OR 0.4, $p = 0.008$).
7. Prevalence of *G. duodenalis* in red foxes (27.4%) and raccoon dogs (30.8%) was higher than in cattle (8.4%) and domestic dogs (10.7%), however, these animals shed lower cyst loads in the environment (3133 CPG and 14,008 CPG, respectively), suggesting that wild canids may act as frequent *G. duodenalis* carriers.
8. Canine-specific *G. duodenalis* assemblages C and D were detected in wild canids, demostrating their role as reservoirs of dog-adapted assemblages.
9. In wild canids, co-infection with *Cryptosporidium* spp. increased the likelihood of *G. duodenalis* infection for red foxes (OR 111.1, $p < 0.001$) and raccoon dogs (OR 16.0, $p < 0.001$), suggesting that red foxes and raccoon dogs are important, multiple zoonotic parasite reservoirs with their relevance within One Health concept.
10. Although wild canids shared the highest *G. duodenalis* prevalence, cattle shed significantly more cysts (15,960,000 CPG-adjusted), increasing the environmental contamination load. The proximity of *G. duodenalis* assemblage A-shedding animals to surface water bodies underscores the potential for environmental contamination and waterborne transmission.

PRACTICAL RECOMMENDATIONS

1. Strengthen farm-level biosecurity: Because *G. duodenalis* cysts have direct life cycle, are small in size (7–10 µm), robust in the environment and are highly prevalent in Latvian cattle herds, it is important for herd owners to implement strict biosecurity protocols. All personnel and veterinarians entering the herd should use personal protective equipment (such as single-use gloves) and boots provided by the herd owner, to minimize introducing cysts in *Giardia*-free herds. Individual calf pens need to be thoroughly cleaned, disinfected and dry before introducing new, naïve calves, to prevent age-related transmission.
2. Promote personnel, food, and water hygiene: Due to the high numbers of *G. duodenalis* cysts shed by all included animals, especially cattle, hygiene practices around fresh produce should be prioritized. Berries, fruits, vegetables and leafy greens grown near cattle herds, are fertilized with manure, or grow in fields that are easily accessible to canids, should be washed thoroughly using clean water, under pressure to help remove the cysts. As *G. duodenalis* is a waterborne parasite, swimming in recreational waters near cattle herds or grazing pastures, especially after heavy rainfalls, when run-off contamination risk is high, should be avoided due to potential waterborne transmission. Additionally, thorough public hygiene education campaigns focusing on proper produce washing and informational signs about possible *G. duodenalis* contamination near recreational swimming waters should be considered.
3. Increase zoonotic surveillance and awareness: Because zoonotic *G. duodenalis* assemblage A was found in cattle and domestic dogs, it should be important to diagnose and monitor the presence of *G. duodenalis* in both species, especially in cases of human diarrhea outbreaks. The detection of co-infection with *Cryptosporidium* spp. in canids underlines the need for broader diagnostic screening in veterinary and public health settings. Currently, *G. duodenalis* is not under any official environmental or veterinary surveillance in Latvia.
4. Implement and promote the "One Health" framework: Because *G. duodenalis* is highly resistant to environmental conditions, minimizing the risk of contaminating the environment, especially water and food sources, is important. Educational campaigns to companies and agricultural workers, on proper manure management should be prioritized to minimize cyst viability before manure is used for field fertilization. Minimizing manure and slurry run-offs from the cattle facilities is also important, especially near open water sources. Providing clean drinking water in the pastures rather than using surface water sources is strongly recommended, to minimize transmission risk in both cattle and humans.

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APPENDIX

Appendix 1

Example of the questionnaire about individual animals

Sample ID number	Cattle ID number	Age (months)	Sex	Breed	Bought	Consistency of feces

Appendix 2

Agreement form and questionnaire for the cattle study

ID NR_____ DATUMS_____

PIEKRIŠANAS FORMA

Doktorantūras pētījumam tiek aicināti ievākt individuālus fekāliju paraugus no dažāda vecuma govīm un teļiem (aptuveni 20 grami) no taisnās zarnas. Dzīvnieki, no kuriem ir ievāktas fekālijas, tiks atzīmēti tabulā (*Aptaujas tabula par individuāliem dzīvniekiem*).

