Norges miljø- og biovitenskapelige universitet

Thesis 2022 (15 ECTS credits)
Department of Paraclinical Sciences (PARAFAG)

Prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in Lambs in South-West of Norway: A Longitudinal Study

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Abstract

Title: Prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in Lambs in South-West of Norway: A Longitudinal Study

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Cryptosporidium spp. and *Giardia duodenalis* are protozoan parasites that infect a variety of vertebrates, and most of the common farm animals are susceptible to infection. The pathogens have been reported globally with regional variations. Most commonly, it is the youngest animals that show clinical signs of the disease, seen in form of diarrhea. *Cryptosporidium parvum* is zoonotic and known to be a major cause of diarrhea in calves and is also known to affect small ruminants. *Giardia* is found in many animals, with zoonotic potential of some of the assemblages. Data were collected from each farm using a questionnaire to identify risk factors. The aim of this study was to investigate *Cryptosporidium* spp. and *Giardia duodenalis* infections in lambs in South-West of Norway. A total of 507 fecal samples were collected from 17 different flocks of lambs in South-West of Norway (11 in Sunnhordland and 6 in Dalane) aged from 2 days to 6 weeks. The samples were analyzed using immunofluorescent antibody staining, followed by PCR and sequencing of positive samples for molecular characterization. Of these, 63 (22.4%) were positive for *Cryptosporidium* spp. and 61 (21,7%) positive for *G. duodenalis* by IFAT/microscopy. Molecular methods on selected samples identified two species of *Cryptosporidium* (*C. parvum* and *C. ubiquitum*) and *Giardia duodenalis* assemblage E. Most of our positive samples were from asymptomatic lambs. *Cryptosporidium* spp. infection peaked at 2-3 weeks of age, while *G. duodenalis* infection peaked at 6 weeks of age. The finding suggests lambs might be capable of harboring potentially zoonotic *Cryptosporidium* spp. Further research is needed to better understand the epidemiology of the disease in the study areas.

Definitions and abbreviations

Bp	Base pair, two nucleotides on opposite complementary DNA or RNA strands
Cyst	The infective stage of *Giardia spp.*
DAPI	4', 6-diamidino-2-phenylindole, used for staining before fluorescence imaging to identify nuclei
FITC	Fluorescein isothiocyanate, a marker substance bound to antibodies
Genotype	A classification group within species based on molecular markers
Genus	A taxonomic category that ranks above species and below family
IFAT	Immunofluorescent antibody test
Prepatent period	The period between infection with a parasite, to the production of (oo)cysts or eggs
Infectious dose	The quantity of a pathogen (here: measured in number of a sporulated oocysts or cysts) necessary to cause infection in a susceptible host
Morphology	The structure, shape, size, and arrangements of the parts of an organism
Nested PCR	A modified PCR run in two rounds using two sets of primers, resulting in a smaller DNA-segment, and thus increasing sensitivity and specificity
Pathogen	A parasite, virus or bacterium that can cause disease
PCR	Polymerase chain reaction used to amplify small segments of DNA
Prevalence	The proportion of a chosen population found to be positive at a specific time
Protozoa	A group of eukaryotic single-celled microscopic organisms, such as *Cryptosporidium* spp. and *Giardia* spp.
Sensitivity	Describes the accuracy of a test, and refers to the probability of a positive test, when the sample is positive (true positive rate)
Shedding	Refers to *Cryptosporidium* spp. oocysts and *Giardia spp.* cysts being secreted by an animal, and thus being available by collection of stool samples
Specificity	Describes the accuracy of a test, and refers to the probability of a negative test, when the result is negative (true negative rate)
Sporulated oocyst	The infective stage of *Cryptosporidium* spp.
Trophozoite	An active, motile stage in the life cycle of certain protozoa
Ubiquitous	Referring to something that seems to be found "everywhere"
Virulence	Microbial traits that promote host damage
Zoonosis	An infectious agent that can be transmitted from animals to humans, or from humans to animals

Introduction

Cryptosporidium spp.

Cryptosporidium spp. is a genus of ubiquitous protozoan parasites with more than 40 identified species and genotypes (Ryan et al., 2021) that cause diarrhea in animals and humans, with young animals being most frequently affected. Cryptosporidiosis has been documented in ruminants globally (Santin, 2020). There are many different species of *Cryptosporidium*, some have the ability to infect more than one species, and also be zoonotic, whereas others are considered to be more host-specific. Three species of *Cryptosporidium* are considered dominant in small ruminants: *C. parvum*, *C. ubiquitum* and *C. xiaoi* (Guo et al., 2021; Santin, 2020). Infection in neonatal to young animals with *Cryptosporidium* that leads to clinical symptoms are of great economic significance to the farmers as the diarrhea most likely will stunt its ability to thrive and grow, or in some cases lead to death (Díaz et al., 2015).

Morphology and lifecycle

Cryptosporidium spp. have a complex lifecycle (Figure 1) with both sexual and asexual stages, as well as invasive stages (Leitch & He, 2012). The parasite undergoes excystation (a) in the intestines after being ingested by a host in the infective stage and releasing four sporozoites (b). The sporozoites invade the intestinal epithelium by creating a parasitophorous vacuole (c) that allows them to maintain an extracytoplasmic, but intracellular, location (Foster & Smith, 2009). From here, the sporozoites will first undergo asexual reproduction (d, e) to form merozoites, and then undergo sexual reproduction as type II meronts (f) differentiate into male (microgamonts) and female (macrogamonts). The microgamont (g) and the macrogamont (h) will fuse into zygotes (i) which will further develop into thick-walled

oocysts (j) that undergo sporogony to form sporulated oocysts (k) containing four sporozoites in the intestine before being excreted into the environment together with the feces (Santin, 2020). These sporulated oocysts, ranging from 3-6 µm (Bouzid et al., 2013) will be immediately infective to other hosts. Approximately 20% of the zygotes form thin-walled oocysts (l) that represent the autoinfective life cycle of *Cryptosporidium*, maintaining the parasite infection in the host (Bouzid et al., 2013).

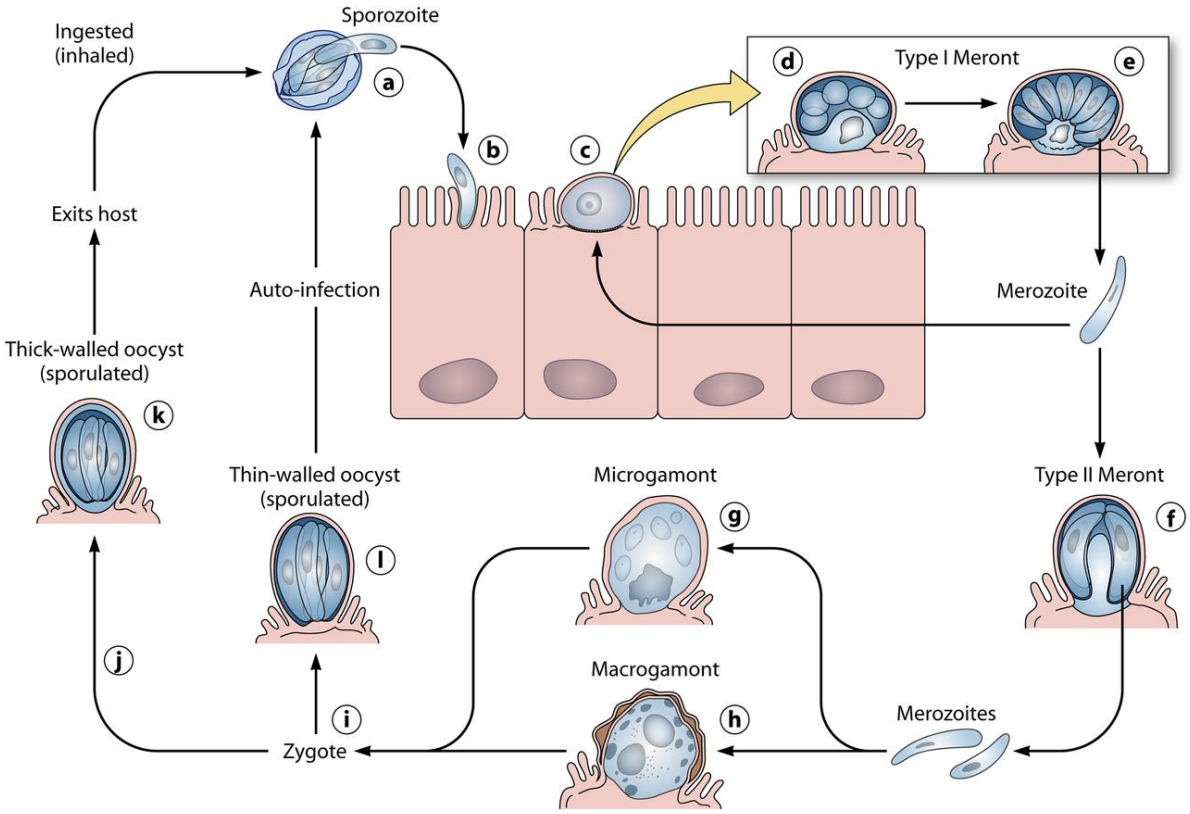

Figure 1: Life cycle of *Cryptosporidium. Image: Current WL, Garcia LS. Cryptosporidiosis. Clin Microbiol Rev. 1991*

Route of transmission and survival of oocysts

The parasite spreads via the fecal-oral route, where it can directly infect through ingestion of oocysts from feces or indirectly through contaminated items such as water or food (Kifleyohannes & Robertson, 2020; Robertson et al., 2006a; Santin, 2020; Utaaker et al., 2017). *Cryptosporidium* oocysts can endure harsh conditions, and hence survive in the

environment for a long period of time. They are resilient to many common disinfectants such as chlorine, and able to survive in barns, even after extensive cleaning (Utaaker et al., 2017).

An infected host, especially a neonatal animal, will excrete large numbers of oocysts. For example, neonatal calves can shed more than 2 million oocysts a day on a span of 1-2 weeks (Nydam et al., 2001; Santin, 2020). This, combined with the low infectious dose of about 10-30 oocysts (Santin, 2020), makes *Cryptosporidium* ssp. a very difficult parasite to manage once there is an outbreak on the farm. The parasite can also have an auto-infective cycle, in which the oocysts that have sporulated in the intestine are released into the host, which will be reinfected (Santin, 2020).

Disease and virulence

Cryptosporidium has been considered an important cause of neonatal diarrhea in calves since the 1970s, and has also been recognized in lambs (Dessì et al., 2020; Paz E Silva et al., 2014; Robertson et al., 2010). The prepatent period is 2-5 days in lambs, and 2-7 days in calves (Witola, 2021). The most common symptom associated with cryptosporidiosis is diarrhea, but fever, malabsorption and lack of appetite also occur (Santin, 2020). In ruminants, the disease is mostly found in neonatal animals which will become infected and begin excreting oocysts shortly after being born. Older animals can have subclinical infections, and hence be a source of infection for newborns (Causapé et al., 2002). Ewes around the time of parturition will shed more oocysts due to suppressed immunity (Ortega-Mora et al., 1999; Xiao et al., 1994). One study found that the highest prevalence of shedding lambs will be at 21 days old, while others determine the peak to be in lambs younger than 14 days old (Santín et al., 2007). It is also possible for animals to be asymptomatic carriers that excrete oocysts in smaller numbers (Santín et al., 2007).

Several putative virulence factors have been identified in *Cryptosporidium* spp., most commonly in *C. parvum*. They include excystation, motility, adhesion and invasion of the intestinal epithelium, intracellular multiplication and survival in the host (Bouzid et al., 2013). *Cryptosporidium spp*. normally does not cause systemic infections, rather, the parasite establishes a membrane-bound compartment in which it attaches to the apical surface of the intestinal epithelium, thus causing significant absorptive and secretory abnormalities. The loss of function in the gut, commonly causing diarrhea, could be the result of direct damage to the epithelium, or indirectly through the damaging effect of cytokines and inflammatory cells recruited by the host immune system. The exact mechanism for cell damage during *Cryptosporidium* infection remains unknown. However, we know that several phospholipases, proteases, and hemolysins cause direct tissue damage (Bouzid et al., 2013; Okhuysen & Chappell, 2002).

Zoonotic potential

The most common species of zoonotic *Cryptosporidium* found in humans with clinical infection is *C. parvum*. Other species, such as *C. ubiquitum,* are considered as emerging zoonotic pathogens, especially in industrialized countries (Fayer et al., 2010; Leitch & He, 2012; Li et al., 2014). Infections with *Cryptosporidium* in humans are characterized by self-limited, profuse, watery diarrhea that, in healthy persons with normal immune systems, can last up to 3 weeks (Leitch & He, 2012). In people who are immunocompromised, an infection can lead to life-threatening malnutrition and wasting (Santin, 2020).

In Wales and England between 2009-2017, 42 % of the 178 reported *Cryptosporidium* outbreaks were determined to be due to animal-contact, all with farm animals and with *C. parvum*. Lambs were most commonly indicated as the sources of infection (Chalmers et al.,

2019). In the United States during the same time period (2009-2017), 86 (19.4%) of the 444 reported outbreaks were related to animal-contact, although most were related to cattle (Gharpure et al., 2019). Two outbreaks linked to children visiting sheep farms have been documented in Norway (Lange et al., 2014)

The public health significance of *Cryptosporidium* spp. in sheep has gained more attention due to the availability of molecular methods (Díaz et al., 2015; Santín et al., 2007). It is now possible to identify subtypes of *C. parvum* and *C. ubiquitum* that can be categorized as human-specific, animal-specific and zoonotic subtypes (Santin, 2020).

Cryptosporidiosis in sheep

Three species of *Cryptosporidium* are common in sheep, although others have been reported occasionally (Wang et al., 2010). Most studies report a predominance of *C. ubiquitum* and *C. xiaoi* worldwide, however, *C. parvum* has been found to be the dominant species in Europe (Díaz et al., 2018; Papanikolopoulou et al., 2018). There also appears to be some degree of co-infection with several *Cryptosporidium* in some studies, where several species were identified from the same sheep (Santín et al., 2007). Fewer studies on *Cryptosporidium* have been conducted on sheep than on cattle, and an age distribution similar to that of cattle has not yet been established, particularly information on post-weaned and adult sheep are very limited (Díaz et al., 2018).

In a study from northern Greece where the sampled lambs were around 7-10 days old, *C. parvum* was the dominant species (Papanikolopoulou et al., 2018). Another study from northern Spain with lambs younger than 35 days found similar results (Díaz et al., 2015). Both of these studies were conducted on diarrheic lambs, supporting the trend that most

clinically ill lambs appear to be infected with *C. parvum* (Santin, 2020). *C. parvum* have been reported in lambs with no clinical disease as well (Paraud & Chartier, 2012).

C. ubiquitum has been found to be the predominant species in non-diarrhetic, post-weaned lambs in Norway (Robertson et al., 2010), and the UK (Elwin & Chalmers, 2008). This species has also been found in non-diarrhetic neonatal/pre-weaned lambs in the United States (Santín et al., 2007), and Belgium (Geurden et al., 2008). One study in China have found *C. ubiquitum* in similar numbers in all age groups: preweaned and postweaned lambs, adult sheep, and ewes preparturition and 0–5 weeks post-parturition sheep (Wang et al., 2010).

C. xiaoi is considered to be the most host-specific of the three species, infecting mostly small ruminants. Attempts to infect cattle and mice have been unsuccessful. *C. xiaoi* have been found both in asymptomatic and symptomatic lambs, usually younger than one month (Fayer & Santín, 2009). It is not found to be zoonotic (Fan et al., 2021).

Giardia duodenalis

Giardia duodenalis is a species of flagellate protozoans which has been reported from most continents and identified in all common agricultural animals. The parasite, sometimes described as *G. intestinalis* and *G. lamblia,* is the species with the greatest zoonotic potential (Veterinærinstituttet, 2022*)*. Other *Giardia*-species exist in animals such as marsupials and amphibians (Santin, 2020), but will not be described further as they are not relevant to ruminants/this study. Hence, all mentions of *Giardia* further on references *G. duodenalis.* Although no direct zoonotic transmission in natural elements has been proven (Cai et al., 2021; Dixon, 2021), around 280 million human cases of giardiasis are reported annually, with higher numbers in developing countries (Einarsson et al., 2016). *Giardia* is also considered to have economic consequences for farmers as it potentially stunts growth, cause diarrhea or

failure to thrive in young ruminants (Geurden et al., 2010; Santin, 2020).

Morphology and lifecycle

G. duodenalis has several genotypes, called assemblages, where only some are considered zoonotic. Eight assemblages (A-H) are recognized, where each assemblage again features several subtypes (Cai et al., 2021; Dixon, 2021). The assemblages are morphologically indistinguishable from each other and distinctions are made based on molecular characteristics (Santín et al., 2007).

Assemblages	***Commonly used name***	***Species susceptible***
A	*Giardia duodenalis*	Humans, non-human primates, livestock, , dogs, cats, other mammals
B	*Giardia enterica*	Humans, non-human primates, dogs, cats, other mammals
C	*Giardia canis*	Dogs and wild canines
D		Dogs and wild canines
E	*Giardia bovis*	Ruminants (cows, goats, sheep)
F	*Giardia cati*	Cats
G	*Giardia simondi*	Rats
H		Marine mammals (seals)

Table 1: *Giardia* spp. assemblages and susceptible species.. *Table modified from Granum, Per Einar: Matforgiftning: smitte gjennom mat og vann (2015)*

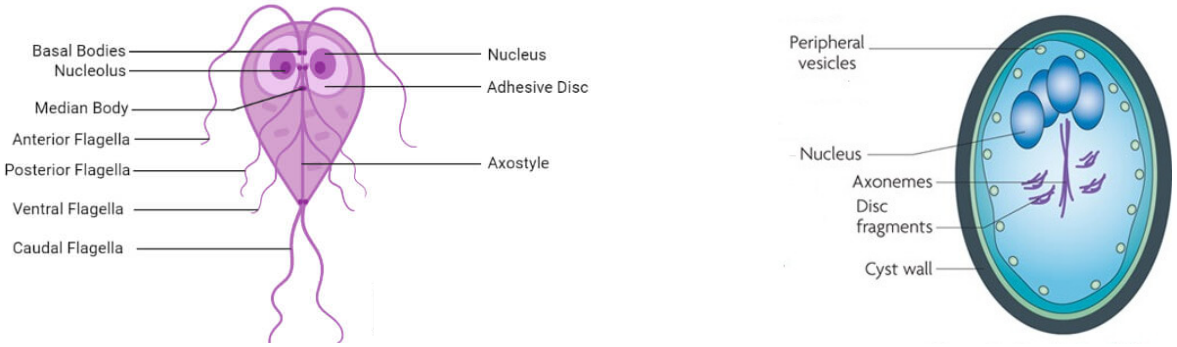

Figure 2: *Giardia* morphology*:* Trophozoite to the left and cyst to the right. Image (left): The Biology Notes, (right): Nature reviews Microbiology

The lifecycle of *G. duodenalis* (figure 3) consists of only two stages: the cyst (the infective stage, measuring 8-12 µm) and the trophozoite (the motile stage). The cysts are ingested by the host, where they will go on to excyst in the duodenum. Two trophozoites are released, and these will continue to undergo repeated mitotic division on the mucosal surface in the small intestine. Conditions present in the gut, such as bile salts, will trigger the trophozoites to develop into cysts that are passed in the feces and are immediately infectious at shedding (Dixon, 2021; Santin, 2020).

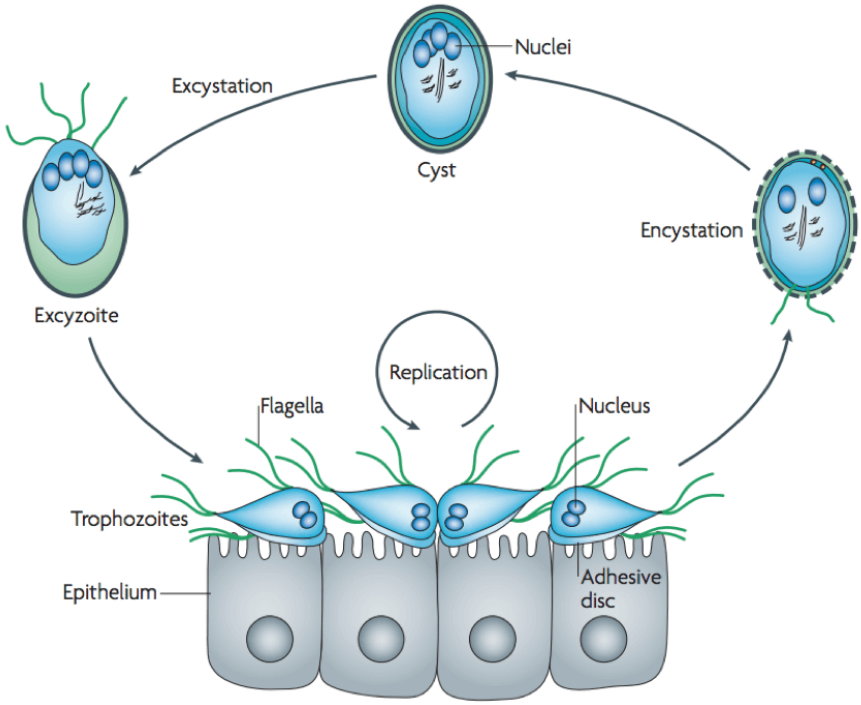

Figure 3: Life cycle of *Giardia duodenalis. Image by Ankarklev J., 2010.*

Route of transmission and survival of cysts

The organism is transmitted through the fecal-oral route and occurs either via direct contact with infected individuals or indirectly through ingestion of food or water contaminated with cysts (Dixon, 2021). Animals of all ages can be infected; however, clinical disease is mainly seen in young animals. Young animals also have the highest cyst excretion rates that continue

to contaminate the environment (Constable et al., 2017; Santin, 2020). The age of the animal is a determinant of infection, as cysts excretion rates are much higher in young animals than in adults. Lambs have been found to shed moderate amounts of *Giardia* from around 2 weeks of age, with a peak at 4 weeks, while numbers remained high even at 6-8 weeks old (Xiao et al., 1994). Ewes have also been found to have an increased excretion period which starts around 2 weeks prepartum, with a peak at 0-4 weeks postpartum before it declines at around 6-8 weeks postpartum (Xiao et al., 1994).

The parasite is quite resilient in the environment, especially in cooler temperatures and moisture, and combined with large numbers excreted to the environment, it has the potential to be a very "successful" parasite. The cysts are also immediately infectious when released into the environment, and have a low infectious dose (Santin, 2020), indicating that it is highly infectious.

Disease and virulence

Giardiasis in ruminants is common. It is characterized by diarrhea, weight loss, malabsorption and poor growth, but there is also a large number of asymptomatic carriers (Santin, 2020). Most infections are self-limiting, but the damage to the intestinal mucosa can be chronic. Recurrence is rather uncommon (Dixon, 2021). Some host remain asymptomatic, and this is believed to be due to a combination of nutritional status, immunity, variance in virulence among strains, coinfections with other enteric pathogens, and the gut microbiota (Dixon, 2021).

The interplay between the host and the parasite is an important part of the pathogenesis of *G. duodenalis*. Following excystation in the intestines, the trophozoites attach to the intestinal epithelial cells using their adhesive discs. This attachment, one of the major virulence factors

of *Giardia*, causes the shortening of microvilli, reducing the intestinal surface area (Dixon, 2021). The parasite also alters the proteins important for maintaining tight junctions between the enterocytes, an important selective barrier between the intestinal lumen and underlying tissue. The reduced mucosal surface area will lead to malabsorption of water, Na^+, and disaccharides, combined with hypersecretion of Cl^-, all contributing to diarrhea (Einarsson et al., 2016).

Zoonotic potential

Giardia duodenalis assemblage A and B are the genotypes with the most zoonotic potential as they have the broadest host ranges (Feng & Xiao, 2011), whereas the majority of hoofed mammals, such as sheep and cattle, are infected with *G. duodenalis* assemblage E. Human infections are mostly caused by assemblage B, followed by assemblage A, with some mixed infections (Einarsson et al., 2016). Molecular epidemiological surveys in ruminants report assemblage E as the predominant assemblage, followed by assemblage A, and sporadic incidents of assemblage B. Assemblage E is not commonly identified in humans, and hence its zoonotic potential is considered low (Cai et al., 2021; Dixon, 2021; Santin, 2020). No studies have been able to provide definite evidence that cases of human giardiasis have been acquired from ingestion of cysts excreted by non-human hosts under "natural" conditions (Cai et al., 2021; Heyworth, 2016).

As for a more local perspective, in 2004 there was an outbreak of diarrhea in Bergen where *Giardia duodenalis* (and some amount of *C. parvum*) was found to be the cause. It was concluded that the source was a sewage leak from nearby residential areas to the water source, and not due to fecal matter from animals (Robertson et al., 2015).

Giardiasis in sheep

Giardia has been found to be somewhat less prevalent in sheep and goats than in calves, and young animals had higher infection rates than older animals (Cai et al., 2021). Assemblage E is most often reported in sheep, followed by assemblage A (Cai et al., 2021; Santín et al., 2007). Occasionally assemblages B and D has also been reported (Cai et al., 2021). A study from Norway on non-diarrhetic, post-weaned lambs, found predominantly Assemblage E (41 isolates) and one Assemblage B (Robertson et al., 2010)

Studies in Australia have been done on both preweaned and postweaned lambs, and found the parasite present in both age groups (Yang et al., 2009). Ewes have also been found to shed increased amounts of cysts during parturition (Xiao et al., 1994), indicating that this age group is also infected. A study from Spain also found a notable amount of cysts from healthy, adult sheep (Castro-Hermida et al., 2007), indicating that infection is not synonymous with clinical disease for *Giardia*.