Novietnes īpašnieks tiek lūgts aizpildīt pievienoto anketu par dzīvnieku novietni. **Anketa ir anonīma, dati par novietni netiks publiski izpausti un izmantoti.**

Fekālijas tiks izmeklētas uz kriptosporīdiju oocistu un žiardiju cistu klātbūtni, un tiks noteikts invāzijas intensitāte līmenis dzīvniekam. Fekāliju izmeklējums ilgs aptuveni vienu nedēļu, un rezultāti tiks nosūtīti uz norādīto e-pasta adresi.

☐ Es piekrītu, ka tiek ņemti fekāliju paraugi no dažāda vecumā govīm novietnē.

☐ Piekrītu, ka, ja būs nepieciešams, būs iespēja noņemt paraugus atkārtoti viena līdz divu gadu laika periodā, iepriekš par to brīdinot.

☐ Vēlos saņemt rezultātus uz e-pastu_____

Saimniecības nosaukums:_____

Novietnes numurs:_____

Ganāmpulka numurs:_____

The questionnaire was developed within the "TRANSPAR" project and has also been used in a master's thesis: "Vienšūņa *Giardia duodenalis* (Stiles, 1902) sastopamība un ietekmējošie faktori Latvijas liellopos (*Bos taurus*)", Author: Maija Selezņova; Supervisor: assoc. prof., *Dr. biol.* Gunita Deksne. Was defended at the University of Latvia, master's programme "Epidemiology and Medical Statistics", on 09.06.2023.

ID NR_____ DATUMS_____

APTAUJAS ANKETA PAR DZĪVNIEKU NOVIETNI

Lūdzu apvilkt atbilstošo atbilžu variantu.

Iespējams ir viens atbilžu variants, izņemot jautājumus, kur ir norādīts savādāk!

1. **Novads**_____
2. **Pagasts**_____
3. **Dzīvnieku skaits novietnē:**_____
4. **Novietnes tips:**

   [1] Piesietā turēšana [2] Nepiesietā turēšana [3] Cits_____

5. **Saimniecības veids:**

   [1] Industriālā (konvencionālā) [2] Bioloģiskā [3] Cits_____

6. **Vai dzīvniekiem ir iespēja iziet ārpus novietnes ēkas? (pārdzenot, pastaigu laukums, ganības, u.t.t.)**

   [1] Jā (Kādā veidā)_____

   [2] Nē

TEĻI

7. **Atnešanās vieta**

   [1] Atsevišķa atnešanās vieta (dzemdību bokss)

   [2] Guļvieta

   [3] Cits _____

8. **Teļu atšķiršanas vecums no mātes**

   [1] Uzreiz pēc piedzimšanas

   [2] Cits _____

9. **Kad tiek iedots pirmpiens pēc piedzimšanas?**

   [1] līdz 2 h [2] 2- 3 h [3] 3- 4h [4] 4-12h [5] ilgāk par 12 h

10. **Cik daudz pirmpiens tiek vidēji iedots (litros) pirmajā ēdināšanas reizē**

    [1] līdz 1 [2] 1-2 [3] 2-3 [4] 3-4 [5] Cits_____

11. **Cik ilgi tiek dots piens teļiem?**

    [1] līdz vienai nedēļai [2] 1 - 2 nedēļas [3] 2-3 nedēļas [4] 3-4 nedēļas [5] 1 mēnesis [6] 2 mēneši

    [7] 2-3 mēneši [8] ilgāk par 3 mēnešiem [8] Tiek dots piena aizstājējs (cik ilgi?)_____

12. **Vai teļi tiek turēti grupās?**

    [1] Jā [2] Nē *(turpināt ar 15. jautājumu)*

13. **Kādā vecumā teļi tiek pārvietoti uz grupu?**

    [1] līdz vienu nedēļu vecumā [2] 1-2 nedēļu vecumā [3] 2-3 nedēļu vecumā

    [4] 3-4 nedēļas [5] 1 mēneša vecumā [6] 2 mēnešu vecumā

    [7] Cits _____

14. **Cik teļu tiek turēti vienā grupā?**

    [1] 1-5 [2] 5-10 [3] 10-15 [4] 15+ [5] Cits_____

15. **Vai teļiem novērojama diareja?**

    [1] Jā [2] Nē (*pāriet uz 22. jautājumu*)

16. **Kādā vecumā tiek novērota diareja?** (*iespējami vairāki atbilžu varianti*)

    [1] Līdz 7 dienu vecumam [2] 7-14 dienu vecumā [3] 14-30 dienu vecumā [4] virs 30 dienu vecumam

    [5] Cits_____

ID NR_____ DATUMS_____

17.Vai diarejas tiek ārstētas?