The impact of *Giardia* on production in ruminants is somewhat unclear, as several factors work together. Infected animals showing clinical signs will be less likely to thrive, although the effect on asymptomatic carriers is more unclear. Longitudinal studies indicate at some point, most animals become infected with the parasite (Dixon, 2021).

Treatment and preventive measures for cryptosporidiosis and giardiasis

There is no authorized effective treatment, or vaccine, against cryptosporidiosis for farm animals in Europe (EuropeanMedicinesAgency, 2022), thus making preventive measures extremely important. Treatment for *Giardia* is possible with benzimidazoles and metronidazole, and is used in humans, dogs and cats with diagnosed giardiasis (Constable et al., 2017; Einarsson et al., 2016; Tysnes et al., 2014), although no drugs are licensed for

treatment in ruminants. Symptomatic lambs with watery diarrhea and dehydration can be managed with supportive treatment and electrolytes for both cryptosporidiosis and giardiasis (Dixon, 2021; Paraud & Chartier, 2012).

Treating *Giardia* in a farm environment will also prove difficult and cost-prohibitive, as infections are considered chronic combined with high amounts of cysts in the environment and the risk of reinfection, which would require several treatments (Feng & Xiao, 2011; Santin, 2020).

Limiting the spread is still considered the most important measure to manage *Giardia* on the farm, as good management can reduce the number of cysts in the environment, hence limiting the potential for transmission to naive animals (Santin, 2020).

The best way to treat *Cryptosporidium* and *Giardia* is to prevent it by having a proper management structure. Preventive measures can include having designated clothing and shoes worn in the animals' housing environment (Gharpure et al., 2019). Decreased animal density and ensuring adequate amounts of colostrum in neonatal animals, as well as isolating clinically ill animals, will also limit the spread and development of infections (Paraud & Chartier, 2012). Oocysts and cysts can be destroyed using heat or chemical disinfection with hydrogen peroxide. The use of steam or ultraviolet lights is also possible (Santin, 2020), however less applicable in a farm scenario in Norway. Given that the oocysts survive for a long time in a wet environment, preventive measures also include keeping the environment as dry as possible, for example let the barn dry completely after washing and disinfecting before letting the animals back in.

To limit contamination of food and water sources with oocysts, good management of manure is important. Good hand hygiene among those handling young ruminants is important to limit transmission from animal to human, and prevent outbreaks (Santin, 2020).

Diagnostics

Several methods exist, and have been used, to diagnose and detect a *Cryptosporidium* spp. and *G. duodenalis* infection, only the most relevant for this study will be discussed below.

Immunofluorescent Antibody Test (IFAT)

Detection of *Cryptosporidium* spp. and *Giardia*- can be done using immunofluorescent antibody test (IFAT), in which antibodies raised against the oocyst or cyst walls are labelled with a fluorescent tag that can be visualized under a fluorescence microscope. The oocysts appear small, round and smooth under the fluorescence microscope. The procedure detects oocyst with high sensitivity and specificity and is considered the "gold standard" in the diagnostics of cryptosporidiosis and giardiasis. Species determination is, however, usually impossible with this technique (Ahmed & Karanis, 2018).

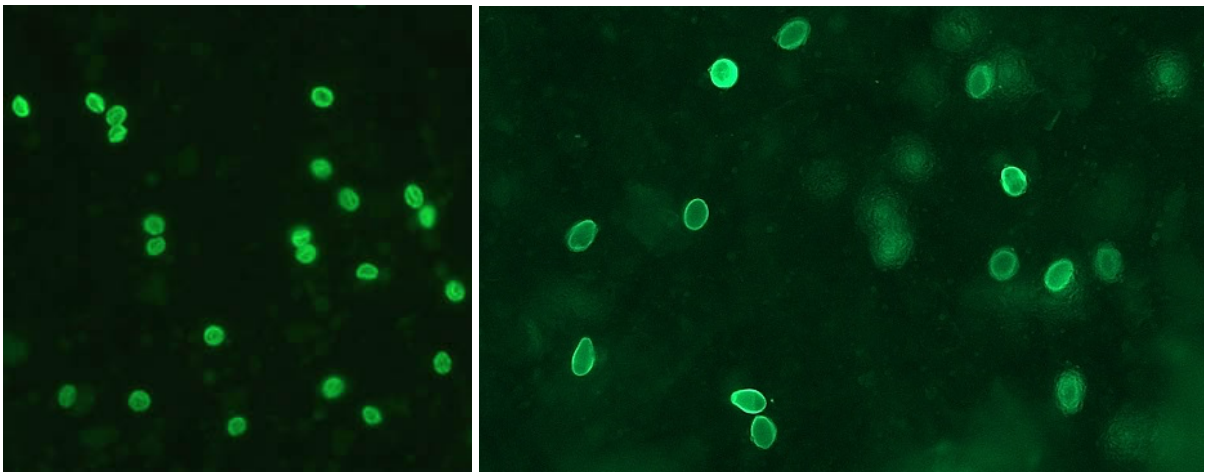

Figure 4: *Immunofluorescence of C. parvum oocysts (left) and G. duodenalis cysts (right) under microscope. Image by the Kansas Department of Health and Environment (left), and L. Robertson, MSD Veterinary Manual (right).*

Giardia-cysts are excreted sporadically, especially in asymptomatic hosts, and hence detection may require examination of several fecal samples (Santin, 2020; Santín et al., 2007). In addition to IFAT, antigen detection immunoassays are also used in diagnostics for giardiasis, as they are considered easy to standardize and show results quickly, but some kits are prone to some false-positive and false-negative results (Ryan et al., 2017).

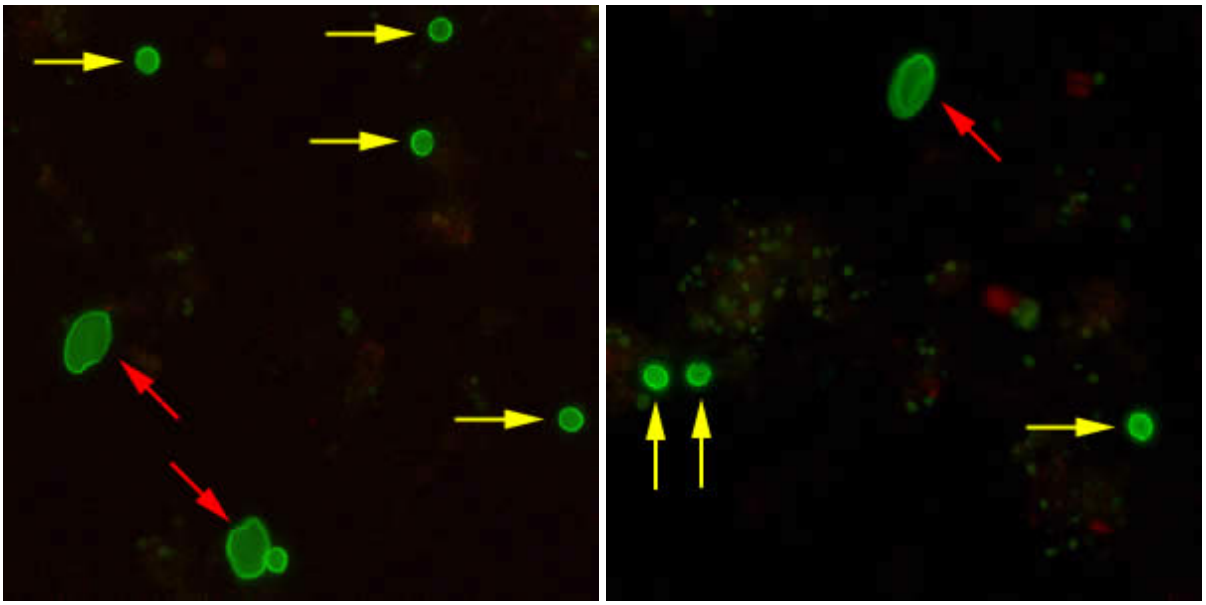

Figure 5: Size and shape difference between *C. parvum* oocysts (yellow arrow) and *G. duodenalis* cysts (red arrow). *C. parvum* oocysts are smaller and rounded. Image: the Kansas Department of Health and Environment.

PCR

Positive IFAT-samples are often brought further to molecular diagnostic tools, such as DNA extraction followed by PCR. PCR allows for large quantity processing, which is very useful in determining the source of a suspected outbreak (Adeyemo et al., 2018; Ahmed & Karanis, 2018). Several studies have reported that PCR-based methods have provided a high sensitivity for detection of *Cryptosporidium* and *Giardia* with a low number of oocysts, but the sensitivity of the enzymatic amplification process for detection of the protozoan in fecal

specimens is linked to a consistent removal of inhibitors and/or DNA degrading substances (Díaz et al., 2015; Yu et al., 2009). The sensitivity and reliability of PCR detection from stool samples are also dependent on the purity and the quality of the DNA, and a greater number of oocysts is sometimes required to get a positive result by sequencing, as this increases the chance of obtaining sufficient DNA that can be amplified (Castro-Hermida et al., 2007).

Some studies have also found that molecular diagnostic tools, such as PCR assays, are more sensitive than IFAT (Santín et al., 2007). PCR also allows the identification of assemblages and subtypes by using a loci of small subunit rRNA (*Cryptosporidium* and *Giardia*), triose phosphate isomerase (*tpi*) (*Giardia*), glutamate dehydrogenase (*gdh*) (Giardia) and β-giardin (*bg)* (*Giardia*) (Feng & Xiao, 2011). Sequencing of PCR products from *Giardia* or *Cryptosporidium* is not done routinely and is most frequently used for research (Ahmed & Karanis, 2018).

Subtyping using the gp60 gene of *Cryptosporidium* isolates is useful to better understand the dynamics of transmission. The gp60 gene is important in determining the subtype of *Cryptosporidium*, and especially when trying to trace an outbreak. This gene has made it possible to determine if an outbreak is animal contact-related (Chalmers et al., 2019).

Objective

The goal of this study was to investigate *Cryptosporidium* spp. and *Giardia duodenalis* infections in lambs in the chosen study populations in south-west of Norway: Sunnhordland and Dalane.

In particular, the sub-objectives were:

1) To provide data on the occurrence of *Cryptosporidium* and *Giardia* in different age groups of lambs
2) To investigate the species of *Cryptosporidium* and Assemblages of *Giardia* in lamb infections, with consideration of zoonotic potential.

Materials and methods

Study areas, target population and collection of samples

The target population was chosen from 17 volunteer farms in two geographical areas in the south-west of Norway: Sunnhordland (n=11) and Dalane (n=6). Sunnhordland is part of Vestland county, whereas Dalane is part of Rogaland county. Both regions have a large population of sheep, Rogaland being the county with high number of sheep population in Norway (Animalia, 2020). Therefore, we have reason to believe that the participants in this study are representative for the typical Norwegian sheep farm. The climate is relatively mild and damp in these areas, which makes them prone to high parasite pressure as the excreted parasites survive well in the environment.The recruitment of farms was originally based on contact with farmers who experienced diarrhea in their herd, but also based on interest from

farmers to participate in the project despite no current problems with diarrhea. In the end, very few farms with a perceived problem with diarrhea ended up participating in the project.

Figure 6*:* Map of Sunnhordland, from which some of the samples were collected. Map from buisunnhordland.no.

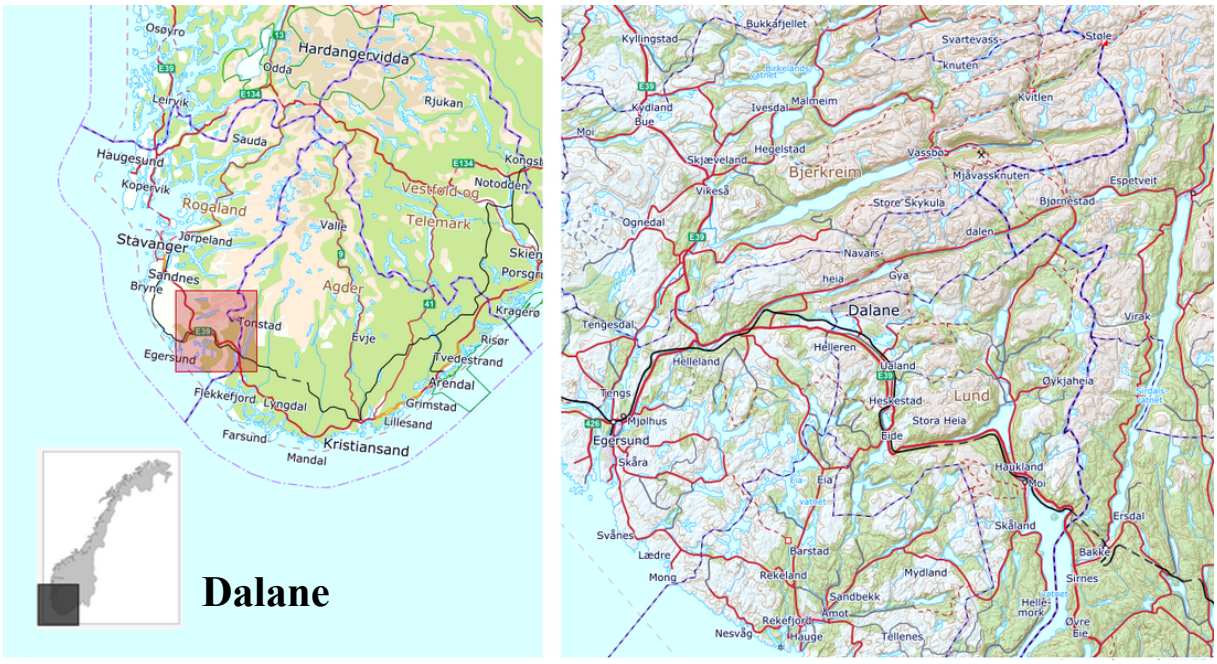

Figure 7: *Map of Dalane, from which some of the samples were collected. Map from Statens Kartverk.*

The age of the lambs was originally selected with the intention of being able to follow an individual at three different ages to see if one or both parasites were more prevalent and see the development of the infections status, thus making this a longitudinal study.

The individuals (lambs) included were of different breeds and both sexes were represented. Optimally twins that were to be raised with their dam was included, but some triplets and single lambs were chosen if the first choice was not available. The lambs included from each farm were chosen based on being the desired age at the time of the first visit. No bottle-fed lambs were included in the study population.

It was planned jointly with the farmers to collect the first samples during the most intensive lambing period. This was done in order to collect samples at a time when there were enough eligible individuals at the desired age. Furthermore, we expected the infection pressure to build up as the animal density increased, to improve our chances of detecting (oo)cyst in the flocks.

Stool samples were collected directly from the rectum of lambs at different ages using a specialized test tupe shaped as a spoon, first 2-3 days of age then 2-3 weeks of age and lastly at around 6 weeks of age. The samples were collected during March to May 2021. The feces were deposited into small, labelled plastic bags and stored at 4 °C until IFAT was conducted.

A questionnaire was filled out for each farm (supplementary file 1) and for each individual lamb (supplementary file 2) participating in this research project. It provided information about herd size, type of grazing, when the lambs were let out, parasite treatment, measures against diarrhea, water supply, cleaning routines, floor type, other animals on the farm, mother-ID, siblings, gender, breed, date of birth, colostrum intake, observed diarrhea, etc.

Detection of oocysts and cysts

We merged samples from siblings and looked at them together, except in cases of clear clinical diarrhea, for which the samples were examined individually. Approximately 20 µl of the fecal sample were placed on a microscope slide and a thin smear was prepared, then air dried in room temperature and fixed with a drop of methanol. Fixed slides were stained with FITC-conjugated monoclonal antibodies (mAbs) and nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI). A coverslip was placed over the sample and they screened using a fluorescence microscope using filter blocks appropriate for FITC (blue - emission 490, excitation 525) and DAPI (UV - emission 350, excitation 470). The samples were scored based on the detection of *Cryptosporidium* and *Giardia* (oo)cysts. The results were either classified as negative (no identification of (oo)cysts), + (1-9 (oo)cysts), ++ (10-50 (oo)cysts), or +++ (>51 (oo)cysts) per field of view on 200x magnification). The entire sample was examined for (oo)cysts before concluding with a negative result. Samples positive for cysts or oocysts were examined for DAPI-staining to identify intact nuclei. No DAPI-positive samples were found. The positive samples classified as ++ or +++ were further analyzed for the identification of species using molecular methods. If a pooled sample were classified as ++ or +++, it was analyzed again as individual samples to identify which lambs were to be included in molecular characterization. Several studies have reported that microscopic methods, such as IFAT, are more sensitive than molecular methods for samples containing a low number of parasites (Castro-Hermida et al., 2007; Díaz et al., 2013).

Molecular identification

The DNA-extraction were executed using PowerSoil DNA Isolation Kit, following the manufacturers procedure. Only samples containing ++ (oo)cysts or more were selected to

increase the chance of getting sufficient DNA for PCR. We added 250 µl of each fecal sample to power bead tubes and gently vortexed. Then the tube was mixed with 60 µl of the lysis solution C1 and vortexed briefly before subjecting the sample to bead beating to release the DNA by breaking the (oo)cysts. This was done using FastPrep-24 5G (MP Biomedicals) in two cycles of 4m/s for 60s with a 45s pause. After cycles of adding C2-C5 (followed by incubation at 4°C for 5 minutes, centrifugation and transfer to new tubes), the DNA was eluted in 50 µl C6, centrifuged at 10,000x g for 30 seconds and stored at -20 °C until PCR was conducted.

The extracted DNA from the IFAT-positive samples for *Cryptosporidium* oocysts were further prepared for PCR targeting the *Cryptosporidium* Small Subunit (SSU)rRNA gene. Primers and reaction cycles, as well as other details are provided in supplementary file 3. Each sample was prepared in duplicates to increase the chance of getting a good quality band for sequencing. We also included one negative control (containing no DNA, only nuclease-free water) and two positive controls (previously sequenced by Norwegian University of Life Sciences and confirmed positive for *Cryptosporidium*). The samples were run using iCycler Applied Biosystems (supplementary file 3).

The extracted DNA from the IFAT-positive samples for *Giardia duodenalis* were further prepared for nested PCR targeting the Beta-Giardin (BG) gene. The nested PCR were consisted of two sequential amplification reactions. Primers and reaction cycles, as well as other details are provided in supplementary file 4 and 5. Each sample was prepared in duplicates. We also included one negative control (containing no DNA, only nuclease-free water) and two positive controls (previously sequenced by Norwegian University of Life Sciences and confirmed positive for *G. duodenalis*). The samples were run using iCycler Applied Biosystems.

The PCR-products for both *Cryptosporidium* and *G. duodenalis* were examined with gel electrophoreses (2% agarose gel, stained with SYBERsafe DNA gel stain) and the results were visualized under UV illumination. DNA-ladder (Thermo Scientific) was used for measurement of fragment size. *Cryptosporidium* results compatible with 860 bp were identified as positives, whereas *Giardia* results compatible with 511 bp was identified as positives.

Cleaning of the PCR products prior to sequencing was executed using ExoSAP-IT Express PCR Product Cleanup (Affymetrix, Inc.), thus removing primers and nucleotides. The cleaned samples were sent for DNA-sequencing in Germany (Eurofins Genomics), for both forward and reverse sequences. The sequences were then checked using Geneious Prime software, and the results were blasted in an online GenBank for comparison. No subtyping was done.

Results

Occurrence of *Cryptosporidium* spp. and *G. duodenalis* by IFAT

Of the 507 individual samples collected, some were analyzed individually, and some were pooled (within farm and age group). This resulted in a total of 281 pooled or individual samples. Of the 281 pooled samples investigated, 63 (22.4%) were found to be *Cryptosporidium* positive and 61 (21.7%) samples were found to be *G. duodenalis* positive by IFAT/microscopy (table 2). Among these, 22 (7.8%) samples were found to have a mixed infection with both *Cryptosporidium* ssp. and *G. duodenalis* (supplementary file 8 and 9).

	LAMB AGE - 2-3 DAYS			LAMB AGE - 2-3 WEEKS			LAMB AGE - 6 WEEKS		
FARM	n	n crypto (%)	n giardia (%)	n	n crypto (%)	n giardia (%)	n	n crypto (%)	n giardia (%)
A	9	0	0	10	5 (50)	2 (20)	10	3 (30)	8 (80)
B	6	0	0	6	1 (17)	1 (17)	8	2 (25)	2 (25)
C	8	0	0	8	1 (13)	4 (50)	8	1 (13)	6 (75)
D	5	0	0	6	1 (17)	1 (17)	6	0	1 (17)
E	5	0	0	5	1 (20)	1 (20)	5	0	0
F	5	0	0	5	2 (40)	1 (20)	5	1 (20)	0
G	5	0	0	4	2 (50)	1 (25)	4	0	3 (75)
H	6	1 (17)	1 (17)	6	0	0	7	0	7 (100)
I	8	0	0	8	6 (75)	3 (38)	7	2 (29)	1 (14)
J	7	0	0	7	1 (14)	3 (43)	6	2 (33)	1 (17)
K	11	1 (9)	0	10	3 (30)	4 (40)	11	2 (18)	5 (45)
L	3	3 (100)	1 (33)	8	7 (88)*	0			
M	6	3 (50)	0	3	0	0	3	0	0
N	4	2 (50)	1 (25)	4	1 (25)	0			
O	3	1 (33)	0	3	0	0	3	3 (100)	2 (66)
P	3	2 (66)	0	4	2 (50)	1 (25)			
Q	3	1 (33)	0	3	0	0	1	0	0
TOTAL	**97**	**14 (14)**	**3 (3)**	**100**	**33 (33)**	**22 (23)**	**84**	**16 (19)**	**36 (43)**

Table 2: Positive pooled samples with IFAT/microscopy. A-K: Sunnhordland. L-Q: Dalane.

*Originally 100% of the pooled samples were positive for *Cryptosporidium* spp., but were run again as individual samples to identify which were to be included in molecular characterization.

Occurrence of infection by location and age group

Most of the *Cryptosporidium*-positive samples were identified in lambs at age 2-3 weeks (33% positive), although there were some lambs shedding oocysts at 2-3 days and six weeks of age as well (table 2 and figure 8). There is a noticeably higher prevalence of *Cryptosporidium* spp. at 2-3 days of age in Dalane, as opposed to Sunnhordland (table 2). There are most *G. duodenalis* cysts identified at age six weeks, and there seem to be a growing number of *G. duodenalis* cysts present by each sampling occasion.

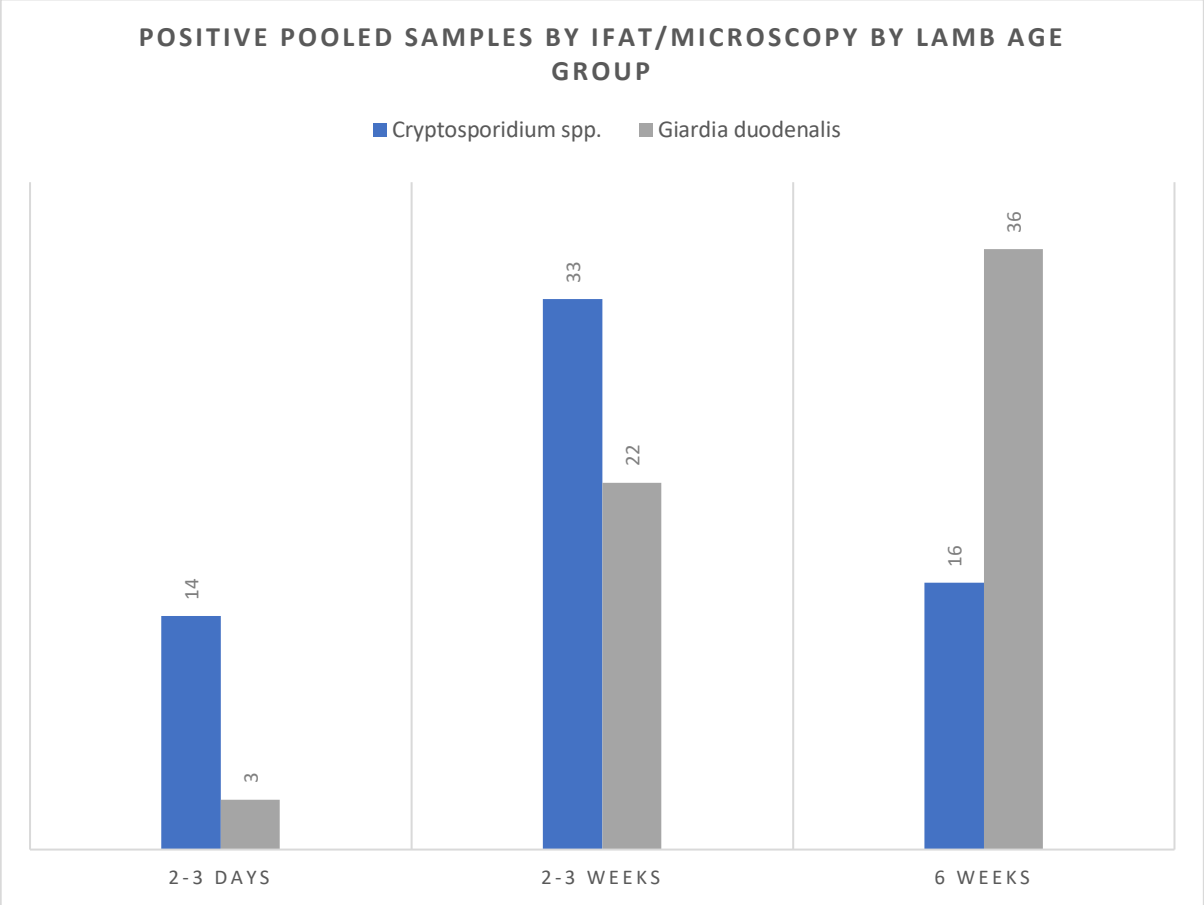

Figure 8: *Positive samples by IFAT/microscopy.*

Intensity of infection and molecular identification

Only samples containing 10 (oo)cysts per field of view (++) or more (+++/++++) were further analyzed, which means 107 (86%) positive IFAT-samples containing only 1-9 (oo)cysts per field of view (+) were not run by PCR or sequenced (table 3).

	n + (%)	n ++ (%)	n +++ (%)	n ++++ (%)	**In total**
Cryptosporidium spp.	53 (84,1)	3 (4,8)	4 (6,3)	3 (4,8)	**63**
G. duodenalis	54 (88,5)	6 (9,8)	0	1 (1,6)	**61**
In total	**107 (86,3)**	**11 (8,9)**	**4 (3,2)**	**2 (1,6)**	**124**

Table 3: Positive IFAT-samples sorted by intensity of infection.