[1] Jā [2] Nē

18. Vai diareju ārstēšana palīdzēja to samazināt?

[1] Jā [2] Nē

19. Kuros kalendāros mēnešos tiek novērota pastiprināta diarejas esamība?_____

20. Cik procentu no visiem teļiem, novēro diareju ganāmpulkā?

[1] 1-2% [1] 2-5% [2] 5-10 % [3] virs 10%

21.Vai teļi, kuriem novēro diareju, tiek izolēti?

[1] Jā (Cik ilgi?)_____

[2] Nē

PASTAIGU LAUKUMI UN GANĪBAS

22. Vai dzīvnieki ir pieeja pastaigu laukumiem, kuri atrodas ārpus novietnes ēkas?

[1] Jā [2] Nē (*pāriet uz 24. jautājumu*)

23. Vai pie pastaigu laukumiem ir ūdens krātuve, kurai dzīvniekiem ir pieeja?

[1] Jā [2] Nē

24.Vai dzīvnieki tiek ganīti ganībās?

[1] Jā [2] Nē (*pāriet uz 35. jautājumu*)

25.Kad tiek uzsākta ganību sezona?

[1] Martā [2] Aprīlī [3] Maijā [4] Cits_____

26.Kad tiek beigta ganību sezona?

[1] Septembrī [2] Oktobrī [3] Novembrī [4] Cits_____

27.Kas ir ietekmējošais faktors ganību sezonas iesākšanu un nobeigšanu?_____

_____

28.Cik bieži tiek mainīti ganību aploki?

[1] 1 reizi mēnesī [2] Ik pa 2 mēnešiem [3] Ik pa 3 mēnešiem [4] Netiek mainīti [5] Cits_____

29. Vai pie ganībām atrodas ūdens krātuve? (*upe, dīķis, ezers, grāvis*)

[1] Jā (kāda?) _____ [2] Nē (*pāriet uz 32. jautājumu*)

30.Cik tālu no ganību aploka atrodas šī ūdens krātuve?_____

31.Vai dzīvniekiem ir pieeja šai ūdens krātuve?

[1] Jā [2] Nē

32. Vai ūdens krātuve ir savienota ar citu ūdens krātuvi? (*aiztek uz upi, ezeru, u.t.t.*)

[1] Jā (Kādu?) _____ [2] Nē

33.Dzirdināšanas veids ganību aplokos? (*iespējami vairāki atbilžu varianti*)

[1] Atklāta ūdens krātuve (kāda?)_____

[2] Dzeramais ūdens, kas tiek pievests

[4] Nav

[4] Cits _____

ID NR_____ DATUMS_____

NOVIETNES MENEDŽMENTS

34.Kādā veidā tiek tīrīta novietne no kūtsmēsliem?

[1] Automatizēti [2] Manuāli (ar ko?)_____

[3] Abi varianti

35. Ja tiek izmantota manuālā kūtsmēslu tīrīšana, vai šie palīgmateriāli/tehnika tiek tīrīta?_____

_____

36.Cik bieži tiek tīrītas guļvietas no kūtsmēsliem?_____

37.Cik bieži tiek tīrīti teļu individuālie aizgaldi/teļu grupu aizgaldi no kūtsmēsliem?_____

38.Lūdzu, raksturojiet kūtsmēslu krātuves atrašanās vietu

[1] Kaudzē pie novietnes [2] Atvērta tipa mēslu bedrē [3] Slēgta tipa mēslu bedrē

[4] Cits_____

39.Kā tiek utilizēti kūtsmēsli/virca?

[1] Savāc firma

[2] Paši izmanto (Kur tiek izmantota?) _____

[3] Pārdod _____

40.Vai kūtsmēsli/virca tiek apstrādāta pirms tās utilizēšanas/izmantošanas?

[1] Jā (kādā veidā?)_____

_____

[2] Nē

41.Cik bieži kūtsmēsli/virca tiek izvesta no saimniecības teritorijas_____

42.Vai kūtsmēslu krātuves tiek tīrītas pēc kūtsmēslu aizvešanas/izmantošanas (mazgātas)?

[1] Jā (kādā veidā?)_____ [2] Nē

43.Vai pēc teļu uzturēšanās individuālajos boksos un grupu boksos tiek dezinficēti?

[1] Jā [2] Nē (*pāriet uz 45. jautājumu*)

44.Kādā veidā tiek dezinficēti individuālie boksi? _____

_____

45.Kādā veidā tiek dezinficēti teļu grupu boksi?_____

_____

46.Vai liellopi novietnē tiek attārpoti?