Due to low oocyst numbers, only 10 (15,9%) of the *Cryptosporidium*-positive samples were further analyzed using molecular methods (table 3). Of these, 8 (80%) were positive by PCR and thereby sequenced. The sequenced results indicated the presence of *C. ubiquitum* and *C. parvum* in three (37,5%) and five (62,5%) of the sequenced results, respectively, as presented in table 4.

Similarly, only 7 (11,5%) of the *G. duodenalis*-positive samples were further analyzed using molecular methods (table 3). Of these, 2 (28,6%) were positive by PCR and sequenced. The sequence analysis identified the presence of *G. duodenalis* assemblage E in both (table 4).

		LAMB AGE - 2-3 DAYS		LAMB AGE - 2-3 WEEKS		LAMB AGE - 6 WEEKS	
FARM	**Individual**	*Crypto*	*Giardia*	*Crypto*	*Giardia*	*Crypto*	*Giardia*
A	10010	NO	NO	++	NO	+	+
A	10015	NO	NO	+++	NO	NO	+
A	10016	NO	NO	+++	++	NO	+
H	10021	NO	NO	NO	NO	NO	++
J	10225	NO	NO	+	+	++++	NO
L	11048	+	+	++	NO		
L	11049	+	NO	+++	NO		
L	11106	+	NO	+++	NO		
L	11108	+	NO	++	NO		

Table 4: Sequenced results in relation to farm, individual and IFAT-sampling results (illustrated with plusses according to intensity of infection). Colored boxed are confirmed by PCR*: C. ubiquitum, C. parvum* and *G. duodenalis* assemblage *E.*

One lamb (individual 10016, farm A), presented with no signs of clinical diarrhea, had a mixed infection with *C. parvum* and *G. duodenalis* assemblage E, verified by PCR (table 4), being the only lamb in this project to have a mixed infection verified by molecular methods.

In farm L, seven out of eight lambs were found to be positive for *Cryptosporidium* spp. by IFAT, however only four had more than 10 oocysts, and were sent for sequencing.

Questionnaire results and cases of diarrhea

The most relevant information gathered from the questionnaires is summarized in table 5. Most farmers had no perceived problem with diarrhea in lambs; 2 out of 17 reported previous problems with diarrhea in lambs <3 weeks of age, and 7 out of 17 reported previous problems with diarrhea after the lambs were let out grazing. The cases labelled as diarrhea in this project were noticed during the collection of samples.

51 samples were taken from lambs with noticeable diarrhea. Only 8 out of 51 (15,7%) of the registered cases of diarrhea had *Cryptosporidium* spp. oocysts by IFAT. Similarly, only 13 out of 51 (25,5%) had *Giardia duodenalis* cysts visible by IFAT. Furthermore, 31 out of the 51 (60,8%) of the cases labelled as diarrhea had no (oo)cysts visible by IFAT (supplementary file 8 and 9). Contingency table analysis indicates no statistically significant association between either *Cryptosporidium* infection or *Giardia* infection and diarrhea (P=0.079), but the proportion of lambs with diarrhea is low. Furthermore, only one lamb (10015) in farm A had a clinical diarrhea combined with a positive PCR result for *C. ubiquitum.* Lamb 10225 in farm J also had clinical diarrhea, and were positive by PCR for *C. parvum* (table 4).

Farm	Flock size	Let out from lambing pen (age of lambs)	Let out for grazing (age of lambs)	Perceived problem with diarrhea in lambs		Other animals on the farm
				<3 weeks	>3 weeks	
A	27	3 days	2-3 weeks	No	Yes	Horse, dog, free-range pigs
B	100	3-4 days	3-4 days	No	No	Cattle, poultry, dog, cat
C	80	2-3 days	As soon as possible	No	No	Cattle, goats, poultry, cat*
D	50	3-5 days	2-3 weeks	No	No	Hen
E	180	3-4 days	3-4 weeks	No	No	Dog
F	39	7 days	2 weeks	No	Yes	No
G	35	2-3 days	As soon as possible	No	Yes	Cat
H	70	1 day	2 weeks	No	Yes	Dog, cat
I	280	1-2 days	1 week	No	No	Pigs, dog, cat
J	300	2-5 days	3 weeks	No	No	Dog, cat
K	200	1-7 days	2-3 days	No	No	Dog, cat
L	60	3 days	As soon as possible	No	No	Cattle**
M	350	1 day	As soon as possible	Yes	Yes	Goats, cattle
N	170	2 days	2-4 weeks	Yes	No	Cattle***
O	140	3-4 days	As soon as possible	No	Yes	Cattle
P	144	2 days	As soon as possible	No	Yes	Cat
Q	160	2-3 days	2-3 weeks	No	No	Cattle, pigs

Table 5: The most relevant results from the questionnaires. A-K: Sunnhordland. L-Q: Dalane.

*Goats were in the same barn as the sheep. **Diarrhea in calves, found to be *Cryptosporidium* ssp. (personal communication with farmer). ***Farmer suspected Cryptosporidiosis in calves, but not confirmed diagnostically.

There is a great variation in flock size, ranging from 27 to 350 ewes. Most farms follow regular parasite treatment protocols, and most clean the barn with a pressure washer and cold water once a year before the sheep are let back inside in the fall. One farm (A) reported use of soap in relation to the annual wash, and one farm (C) reported washing of the barn several times a year.

The response "as soon as possible" represents the farmers wanting to wait until the right conditions before letting their sheep out. The right conditions included factors such enough grass so that supplementary feeding was unnecessary and warm enough temperatures during nighttime to avoid hypothermia in lambs. Hence, this response may be somewhat misleading in terms of the lambs being older than three weeks. A trend in Sunnhordland is that the majority were let out within the age of two weeks, whereas the farmers in Dalane preferred to wait for the right conditions. Most farms (A-Q) had let their flocks out at the time of the third sampling.

From personal communication with the farmer, it was revealed that Farm L recently had an outbreak of *Cryptosporidium* ssp. in their calves. During the sampling, it was noted that one had to pass through the calf area to enter the sheep housing. Farm N suspected that they might have a current outbreak of *Cryptosporidium* ssp. in their calves, but has not been confirmed diagnostically.

Discussion

Cryptosporidium and *Giardia* infection

The main findings of this study are that both *Cryptosporidium* and *Giardia* occur at all ages of the lambs in the study area, although their distribution across age groups varies. The species and assemblages, however, are not identified in all IFAT-positive lambs as only a few oocysts were found (insufficient for molecular characterisation) in 86,3% of the samples (table 3).

The IFAT-screening indicated the presence of both *G. duodenalis* and *Cryptosporidium* spp. in some lambs by 2-3 days of age, but only in small amounts (+), and therefore screening was not done individually. Because of the low number of oocysts (less than 10), hence the low chance of getting enough DNA for genotyping, none of these samples were run by PCR. Therefore, we do not know which species of *Cryptosporidium* and assemblages of *G. duodenalis* were present in this age group. Few studies appear to have been conducted on lambs as young as 2-3 days old, however studies on lambs ranging from 5-21 days have identified *C. parvum*, *C. ubiquitum*, and *C. xiaoi* (Bordes et al., 2020; Kaupke et al., 2017; Santín et al., 2007). Similarly, *G. duodenalis* assemblage E have been identified in lambs as young as seven days old (Santín et al., 2007).

The prevalence of *Cryptosporidium* ssp. is the highest at 2-3 weeks of age. In total 33 (33%) of the pooled samples were positive by IFAT in this age group (table 2), and PCR confirmed both *C. parvum* and *C. ubiquitum* (table 4). There is also a beginning trend of an increasing *G. duodenalis* infection (Figure 8). Assemblage E is the most commonly identified assemblage in ruminants (Cai et al., 2021). Though other studies have found *C. xiaoi* to be uncommon in Europe compared to *C. parvum* and *C. ubiquitum*, it does occur occasionally (Guo et al., 2021). A study from Poland found *C. xiaoi* to be the most dominant species in all age groups

tested, ranging from one day to nine weeks of age (Kaupke et al., 2017) and a longitudinal study from 2010 have identified this species in Norwegian sheep as well (Robertson et al., 2010). Although we did not find *C. xiaoi* from our sequenced samples, we cannot exclude the possibility of its presence in the samples for which we have no data from molecular characterization.

There were still *Cryptosporidium* oocysts and *Giardia* cysts being shed from some lambs at six weeks of age, with one farm (H) having identified *Giardia* cysts in all (seven) pooled samples. This is the age group where most pooled samples identified *Giardia* cysts by IFAT (Figure 8). Only one sample came back positive by PCR as *G. duodenalis* assemblage E in farm H. A previous longitudinal study from Norway done initially on 5-6 weeks old lambs concluded that the prevalence of both *Cryptosporidium* and *Giardia* increased after a month, together with the amounts of co-infections (Robertson et al., 2010). This concurs with our findings for *Giardia*, as its prevalence increases from 2-3 weeks to 6 weeks (figure 8), but not for *Cryptosporidium*. However, our data set from 6 weeks is lacking as some farmers were unavailable for a third sampling, hence it is difficult to say anything conclusive about the prevalence. Furthermore, this study only identified *C. ubiquitum* and *C. xiaoi* as the species present (Robertson et al., 2010), whereas our study also identified *C. parvum* (but not *C. xiaoi)*. In general, there seem to be less of an apparent distribution of *Cryptosporidium*-species by age in sheep, than in large ruminants (Guo et al., 2021).

The prevalence of both *Cryptosporidium* spp. and *G. duodenalis* has been reported with considerable variation worldwide due to differences in study design, geographical and seasonal differences, management system and age of lambs participating (Geurden et al., 2008), indicating that studies done on *Cryptosporidium* spp. and *G. duodenalis* in lambs are not automatically comparable between countries and regions. No studies have been conducted

to systemically map out potential risk factors associated with Norwegian sheep farming.

Association between clinical symptoms and positive samples

Most of our sampled flocks had some animals with diarrhea, if not at sampling time, then in previous lambing seasons. The farmers were asked to pay attention if some of the lambs were developing diarrhea during the lambing season, as one early idea behind this study was to investigate whether diarrhea in lambs might be associated with infection with *Cryptosporidium* and/or *Giardia*. This was not followed through, due to lack of reported cases of diarrhea from farmers.

Clinical diarrhea is a difficult term to describe objectively because people have different subjective criteria for the term. There is also a wide range of severity of diarrhea, differing from light, for example in lambs at 2-3 days age resulting from high colostrum intake, to profound watery diarrhea with clinical dehydration. There are also other factors that contribute to diarrhea besides *Cryptosporidium* and *Giardia*, for example other parasites, bacteria such as *E.coli*, viruses such as Rota-virus, management (feeding, stress, bottle feeding), anatomical causes and toxins (Castro-Hermida et al., 2007; Paraud & Chartier, 2012). It is possible that nobody noticed the diarrhea when/if it occurred. Co-infections with other agents are also possible (Dahmani et al., 2020).

Furthermore, asymptomatic carriers of both *Cryptosporidium* and *Giardia* has been identified, both in this study and in previous studies. A small number of *Cryptosporidium* were identified in asymptomatic adult sheep in Northern Spain, however they were unable to identify which species due to the low concentration of oocysts in each sample (Castro-Hermida et al., 2007). In France, *C. parvum* was found in both lambs (5-17 days) and ewes, without them displaying symptoms (Bordes et al., 2020). In our study, Farm L had a prevalence of 91% of

Cryptosporidium ssp. (table 2), although no clinical symptoms were recorded. At this farm *C. parvum* was identified in lambs 2-3 weeks old (table 4), supporting that asymptomatic infections of this parasite in lambs is possible.

Zoonotic potential

Sheep can harbor both zoonotic *Cryptosporidium* species and potentially zoonotic *Giardia* (Cai et al., 2021; Guo et al., 2021). *C. parvum* was found in four of the sampled flocks (table 4) and was the most common species of *Cryptosporidium* found from molecular methods. *C. parvum* has a wide range of possible hosts, both in livestock and in wild animals. We have not subtyped *C. parvum*, but we assume that they are zoonotic to humans, as non-zoonotic *C. parvum* have mainly been reported in parts of Africa (Kifleyohannes et al., 2022; Robertson et al., 2020). Previous studies done in Norway on outbreaks in school children concluded with zoonotic *C. parvum* after contact with small ruminants (Lange et al., 2014; Robertson et al., 2006a). *C. ubiquitum*, found in three of the sampled flocks (table 4), has been recognized as an emerging zoonotic species the latest years, with the potential to infect a broad range of species (Fayer et al., 2010; Li et al., 2014). Therefore, we can assume that all *Cryptosporidium* verified by PCR in this study are zoonotic.

G. duodenalis assemblage E have mostly been found to infect ruminants (Cai et al., 2021). However, a recent study from New Zealand reported *G. duodenalis* assemblage E in human isolates. Assemblage E has also been found in rural areas with high densities of ruminants in Australia (Zahedi et al., 2017), Egypt (Abdel-Moein & Saeed, 2016; Foronda et al., 2008; Helmy et al., 2014) and Brazil (Fantinatti et al., 2016), indicating that the route of transmission in humans is potentially zoonotic. However, there is a lack of clarity in some of these publications, leading them somewhat open to doubt; in addition, carriage without

infection is a possibility. Infections in humans in Norway has so far only been found to be assemblage A and B (Robertson et al., 2006b). Therefore, we can assume that the *G. duodenalis* verified by PCR in this study are not zoonotic.

Risk Factors for *Cryptosporidium* and *Giardia* in lambs

There was a noticeably higher prevalence of *Cryptosporidium* oocysts identified by IFAT in lambs at 2-3 days of age in Dalane, as opposed to Sunnhordland. This may be due to a bigger flock size in the farms in this area (table 5), resulting in a higher infectious pressure and an earlier peak of infection in these farms, which has been considered a risk factor previously (Santin, 2020). The lambs were, on average, let outside much earlier in Sunnhordland (table 5), and if the weather allowed it; already in a few days after lambing, possibly contributing to a lower infectious pressure inside the barn in these farms. The lambs from the two farms in Sunnhordland that had verified Cryptosporidium ssp. by 2-3 days of age were also let out of the lambing pen after 1 day (table 5), hence being exposed to infection at an earlier stage.

A study from northeastern Spain analyzed potential risk factors for *C. parvum* in lambs, and found a correlation between the presence of diarrheic lambs (79,4%) and high cryptosporidal infection rates (Causapé et al., 2002) Our study, however, does not provide supporting evidence; most lambs with *Cryptosporidium* spp. oocysts and *G. duodenalis* cysts were asymptomatic and of the lambs with diarrhea only 15,7% were shedding *Cryptosporidium* and only 25,5% were shedding *Giardia*.

The same study from Spain also found several factors associated with a decreased risk of *C. parvum* in lambs, including low numbers of lambs in the farm and cleaning of the lambing area, and Cryptosporidium infection was also detected in 16 ewes (7.8%) which excreted few

oocysts and without diarrhoea (Causapé et al., 2002), indicating that ewes play a role in maintaining and spreading of the parasite in a herd. Another study that supports this theory found that some ewes were shedding *C. parvum* oocysts at the start of lambing (Xiao et al., 1994). The main findings of that study, however, were a periparturient rise in excretion of *G. duodenalis* cysts in ewes from 2 weeks prepartum, with a peak at zero and 4 week postpartum, and back to normal levels 6 and 8 weeks postpartum. This is considered to be the major source of giardiasis in lambs, and is believed to be because of a drop in immunity in the ewes before lambing (Ortega-Mora et al., 1999; Xiao et al., 1994).

Limitations of this study

Because of the size of the lamb in 2-3 days of age, it was not easy to collect feces. If we were able to get some during the first round of sampling, there were usually small amounts. Some incidents of blood from rectum were also registered, meaning that we had to stop. Some individuals had no available feces at the sampling occasion, and there were no other lambs in the right age group available for choice. If this was the case, the first sample was labelled "empty", and we came back at 2-3 weeks of age to collect a new sample. Since this is a longitudinal study, the main goal was to follow the same individual, and therefore there were not found substitutes when the lambs were empty during the second or third sampling occasion. In some cases of empty rectum, we returned the next day to try again.

The study was originally planned as a longitudinal study. Therefore, two of the originally participating farms, where the lambs were not ID-marked by the first sampling occasion, were not included nor analyzed, due to not being able to identify the individual at the next sampling. Two farms (N and P) were not available for collection by the third sampling occasion. Unfortunately, the third round of samples from one farm (L) were lost in the mail

and did never reach the laboratories at NMBU Ås. There were also several cases of lambs being let out grazing or unavailable due to other causes, such as death. The combination of the causes listed above explains why the amount of sampling material turned out smaller than planned. It would have been useful to have more information regarding the situation around 6 weeks of age, especially in Dalane, to follow the development of infection with *Cryptosporidium* and *G. duodenalis.*

This study cannot conclude what happens between the sampling occasions. The lambs may have had an infection in between sampling occasions, which means we were not able to collect (oo)cysts. Lambs we were not able to collect feces from at some point, may also have had (oo)cysts. Some studies report sporadic shedding of *Giardia* (Santín et al., 2007), while others report 100% infection rate with time (Santin, 2020). There may be a chance that the lambs weren't shedding at the sampling occasion. In some of our farms the peak of *G. duodenalis* infection and secretion of cysts may also occur later than 6 weeks of age.

Although there were positive IFAT samples for both *Cryptosporidium* and *Giardia* (table 3) at age 2-3 days, the concentration of oocysts were deemed too low for molecular sequencing. Henceforth, we can say that there is most likely some degree of infection at this stage, but cannot conclude as to which species of *Cryptosporidium* or assemblage of *Giardia* that are present, only assume based on other studies done on neonatal lambs, goat kids and calves.

Conclusion

This study found two species of *Cryptosporidium*, *C. parvum* and *C. ubiquitum*, as well as *Giardia duodenalis* assemblage E infecting lambs among 17 flocks in South-West of Norway. Most of our positive samples came from asymptomatic lambs. *Cryptosporidium* spp. infection peaked at 2-3 weeks of age, while *G. duodenalis* infection peaked at 6 weeks of age. The *Cryptosporidium* species identified in this study are most likely zoonotic, whereas the *G. duodenalis* identified are most likely not. To improve this study; a cross sectional study at the three different stages, instead of a longitudinal study, would be more effective in describing the status of infection in lambs in this region.

Acknowledgements

Firstly, we would like to thank all the dedicated farmers who participated and provided their time (and patience) with us during collection of the stool samples. Secondly, we would like to thank Animalia, Bjerkreim og Gjesdal Avløysarlag, and Bømlo Sau og Geit for distribution of information to get in touch with local farmers. Finally, we would like to thank our patient advisors for their help with authoring this paper. A special thanks is required to Tsegabiran Kifleyohannes Tesama, for the incredible work of processing all the samples and her outstanding guidance in the lab. This paper would not have been completed without your contributions.

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Supplementary

Supplementary file 1. Herd information form, Sunnhordland

Used for both Sunnhordland and Rogaland.

Besetningsskjema Sunnhordland

Norwegian University of Life Sciences

Eier: _____ Tlf eier: _____

Adresse: _____

_____

Besøk av andre som ikke normalt steller i fjøset: JA / NEI _____

Antall vinterfôra søyer: _____ Forventa lammetall: _____

Lamming start: _____ Lamming slutt: _____

Intensiv lammingsperiode: _____

Når slippes lam ut i fellesbinge? _____

Når slippes lam ut på beite? _____

Beitetype (kulturbeite, utmarksbeite, fjellbeite): _____

Diaréproblematikk hos unge lam (<3 uker)? JA NEI

Diaréproblematikk hos eldre lam (>3 uker)? JA NEI

Parasittbehandling: JA / NEI Alder: _____ Type: _____

Andre tiltak mot diaré: _____

_____

Type underlag i fjøset: _____

Vannforsyning (brønn, elv, kommunalt): _____

Andre dyr på gården: _____

Tilleggsinfo:

_____

_____

_____

_____

Supplementary file 2. Individual information form

Used for both Sunnhordland and Rogaland.

Individskjema Sunnhordland

Norwegian University of Life Sciences

Besetning: _____ Mors individnummer: _____

Individnummer: _____ Søsken (antall og nr):_____

Rase:_____ Kjønn: _____

Fødselsdato:_____ Fødselsvekt:_____

Råmelk (sett ring rundt aktuelle):

Drikker selv Sonde Fått fra flaske

Råmelk fra egen mor Råmelk fra ku Råmelk fra annen søye Usikker

Tilleggsinfo _____

_____

_____

_____

_____

_____

Diaré observert?

JA NEI

(Fylles ut av prøvetaker, dato):

- 2-3 dagers prøve: _____
- 2-3 ukers prøve: _____
- 6 ukersprøve: _____

Supplementary file 3. PCR: Cryptosporidium SSU

PCR maskin: iCycler Applied Biosystems

dato: 6.1.2022

Crypto SSU

Cryptosporidium Small Ribosomal Subunit	
Primer 1: **SsuF3;** **GGAAGGGTTGTATTTATTAGATAAAG**	Primer 2: **SsuR3** **AAGGAGTAAGGAACAACCTCC A**
DNA polymerase**: DreamTaq hot start**	Polymerase mengde: 2,5 **U**
$MgCl_2$ konsentration: **2,5 mM**	Forventet PCR produkt: **860bp**

Antall Prøver	1	24,00
	Volum µL	
PCR vann	8,50	204,00
Forward primer	1,00	24,00
Reverse primer	1,00	24,00
Mastermix	12,50	300,00
Total volum	23,00	552,00

Bemerkninger:
Positiv kontroll, USA, 2,0 µl
Templat, 2 µl DNA

PCR no.	**DNA**	**H_2O**	
1. neg.kontr		2,0	
2. 10015			
3. 10015			
4. 10016			
5. 10016			
6. 10225			
7. 10225			
8. 10037			
9. 10037			
10. 10010			
11. 10010			
12. 10605			
13. 10605			
14. 10613			
15. 10613			
16. 10021			
17. 10021			
18. 10019			
19. 10019			
20. 10093			
21. 10093			
22. Pcry			
23. Pcry			

Primer kons.: 10 pmol/µl

Temp.°C	Tid	Prog. h min
95	3 min	
94	45 sek.	50 X
55	45 sek.	
72	60 sek.	
72	10 min	
4	PAUSE	

Supplementary file 4. Nested PCR: Giardia BG 1st

PCR maskin: iCycler – Applied biosystems

date: 7.01.2022

Tsega testing Giardia BG 1st round

Giardia Beta-Giardin

Primer 1: **G7 AAGCCCGACGACCTCACCCGCAGTGC**	Primer 2: **G759 GAGGCCGCCCTGGATCTTCGAGACGAC**
DNA polymerase**: husk hot start**	Polymerase mengde: 2,5 **U**
$MgCl_2$ konsentration: **2,5 mM**	Forventet PCR produkt: 753

Antall Prøver	1	19,00
	Volum µL	
PCR vann	8,50	161,50
Forward primer	1,00	19,00
Reverse primer	1,00	19,00
Mastermix	12,50	237.5
Total volum	23,0	437,00

Bemerkninger:
Positiv kontroll, USA, 1,0 µl
Templat, 2 µl DNA

PCR no.	DNA	H_2O		
1. neg.kontr		2,0		
2. 10016				
3. 10016				
4. 10019				
5. 10019				
6. 10021				
7. 10021				
8.10605				
9. 10605				
10. 10613				
11.10613				
12. 10037				
13. 10037				
14. 10093				
15. 10093				
16. GS				
17. WB				

Primer kons.: 10 pmol/µl

Temp.°C	Tid	Prog. h min
95	3 min	
94	30 sek.	35 X
60	30 sek.	
72	60 sek.	
72	10 min	
4	PAUSE	

Supplementary file 5: Nested PCR: Giardia BG 2nd

PCR maskin: **Applied Biosystems** dato: 7.01.2022

Tsega testing Giardia BG 2ND round

Giardia Beta-Giardin

Primer 1: GAACGAGATCGAGGTCCG	Primer 2: CTCGACGAGCTTCGTGTT
DNA polymerase**: husk hot start**	Polymerase mengde: 2,5 **U**
$MgCl_2$ konsentration: **2,5 mM**	Forventet PCR produkt: 511

Antall Prøver	1	18,00
	Volum µL	
PCR vann	8,50	153,00
Forward primer	1,00	18,00
Reverse primer	1,00	18,00
Mastermix	12,50	225,00
Total volume	23,0	414,00