[1] Jā [2] Nē

47.Cik bieži tiek attārpoti liellopi novietnē?

[1] 1x gadā [2] 2x gadā [3] Cits _____

48.Vai novietnē ir novērota grauzēju klātbūtne?

[1] Jā [3] Nē (*pāriet uz 51. jautājumu*)

49.Vai novietnē tiek veikta grauzēju kontrole?

[1] Jā [2] Nē

50.Grauzēju kontroles veids (*iespējami vairāki atbilžu varianti*)

[1] Ķīmiskā (indes) [2] Mehāniskā (slazdi) [2] Kaķis [3] Cits_____

51.Vai dzīvnieku personālam tiek nodrošināts maiņas apavi?

[1] Jā [2] Nē

ID NR_____ DATUMS_____

52. Vai veterinārārstam un citām "trešajām" personām, ieejot novietnē, tiek nodrošināts maiņas apavi vai dezinfekcijas paklājs?

[1] Jā [2] Nē

53. Vai novietnes personālam ir bijušas sūdzības par nezināmas izcelsmes diareju, kura ilgst ilgāk par 3 dienām?

[1] Jā [2] Nē

54. Vai darbiniekiem ir iespēja dezinficēt rokas?

[1] Jā [2] Nē

55. Vai novietnes teritorijā atrodas sausā tualete?

[1] Jā [2] Nē

BAROŠANA

56. Kāds barības veids tiek izmantots dzīvnieku barošanai? (*iespējami vairāki atbilžu varianti*)

[1] Totāli miksēta barība [2] Siens [3] Svaiga zāle [4] Skābbarība [5] Skābsiens

[6] Cits_____

NOVIETNES APKĀRTNE

57. Kas atrodas 500-1000m rādiusā ap novietni *(iespējami vairāki atbilžu varianti)*?

[1] Citas apdzīvotas mājas [2] Ceļš/dzelzceļš [3] Pļavas [4] Mežs [5] Krūmāji

[6] Ūdens krātuve [7] L/S zeme

58. Lūdzu, novērtējiet attālumu līdz tuvākajai saimniecībai, kurā ir vairāk par 10 govīm_____

59. Vai ap novietnes teritoriju atrodas ūdens krātuve (dīķis, ezers, upe, grāvji)?

[1] Jā (Kāda veida un cik tālu no novietnes?)_____

[2] Nē

60. Vai saimniecībā ir citi lauksaimniecības dzīvnieki? (*iespējami vairāki atbilžu varianti)*

[1] Zirgi [2] Aitas [3] Kazas [4] Cūkas [5] Mājputni [6] Truši [7] Nē

61. Vai novietnē ir sastopami citi mīļdzīvnieki? (*iespējami vairāki atbilžu varianti*)

[1] Kaķi [2] Suņi [3] Nē [4] Citi_____

62. Vai novietnes/ganību apkārtnē ir novēroti savvaļas dzīvnieki?

[1] Jā (*lūdzu, aizpildiet tabulu, ievelkot krustiņu pie attiecīgā laika*) [2] Nē

Meža dzīvnieku sugas	**Laiks**				
	Katru dienu	Vienu reizi nedēļā	Vienu reizi divās nedēļās	Vienu reizi mēnesī	Retāk nekā vienu reizi mēnesī
Mežacūkas					
Savvaļas atgremotāji					
Savvaļas plēsēji					
Savvaļas putni					

Appendix 3

Description of the study for domestic dogs

Kontaktinformācija: Margarita Terentjeva, e-pasts: margarita.terentjeva@llu.lv; tālr.: 29 179 010
Maira Mateusa, e-pasts: maira.mateusa@bior.lv; tālr.: 28 656 424

Aicinājums piedalīties pētījumā par *Cryptosporidium* spp. un *Giardia duodenalis* sastopamību suņiem Latvijā

Institūts "BIOR", laika posmā no 2022. gada **1. janvāra līdz 31. decembrim (vai līdz būs ievākts pētījumam pietiekošs paraugu apjoms)**, ir uzsācis pētījumu par kriptosporīdiju (*Cryptosporidium* spp.) un žiardiju (*Giardia duodenalis*) sastopamību suņos Latvijā. Tiek aicināti atsaukties visi interesenti, kā arī veterinārārsti, suņu audzētavas un dzīvnieku patversmes.