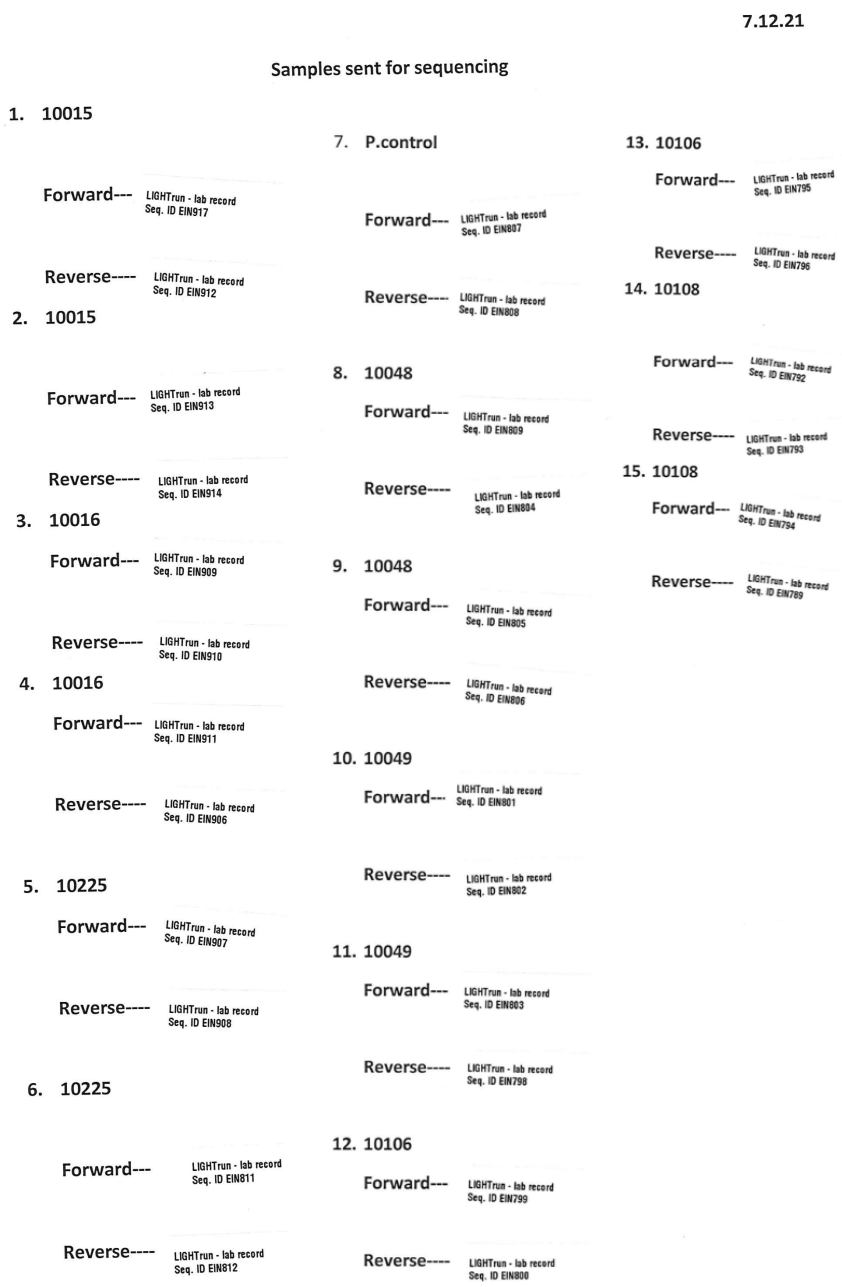
Bemerkninger:
Positiv kontroll, USA, 1,0 µl
Templat, 2 µl DNA

PCR no.	DNA	H_2O		
1. neg.kontr		2,0		
2. 10016				
3. 10016				
4. 10019				
5. 10019				
6. 10021				
7. 10021				
8.10605				
9. 10605				
10. 10613				
11.10613				
12. 10037				
13. 10037				
14. 10093				
15. 10093				
16. GS				
17. WB				

Primer kons.: 10 pmol/µl

Temp.°C	Tid	Prog. h min
95	3 min	
94	30 sek.	40 X
60	30 sek.	
72	60 sek.	
72	10 min	
4	PAUSE	

Supplementary file 6: Samples sent for sequencing



7.12.21

Samples sent for sequencing

1. 10015
   - Forward--- LIGHTrun - lab record Seq. ID EIN917
   - Reverse---- LIGHTrun - lab record Seq. ID EIN912
2. 10015
   - Forward--- LIGHTrun - lab record Seq. ID EIN913
   - Reverse---- LIGHTrun - lab record Seq. ID EIN914
3. 10016
   - Forward--- LIGHTrun - lab record Seq. ID EIN909
   - Reverse---- LIGHTrun - lab record Seq. ID EIN910
4. 10016
   - Forward--- LIGHTrun - lab record Seq. ID EIN911
   - Reverse---- LIGHTrun - lab record Seq. ID EIN906
5. 10225
   - Forward--- LIGHTrun - lab record Seq. ID EIN907
   - Reverse---- LIGHTrun - lab record Seq. ID EIN908
6. 10225
   - Forward--- LIGHTrun - lab record Seq. ID EIN811
   - Reverse---- LIGHTrun - lab record Seq. ID EIN812
7. P.control
   - Forward--- LIGHTrun - lab record Seq. ID EIN807
   - Reverse---- LIGHTrun - lab record Seq. ID EIN808
8. 10048
   - Forward--- LIGHTrun - lab record Seq. ID EIN809
   - Reverse---- LIGHTrun - lab record Seq. ID EIN804
9. 10048
   - Forward--- LIGHTrun - lab record Seq. ID EIN805
   - Reverse---- LIGHTrun - lab record Seq. ID EIN806
10. 10049
    - Forward--- LIGHTrun - lab record Seq. ID EIN801
    - Reverse---- LIGHTrun - lab record Seq. ID EIN802
11. 10049
    - Forward--- LIGHTrun - lab record Seq. ID EIN803
    - Reverse---- LIGHTrun - lab record Seq. ID EIN798
12. 10106
    - Forward--- LIGHTrun - lab record Seq. ID EIN799
    - Reverse---- LIGHTrun - lab record Seq. ID EIN800
13. 10106
    - Forward--- LIGHTrun - lab record Seq. ID EIN795
    - Reverse---- LIGHTrun - lab record Seq. ID EIN796
14. 10108
    - Forward--- LIGHTrun - lab record Seq. ID EIN792
    - Reverse---- LIGHTrun - lab record Seq. ID EIN793
15. 10108
    - Forward--- LIGHTrun - lab record Seq. ID EIN794
    - Reverse---- LIGHTrun - lab record Seq. ID EIN789

18.1.22

Samples sent for sequencing

1. 10225

   Forward--- LIGHTrun - lab record Seq. ID EIN971

   Reverse---- LIGHTrun - lab record Seq. ID EIN966

2. 10225

   Forward--- LIGHTrun - lab record Seq. ID EIN967

   Reverse---- LIGHTrun - lab record Seq. ID EIN968

3. 10010

   Forward--- LIGHTrun - lab record Seq. ID EIN963

   Reverse---- LIGHTrun - lab record Seq. ID EIN964

4. 10016

   Forward--- LIGHTrun - lab record Seq. ID EIN965

   Reverse---- LIGHTrun - lab record Seq. ID EIN960

5. 10019

   Forward--- LIGHTrun - lab record Seq. ID EIN961

   Reverse---- LIGHTrun - lab record Seq. ID EIN962

6. 10021

   Forward--- LIGHTrun - lab record Seq. ID EIN957

   Reverse---- LIGHTrun - lab record Seq. ID EIN958

1. Nothrus

   Forward--- LIGHTrun - lab record Seq. ID EIN788

   Reverse---- LIGHTrun - lab record Seq. ID EIN787

2. Nothrus

   Forward--- LIGHTrun - lab record Seq. ID EIN786

   Reverse---- LIGHTrun - lab record Seq. ID EIN791

Supplementary file 8: Raw data Sunnhordland

Farm	Individual	Mother	Diarrhea	1st sample	CODE	Crypto	Giardia	2nd sample	CODE	Crypto	Giardia	3rd sample	CODE	Crypto	Giardia
A	10010	70025	Ja	01.04.2021	F	NO	NO	24/04/2021	023	++	NO	15/05/2021	097	+	+
	10012	80014	Ja	01.04.2021	A	NO	NO	24/04/2021	024	+	NO	15/05/2021	098	NO	+
	10013	80014	Nei	01.04.2021	C	NO	NO	24/04/2021	025	NO	NO	15/05/2021	099	+	+
	10014	70008	Ja	01.04.2021	D	NO	NO	24/04/2021	026	+	NO	15/05/2021	100	NO	+
	10015	70008	Ja	01.04.2021	E	NO	NO	24/04/2021	027	+++	NO	15/05/2021	101	NO	+
	10016	70008	Ja	01.04.2021	B	NO	NO	24/04/2021	028	+++	++	15/05/2021	102	NO	+
	10017	70029	Nei	-	-	-	-	24/04/2021	029	NO	NO	15/05/2021	103	NO	+
	10018	70029	Ja	01.04.2021	I	NO	NO	24/04/2021	030	NO	NO	15/05/2021	104	+	+
	10019	80009	Ja	01.04.2021	H	NO	NO	24/04/2021	031	NO	NO	15/05/2021	105	NO	NO
	10020	80006	Ja	01.04.2021	G	NO	NO	24/04/2021	032	NO	+	15/05/2021	106	NO	NO
B	10050	70157	Nei	09.04.2021	J	NO	NO	23/04/2021	033	NO	NO	22/05/2021	107	NO	NO
	10051	70157	Nei	09.04.2021		NO	NO	23/04/2021				22/05/2021			
	10052	70015	Ja	09.04.2021	K	NO	NO	23/04/2021	034	NO	+	22/05/2021	108	+	+
	10053	70015	Nei	09.04.2021	L	NO	NO	23/04/2021	035	NO	NO	22/05/2021	109	NO	NO
	10054	80102	Nei	09.04.2021				23/04/2021				-	-	-	
	10055	70003	Nei	09.04.2021				23/04/2021				22/05/2021	110	NO	NO
	10056	70003	Ja	09.04.2021	M	NO	NO	23/04/2021	036	NO	NO	22/05/2021	111	NO	NO
	10057	80074	Nei	09.04.2021	N	NO	NO	23/04/2021	037	NO	NO	22/05/2021	112	NO	NO
	10058	80074	Nei	09.04.2021				23/04/2021				22/05/2021			
	10059	80042	Ja	09.04.2021	O	NO	NO	23/04/2021	038	+	NO	22/05/2021	113	+	+
	10037			-	-	-		-	-	-	-	22/05/2021	114	NO	NO
C	10071	60161	Ja	10.04.2021	P	NO	NO	24/04/2021	039	NO	NO	22/05/2021	115	NO	Only 1
	10072	60161	Ja	10.04.2021	Q	NO	NO	24/04/2021	040	NO	NO	22/05/2021	116	+	+
	10075	99074	Ja	10.04.2021	R	NO	NO	24/04/2021	041	NO	NO	22/05/2021	117	NO	+
	10076	99074	Nei	10.04.2021	S	NO	NO	24/04/2021	042	NO	+	22/05/2021	118	NO	+
	10085	88072	Nei	10.04.2021				24/04/2021				22/05/2021			
	10086	88072	Nei	10.04.2021				24/04/2021				22/05/2021			
	10087	99046	Ja	10.04.2021	T	NO	NO	24/04/2021	043	+	+	22/05/2021	119	NO	NO
	10088	99046	Nei	10.04.2021	U	NO	NO	24/04/2021	044	NO	NO	22/05/2021	120	NO	+
	10092	99132	Ja	10.04.2021	V	NO	NO	24/04/2021	045	NO	+	22/05/2021	121	NO	NO
	10093	70033	Ja	10.04.2021	W	NO	NO	24/04/2021	046	NO	+	22/05/2021	122	NO	++
D	10126	40410	Nei	10.04.2021	X	NO	NO	24/04/2021	047	NO	NO	18/05/2021	123	NO	NO
	10127	40410	Nei	10.05.2021				24/04/2021				-	-	-	-
	10130	40455	Ja	-	-	-	-	24/04/2021	048	NO	+	18/05/2021	124	NO	NO
	10131	40455	Nei	-	-	-	-	24/04/2021	049	NO	NO	18/05/2021	125	NO	NO
	10132	40455	Nei	10.04.2021	Y	NO	NO	24/04/2021	-	-	-	-	-	-	-
	10133	40455	Nei	10.05.2021				24/04/2021	-	-	-	-	-	-	-
	10134	80816	Nei	10.06.2021				24/04/2021	049	NO	NO	18/05/2021	126	NO	NO
	10136	70720	Nei	10.07.2021	Z	NO	NO	24/04/2021				18/05/2021			
	10137	70720	Ja	10.04.2021	AA	NO	NO	24/04/2021	050	+	NO	18/05/2021	127	NO	NO
	10139	90914	Nei	10.04.2021	BB	NO	NO	24/04/2021	051	NO	NO	18/05/2021	128	NO	Only 1
	10140	90914	Nei	10.04.2021				24/04/2021				18/05/2021			
	10142	90903	Nei	10.04.2021				24/04/2021				18/05/2021			
E	12002		Nei	15/04/2021	CC	NO	NO	03.05.2021	052	+	+	28/05/2021	129	NO	NO
	12003		Nei	15/04/2021				03.05.2021				28/05/2021			
	12004		Nei	15/04/2021				03.05.2021				28/05/2021			
	12005		Nei	15/04/2021	DD	NO	NO	03.05.2021	053	NO	NO	28/05/2021	130	NO	NO
	12006		Nei	15/04/2021				03.05.2021				28/05/2021			