Kriptosporidioze un žiardiāze ir slimības, ar ko var slimot gan cilvēki, gan dažādu sugu dzīvnieki, tajā skaitā suņi. Šīs slimības izpaužas kā akūta caureja, kas var ilgt vienu līdz divas nedēļas, reizēm ilgāk. Bērni, vecāki cilvēki un cilvēki ar samazinātu imunitātes ir uzņēmīgākās cilvēku grupas. Savukārt suņi var saslimt visos vecumos, bet visuzņēmīgākie ir kucēni ap sešu mēnešu vecumu. Suņu fekālijas, kas invadētas ar parazītu (oo)cistām, ir nozīmīgs piesārņojuma avots cilvēkiem. Pat dažas kriptosporīdiju oocistas un žiardiju cistas cilvēkiem var ierosināt saslimšanu. No sabiedrības veselības viedokļa ir būtiski uzraudzīt parazītu sastopamību suņos, jo tie ir vieni no tuvākajiem mājdzīvniekiem cilvēkiem.

Pētījuma mērķis:
Novērtēt *Cryptosporidium* spp. un *Giardia duodenalis* parazītisko vienšūņu sastopamību suņos, raksturot šo vienšūņu ģenētisko daudzveidību un patogēnu infekcijas avotus.

Paraugu ievākšanas procedūra:
1. Fekāliju paraugu **(10-15g, apmēram pilna sauja)** ievākt pēc **iespējas svaigāku** un to ievietot ūdensnecaurlaidīgā iepakojumā (plastmasas vai stikla trauks, plastmasas maisiņš).
2. Līdz nogādāšanai laboratorijā **paraugu glabāt +4 °C** (ledusskapī).
3. **Obligāti, kopā ar fekāliju paraugu, aizpildīt anketu par suņa identifikāciju un paradumiem**.
4. Ievāktos paraugus un anketas nogādāt uz Institūta "BIOR" laboratoriju Rīgā (Lejupes iela 3), vai uz Jums tuvāko Institūta "BIOR" Paraugu Pieņemšanas kabinetu (Rīgā, Balvos, Bauskā, Daugavpilī, Gulbenē, Jēkabpilī, Jelgavā, Krāslavā, Liepājā, Limbažos, Ludzā, Madonā, Ogrē, Preiļos, Rēzeknē, Saldū, Talsos, Tukumā, Valmierā un Ventspilī (darba laikus skatīt BIOR mājas lapā: https://bior.lv/lv/kontakti/paragu-pienemsanas-laiki) **ar norādi "Personīgi Parazitoloģijai"**.
5. Dzīvnieku patversmju, audzētavu un veterināro klīniku gadījumā, lūdzu, sazināties ar pētījuma kontaktpersonām, par nepieciešamību sagādāt iepakojumus paraugu ievākšanai, drukātas anketas u.t.t.

Jūsu ieguvumi no pētījuma:
1. Bez maksas veiksim parazitoloģiskos izmeklējumus, īpašu uzmanību pievēršot *Cryptosporidium* spp. un *Giardia duodenalis* parazītu klātbūtnes noteikšanai.
2. Ja vēlēsieties, nosutīsim individuālus testēšanas rezultātus divu nedēļu laikā, pēc parauga saņemšanas laboratorijā. Šajā gadījumā, aizpildot anketu, obligāti jānorāda e-pasts.

Kontakti:
Ja Jums ir interese piedalīties pētījumā, nodrošināt paraugu ievākšanu, vai ja Jums radušies papildu jautājumi vai neskaidrības, lūdzu sazinieties ar mums:

Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta,
Latvijas Lauksaimniecības universitātes
Asoc. prof., Dr. vet. med.; vadošā pētniece
Margarita Terentjeva
e-pasts: margarita.terentjeva@llu.lv
Tālr.: 29179010

Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta, Latvijas Lauksaimniecības universitātes
Doktorantūras studente, pētniece
Maira Mateusa
e-pasts: maira.mateusa@bior.lv
Tālr.: 28 656 424

Pētījums tiek veikts LZP-2021/1-0055 "Pārtikas parazitāro patogēnu pārnese no dzīvniekiem uz cilvēku: TRANSPAR" ietvaros

Appendix 4

Questionnaire form for the domestic dogs study

Pārtikas paraizītāro patogēnu pārnese no dzīvniekiem uz cilvēku: TRANSPAR
Kontaktinformācija: Margarita Terentjeva, e-pasts: margarita.terentjeva@llu.lv; tālr.: 29 179 010
Maira Mateusa, e-pasts: maira.mateusa@bior.lv; tālr.: 28 656 424

Ievākšanas datums: *dd/mm/gggg*	**Parauga ID** *(aizpilda laboratorijā):*

Dati par saimnieku:

Saimnieka vārds:	Saimnieka uzvārds .....
Adrese (pašvaldība, pasta indekss)	
E-pasts (gadījumā, ja vēlas saņemt izmeklējumu rezultātus)	

Dati par suni:

Suņa vārds:	Šķirne:	Dzimums: ◻ V ◻ S	Vecums (gados): .....

Dzīvesvieta:	◻ Dzīvoklis	◻ Privātmāja	◻ Lauku teritorija	◻ Dzīvnieku patversme

Suna aktivitātes/pastaigu vietas: (iespējamas vairākas atbildes)

Aktivitāšu vietas:	Nekad	Vismaz reizi gadā	Vismaz reizi mēnesī	Vismaz reizi nedēļā	Vismaz reizi dienā	Pastāvīgi uzturas
Pagalms	◻	◻	◻	◻	◻	◻
Pilsētvide	◻	◻	◻	◻	◻	◻
Publisks parks	◻	◻	◻	◻	◻	◻
Pļava	◻	◻	◻	◻	◻	◻
Mežs	◻	◻	◻	◻	◻	◻
Cits	◻	◻	◻	◻	◻	◻

Aktivitāšu veids:				
◻ Pastaigas pilsētā ◻*tikai pie pavadas*	◻ Pastaigas dabā ◻*tikai pie pavadas*	◻ Medības	◻ Sargsuns	◻ Cits.....

Veselības stāvoklis:

Sunim, pēdējos 6 mēnešus, tiek novērota caureja, kas ilgst vairāk par 3 dienām?	◻ Jā	◻ Nē
Vai sunim pēdējos 6 mēnešus novēro periodisku, pārejošu caureju?	◻ Jā	◻ Nē

Attārpošana

Attārpošanas biežums:	Nekad	Mazāk kā reizi gadā	Reizi gadā	Divreiz gadā	Reizi 3 mēnešos	Reizi mēnesī
	◻	◻	◻	◻	◻	◻

Pēdējā attārpošana (datums):	**Attārpošanas medikamenta nosaukums:** .....
Kurš veica attārpošanu? Saimnieks pats ◻ Veterinārārsts ◻ Cits	

Suņa barošana (iespējamas vairākas atbildes)

◻ Termiski neapstrādāta gaļa	◻ Komerciālā barība	◻ Mājas apstākļos gatavota barība	◻ Piekļuve kaušanas atlikumiem	◻ Medījums

Papildu informācija

Ja suns dzīvo lauku mājā/saimniecībā/fermā, vai tur ir: ◻ cūkas, ◻ aitas, ◻ liellopi, ◻ cits....., ◻ **Nav** lauksaimniecības dzīvnieku
Lauksaimniecības dzīvnieku kaušana mājas apstākļos: ◻ Jā, ◻ Nē

Saimnieka apstiprinājums:
Es piekrītu parauga materiāla un anketas atbilžu izmantošanai zinātniskiem mērķiem. Visa iegūtā informācija tiks padarīta par anonīmu. Es piekrītu, ka pētījumu rezultāti var tikt izmantoti zinātniskiem pētījumiem un publikācijām.

DATUMS UN PARAKSTS: _____

Appendix 5

Questionnaire about Individual cattle for *Giardia duodenalis* in cattle in Latvia

Question	Answer	Examined cattle
Total Cattle examined		973
Region	Kurzeme	283
	Latgale	106
	Vidzeme	244
	Zemgale	340
Age groups	0-3	325
	4-24	282
	>24	369
Sex	Female	853
	Male	120
Breed	AB	2
	AI	2
	DS	19
	HE	1
	HM	699
	HS	122
	LB	71
	LI	35
	SV	1
	XP	4
	XX	14
	ZS	3
Diarrhea	Yes	125
	No	848