	12007		Nei	15/04/2021				03.05.2021				28/05/2021			
	12008		Nei	15/04/2021	EE	NO	NO	03.05.2021	054	NO	NO	28/05/2021	131	NO	NO
	12016		Ja	15/04/2021	FF	NO	NO	03.05.2021	055	NO	NO	28/05/2021	132	NO	NO
	12017		Nei	15/04/2021	GG	NO	NO	03.05.2021	056	NO	NO	28/05/2021	133	NO	NO
	12018		Nei	15/04/2021				03.05.2021				28/05/2021			
F	12018	91020	Nei	16/04/2021	HH	NO	NO	03.05.2021	057	NO	NO	28/05/2021	134	NO	NO
	12019	91020	Nei	16/04/2021				03.05.2021				28/05/2021			
	12020	o2036	Ja	16/04/2021	II	NO	NO	03.05.2021	058	NO	NO	28/05/2021	135	NO	NO
	12021	61031	Nei	16/04/2021	JJ	NO	NO	03.05.2021	059	NO	NO	28/05/2021	136	+	NO
	12022	61031	Nei	16/04/2021				03.05.2021				28/05/2021			
	12023	61031	Nei	16/04/2021				03.05.2021				28/05/2021			
	12024	71011	Nei	16/04/2021	KK	NO	NO	03.05.2021	060	+	+	28/05/2021	137	NO	NO
	12025	71011	Nei	16/04/2021				03.05.2021				28/05/2021			
	12026	81027	Nei	16/04/2021				03.05.2021				28/05/2021			
	12027	81027	Nei	16/04/2021	LL	NO	NO	03.05.2021	061	+	NO	28/05/2021	138	NO	NO
G	10102	50696	Nei	18/04/2021	MM	NO	NO	03.05.2021	062	+	NO	28/05/2021	139	NO	+
	10103	60911	Nei	18/04/2021				03.05.2021				28/05/2021			
	10104	60911	Ja	18/04/2021	NN	NO	NO	03.05.2021	063	NO	NO	28/05/2021	140	NO	NO
	10114	70089	Nei	18/04/2021	OO	NO	NO	03.05.2021	064	NO	+	28/05/2021	141	NO	+
	10115	70089	Nei	18/04/2021				03.05.2021				28/05/2021			
	10132	70064	Nei	18/04/2021				03.05.2021				28/05/2021			
	10121	oooo5	Nei	18/04/2021	PP	NO	NO	03.05.2021	065	+	NO	28/05/2021	-	-	-
	10133	60861	Nei	18/04/2021				03.05.2021				28/05/2021	142	NO	+
	10130	60861	Nei	18/04/2021				03.05.2021				28/05/2021			
	10129	60861	Nei	18/04/2021	QQ	NO	NO	03.05.2021	dead	dead	dead	28/05/2021	dead	dead	dead
H	10019	90028	Nei	19/04/2021	RR	NO	NO	04.05.2021	066	NO	NO	31/05/2021	143	NO	++
	10020	90010	Nei	19/04/2021				04.05.2021				31/05/2021			
	10021	90010	Nei	19/04/2021				04.05.2021				31/05/2021	144	NO	++
	10025	60032	Nei	19/04/2021	SS	NO	NO	04.05.2021	067	NO	NO	31/05/2021			
	10026	60032	Ja	19/04/2021	TT	only 1	only 1	04.05.2021	068	NO	NO	31/05/2021	145	NO	+
	10032	70080	Nei	19/04/2021	UU	NO	NO	04.05.2021	069	NO	NO	-	-	-	-
	10033	70080	Nei	19/04/2021				04.05.2021				31/05/2021	146	-	+
	10034	70080	Nei	19/04/2021				04.05.2021				31/05/2021	147	NO	+
	10060	60090	Nei	19/04/2021	VV	NO	NO	04.05.2021	070	NO	NO	31/05/2021	148	NO	+
	10061	60090	Ja	19/04/2021	WW	NO	NO	04.05.2021	071	NO	NO	31/05/2021	149	NO	+
I	10570	oo483	Ja	19/04/2021	XX	NO	NO	05.05.2021	072	NO	NO	31/05/2021	150	+	NO
	10559	oo271	Ja	19/04/2021	YY	NO	NO	05.05.2021	073	+	NO	31/05/2021	151	+	NO
	10560	oo271	Ja	19/04/2021	AAA	NO	NO	05.05.2021	074	+	NO	31/05/2021	152	NO	NO
	10561	80177	Ja	19/04/2021	BBB	NO	NO	05.05.2021	075	+	+	31/05/2021	153	NO	NO
	10605	90383	Ja	19/04/2021	CCC	NO	NO	05.05.2021	076	NO	++	dead	dead	dead	
	10581	80311	Nei	19/04/2021	DDD	NO	NO	05.05.2021	077	+	NO	31/05/2021	154	NO	NO
	10582	80311	Nei	19/04/2021				05.05.2021				31/05/2021			
	10612	60361	Nei	19/04/2021				05.05.2021				31/05/2021			
	10613	60361	Ja	19/04/2021	EEE	NO	NO	05.05.2021	078	+	NO	31/05/2021	155	NO	++
	10614	60361	Ja	19/04/2021	FFF	NO	NO	05.05.2021	079	+	+	31/05/2021	156	NO	NO
J	10111	50028	Nei	20/04/2021	GGG	NO	NO	05.05.2021	080	NO	NO	31/05/2021	157	NO	NO
	10212	80226	Ja	20/04/2021	HHH	NO	NO	05.05.2021	081	NO	+	-	-	-	-
	10214	50565	Nei	20/04/2021	III	NO	NO	05.05.2021	082	NO	NO	31/05/2021	158	NO	NO
	10219	50766	Nei	20/04/2021				-	-	-	-	31/05/2021	159	NO	NO
	10224	80116	Ja	20/04/2021	JJJ	NO	NO	05.05.2021	083	NO	+	31/05/2021	160	+	+
	10225	80116	Ja	20/04/2021	KKK	NO	NO	05.05.2021	084	+	+	31/05/2021	161	++++	NO

	10251	90566	Nei	20/04/2021				05.05.2021				31/05/2021			
	10253	80270	Nei	20/04/2021	LLL	NO	NO	05.05.2021	085	NO	NO	31/05/2021	162	NO	NO
	10255	oo268	Nei	20/04/2021				05.05.2021				31/05/2021			
	10258	ooo69	Nei	20/04/2021	MMM	NO	NO	05.05.2021	086	NO	NO	-	-	-	-
K	10037	90265	Ja	20/04/2021	NNN	NO	NO	07.05.2021	087	NO	NO	01.06.2021	163	++	++++
	10038	90265	Ja	20/04/2021	OOO	only 1	NO	07.05.2021	088	NO	NO	01.06.2021	164	NO	+
	10039	90265	Ja	20/04/2021	PPP	NO	NO	07.05.2021	089	+	+	01.06.2021	165	NO	+
	10047	90089	Ja	20/04/2021	QQQ	NO	NO	07.05.2021	090	NO	+	01.06.2021	166	NO	NO
	10048	90089	Ja	20/04/2021	RRR	NO	NO	07.05.2021	091	NO	+	01.06.2021	167	NO	+
	10060	60020	Ja	20/04/2021	SSS	NO	NO	07.05.2021	092	NO	NO	01.06.2021	168	+	NO
	10061	60020	Ja	20/04/2021	TTT	NO	NO	07.05.2021	093	+	+	01.06.2021	169	NO	NO
	10063	60020	Nei	20/04/2021	UUU	NO	NO	07.05.2021	-	-	-	-	-	-	-
	10070	40280	Ja	20/04/2021	VVV	NO	NO	07.05.2021	094	++	NO	01.06.2021	170	NO	NO
	10441	80457	Ja	20/04/2021	WWW	NO	NO	07.05.2021	095	NO	NO	01.06.2021	171	NO	NO
	10442	80458	Ja	20/04/2021	XXX	NO	NO	07.05.2021	096	NO	NO	01.06.2021	172	NO	NO
	10452			-	-	-	-	-	-	-	-	01.06.2021	-	-	-
	10625											06.01.2021	173	NO	+
				Samples				**Samples**				**Samples**			**In total**
				110				**112**				**107**			**329**

Supplementary file 9: Raw data Rogaland

Farm	Individual	Diarrhea	1st	CODE	Crypto	Giardia	2nd	CODE	Crypto	Giardia	3rd	CODE	Crypto	Giardia
L	11047	no	15/04/21	-		tomt	30/04/2021	178	+++	NO	x			
	11048	no	15/04/21	001	+	+	30/04/2021	177	++	no	x			
	11104	no	15/04/21				30/04/2021				x			
	11107	no	15/04/21				30/04/2021				x			
	11046	no	15/04/21	002	+	NO	30/04/2021	-			x			
	11049	no	15/04/21				30/04/2021	178	+++	NO	x			
	11106	no	15/04/21				30/04/2021				x			
	11108	no	15/04/21	003	+	NO	30/04/2021	179	++	NO	x			
	11109	no	15/04/21				30/04/2021				x			
M	10720	no	04/04/2021	004	NO	NO	18/04/2021	180	NO	NO	19/05/2021	193	NO	NO
	10721	no	04/04/2021	-			18/04/2021				19/05/2021			
	10568	no	04/04/2021	004			18/04/2021				19/05/2021	-		
	10569	no	04/04/2021	-			18/04/2021	181	NO	NO	19/05/2021	-		
	10174	no	04/04/2021	004			18/04/2021				19/05/2021	194	NO	NO
	10173	no	04/04/2021	005	+	NO	18/04/2021				19/05/2021			
	10722	no	04/04/2021	006	+	NO	18/04/2021	182	NO	NO	19/05/2021			
	10723	no	04/04/2021	007	NO	NO	18/04/2021				19/05/2021	195	NO	NO
	10570	no	04/04/2021	008	+	NO	18/04/2021			tomt	19/05/2021			
	10571	no	04/04/2021	009	NO	NO	18/04/2021			tomt	19/05/2021			
N	10140	no	15/04/2021	010	NO	NO	02/05/2021	186	NO	NO	x			
	10142	no	15/04/2021				02/05/2021				x			
	10151	no	15/04/2021	011	+	+	02/05/2021	187	NO	NO	x			
	10152	no	15/04/2021				02/05/2021				x			
	10134	yes	15/04/2021	012	NO	NO	02/05/2021	188	+	NO	x			
	10145	no	15/04/2021	-			02/05/2021	-			x			
	10146	no	15/04/2021	013	+	NO	02/05/2021	189	NO	NO	x			
	10127	no	15/04/2021				02/05/2021				x			
	10144	no	15/04/2021				02/05/2021	-			x			
O	11032	no	14/04/2021	014	NO	NO	30/04/2021	174	No	NO	28/05/2021	197	+	NO
	11031	no	14/04/2021				30/04/2021				28/05/2021			
	11037	no	14/04/2021				30/04/2021				28/05/2021			
	11038	no	14/04/2021	015	NO	NO	30/04/2021	175	No	NO	28/05/2021	-		
	11041	no	14/04/2021				30/04/2021				28/05/2021	-		
	11042	no	14/04/2021				30/04/2021				28/05/2021	198	+	+
	11039	no	14/04/2021				30/04/2021	-			28/05/2021	199	+	+
	11040	no	14/04/2021	016	+	NO	30/04/2021	176	No	NO	28/05/2021	-		
	11029	no	14/04/2021				30/04/2021				28/05/2021	199	+	+
	11030	no	14/04/2021				30/04/2021				28/05/2021			
P	10105	no	12/04/21	-		tomt	02/05/21	183	+	no	x			
	10106	no	12/04/21	017	NO	NO	02/05/21				x			
	10216	no	12/04/21	-			02/05/21	-			x			
	10217	no	12/04/21	-			02/05/21				x			
	10107	no	12/04/21	017	NO	NO	02/05/21	183	NO	+	x			
	10108	no	12/04/21	017	NO	NO	02/05/21				x			
	10091	no	12/04/21	018	+	NO	02/05/21	184	+	no	x			
	10092	no	12/04/21				02/05/21				x			
	10074	no	12/04/21				02/05/21	185	no	no	x			
	10075	no	12/04/21	019	+	NO	02/05/21				x			
Q	10135	no	12/04/21	020	NO	NO	02/05/2021	-			29/05/2021	196	NO	NO
	10139	no	12/04/21				02/05/2021	190	no	no	29/05/2021			
	10143	no	12/04/21				02/05/2021	-			29/05/2021			
	10140	no	12/04/21	021	NO	NO	02/05/2021	191	no	no	29/05/2021			
	10141	no	12/04/21				02/05/2021				29/05/2021			
	10142	no	12/04/21				02/05/2021				29/05/2021	-		
	10145	no	12/04/21	022	+	NO	02/05/2021	192	no	no	29/05/2021			
	10146	no	12/04/21				02/05/2021	-			29/05/2021			
	10160	no	12/04/21				02/05/2021				29/05/2021			

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