Appendix 6

Questionnaire about cattle herds for *Giardia duodenalis* in cattle in Latvia

Question	Answer options	Responses
Herd type:	Tethered	7
	Untethered	16
	Untethered and tethered	9
Farming type:	Industrial	28
	Biological	4
Can cattle leave the herd building? Area, pasture, etc.)	Yes	23
	No	9
Place of calving	Separate calving space	18
	Sleep area	14
Age of calf at the separation from the dam	Right after birth	28
	After 24 hours	2
	After 1 month	2
When is colostrum given after birth?	Up to 2 hours	26
	2- 3 h	2
	3- 4h	3
	4- 12 h	1
How much colostrum is given (in liters) on the first time?	1-2	10
	2-3	16
	3-4	6
How long is milk given to calves?	< 1 week	1
	1 week	1
	2-3 weeks	2
	3-4 weeks	8
	4 weeks	2
	8 weeks	2
	8-12 weeks	11
	> 12 weeks	4
	Milk replacer	1
Are calves held in groups?	Yes	28
	No	4
At what age are calves moved to a group?	Up to one week of age	3
	1-2 weeks	2
	2-3 weeks old	10
	3-4 weeks old	6
	1 month old	3
	2 months old	4
	No grouping	4
How many calves are held in one group?	1-5	11
	5-10	11
	10-15	3
	15+	3
	No grouping	4
Do calves have diarrhea?	Yes	30
	No	2
At what age is diarrhea seen in calves?	Up to 7 days old	10
	1-14 days	9
	7-14	5

Question	Answer options	Responses
At what age is diarrhea seen in calves?	7-60	1
	>30d	4
	No diarrhea	3
Is diarrhea treated?	Yes	26
	No	3
	No diarrhea	3
Did diarrhea treatment help?	Yes	24
	No	5
	No diarrhea	3
In which calendar month does diarrhea flare up?	All Year	21
	Autumn	2
	Autumn and Winter	1
	None	3
	Spring	1
	Spring and Autumn	2
	Winter	2
How many percent of calves have diarrhea?	1-2%	6
	2-5%	3
	5-10 %	3
	Above 10%	17
	No diarrhea	3
Are calves with diarrhea isolated?	Yes	10
	No	22
Can cattle leave the herd house for a walking area?	Yes	15
	No	17
Is there a water body in the walking area that cattle can access?	Yes	7
	No	25
Are cattle pastured?	Yes	19
	No	13
When does the pasture season start?	April	1
	May	18
	No pastures	13
When does the pasture season end?	September	1
	October	13
	November	5
	No pastures	13
Which factors affect the start and end of pasture season?	End of grass	5
	Weather	14
	No pasture	13
How often is the pasture paddock changed?	Never	6
	No pasture	13
	Often	7
	Rarely	3
	Very often	3
Is there water in the pasture? (river, pond, lake, ditch)	Yes	9
	No	10
	No pasture	13
Type of free water available in the pasture	Ditch	4
	Lake	2
	No free water is available	9
	No pasture	13
	Pond	2

Question	Answer options	Responses
	River	2
How far away from pasture is this waterbody?	50	1
	100	1
	In pasture	8
	No free water is available	9
	No pasture	13
Can cattle access this waterbody?	Yes	6
	No	26
Is this waterbody connected to another waterbody? (Flows to a river, lake, etc.)	Yes	7
	No	25
How do cattle drink water in the pasture?	Ditch	1
	Drinking water	17
	None	13
	River	1
How is manure removed from the herd?	Automatically	2
	Manually	11
	Both	19
If manual manure cleaning is used, is the equipment cleaned?	Water	12
	No	20
How often are sleeping areas cleaned from manure?	Less often	1
	Not often	1
	Often	16
	Rarely	3
	Very often	11
How often are individual calf pens/calf group pens cleaned of manure?	Never	1
	No grouping	1
	Often	18
	Rarely	12
Please describe the location of the manure storage	A pile next to the facilities	8
	Open manure pit	16
	Closed manure pit (lagoon)	8
Where is manure/slurry used?	Field fertilization	32
Is manure/slurry processed before its utilization/use?	Yes	10
	No	22
How is manure/slurry processed before usage?	Fermentation	2
	Lagun	8
	No treatment	22
How often is manure/slurry removed from the farm area?	Everyday	3
	Five times a year	1
	Four times a year	1
	Once per year	4
	Three times per year	1
	Twice per year	22
Are manure storages cleaned after manure removal/use (washed)?	Yes	1
	No	31
How are manure storages cleaned?	Water	1
	No cleaning	31
Are the calf boxes disinfected after their stay in the individual boxes and group boxes?	Yes	23
	No	9

Question	Answer options	Responses
How are the individual boxes disinfected?	Disinfectant	21
	Fire	1
	No cleaning	4
	No grouping	5
	Water	1
How are calf group boxes disinfected?	Disinfectant	17
	No cleaning	5
	No grouping	6
	Water	4
Are the cattle dewormed?	Yes	10
	No	22
How often are cattle dewormed	1x a year	5
	Once in a lifetime	5
	No deworming	22
Has the presence of rodents been observed on the herd?	Yes	16
	No	16
Is rodent control carried out on the herd?	Yes	31
	No	1
Type of rodent control	Poison	18
	Cat	13
	No control	1
Is the staff provided with protective equipment (change of shoes)?	Yes	28
	No	4
Are the veterinarian and other "third" persons provided with a change of shoes or a disinfection mat when entering the herd?	Yes	22
	No	10
Have the employees complained about diarrhea of unknown origin lasting longer than 3 days?	Yes	4
	No	28
Can employees disinfect their hands?	Yes	20
	No	12
Is there a dry toilet in the herd area?	Yes	6
	No	26
Are cattle few with totally mixed feed?	Yes	25
	No	7
Are cattle few with hay?	Yes	14
	No	18
Are cattle few with fresh grass?	Yes	4
	No	28
Are cattle few with silage?	Yes	17
	No	15
Are there houses near herd premises?	Yes	26
	No	6
Is there a road/railroad near the herd premises?	Yes	22
	No	10
Are there pastures near the herd premises?	Yes	23
	No	9
Is there a forest near the herd premises?	Yes	19
	No	13
Are there bushes near the herd premises?	Yes	19
	No	13
	Yes	22

Question	Answer options	Responses
Is there a water reservoir (pond, lake, river) near the herd premises?	No	10
Is there agricultural land near the herd premises?	Yes	28
	No	4
Please estimate the distance in meters to the nearest herd with more than 10 cows	100	1
	200	1
	400	1
	500	5
	600	2
	800	1
	900	1
	1000	5
	1500	1
	2000	3
	3000	5
	5000	3
	7000	1
	8000	1
	10000	1
What type of water reservoir (pond, lake, river, ditches) around the herd area?	No water reservoir	7
	Ditch	5
	Lake	6
	Pond	6
	River	8
Are there other farm animals on the farm?	Yes	5
	No	27
Horses	Yes	3
	No	29
Sheep	Yes	1
	No	31
Goats	Yes	2
	No	30
Domestic pigs	Yes	1
	No	31
Domestic birds	Yes	2
	No	29
Are there other pets on the farm?	Yes	29
	No	3
Cats	Yes	24
	No	8
Dogs	Yes	15
	No	17
Have wild animals been observed in the vicinity of the herd/pasture?	Yes	32
Wild boar	Yes	18
	No	14
Wild ruminants	Yes	26
	No	6
Carnivores	Yes	26
	No	6
Wild birds	Yes	27
	No	5

Appendix 7

Responses to each questionnaire that was made to get information about domestic dogs

Question	Answer	Responses
Region	Kurzeme	57
	Vidzeme	206
	Latgale	23
	Zemgale	87
Breed	Breed	218
	No breed	155
Sex	Male	190
	Female	183
Age group	Puppy	65
	Adult	193
	Senior	96
	Geriatric	19
Place of living	City	230
	Countryside	100
	Suburbs	43
Owner or shelter dog	Owner	328
	Shelter	45
Activity in backyard	Yes	54
	No	319
Activity in city	Yes	160
	No	213
Activity in public park	Yes	175
	No	198
Activity in medow	Yes	254
	No	119
Activity in forest	Yes	256
	No	117
Activity in city without leash	Yes	214
	No	159
Activity in city with leash	Yes	152
	No	221
Activity outside city with leash	Yes	302
	No	71
Activity outside city without leash	Yes	119
	No	254
Diarrhea in the last six months	Yes	39
	No	174
	Not answered	150
Intermittent diarrhea in the last six months	Yes	54
	No	159

Question	Answer	Responses
Intermittent diarrhea in the last six months	Not answered	160
Deworming frequency	Regularly	250
	Rarely	97
	Never	11
	Not answered	15
Feeding raw food	Yes	180
	No	193
Feeding home-made food	Yes	159
	No	214
Feeding game meat	Yes	47
	No	326
Access to farm animals	Yes	34
	No	339
Which animals does the dog has access to?	None	339
	Chickens	12
	Horses	3
	Ruminants	18
	Pigs	1
Farm animal slaughter at home	Yes	14
	No	359
Which animals are slaughtered at home	None	359
	Ruminants	6
	Chickens	7
	Pigs	1
Does the dog have access to slaughter byproducts?	Yes	29
	No	344

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