Presence and pathogenicity of Paramphistomidae in ruminants in The Netherlands.

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Final Report

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1. Summary

A study has been performed to get an impression about the presence and pathogenicity of rumen fluke in ruminants in the Netherlands. The study included a data analysis of results from parasitological examinations performed at GD Animal Health Service (GD) and a slaughterhouse survey. During eight visits 116 cows were examined: a general impression of the animals was obtained, rumens were inspected for the presence of flukes and samples were taken from duodenum and feces to be examined for larval stages and eggs, respectively. Two methods, a modified Dorsman and a CSF-technique, were used for examination of the feces for the presence of rumen fluke eggs. In the data analysis a herd prevalence of 15.8% in cattle herds and 8% in sheep herds was found. More positive herds were found in the Western part of the Netherlands. A prevalence of 23.3% in slaughtered cattle and >4.9% in slaughtered sheep 3 was found in the slaughterhouse survey. A relation between the estimated number of flukes in the rumen and the number of eggs in the feces when using a modified Dorsman technique was found. With visual inspection of the rumen as “gold standard” a sensitivity and specificity of resp. 82.6% and 83.3% was found for the Dorsman. Concluding that this is a suitable method for detecting rumen fluke infection. Non-dairy cattle had more often rumen fluke infection than dairy cattle, a possible explanation is a difference in grazing pastures. The study was not suitable for evaluation of the pathogenicity. More research is needed to determine a proper prevalence (herd and animal), rumen fluke related problems in herds and the species of the family Paramphistomidae in the Netherlands.

2. Introduction

Rumen flukes or paramphistomes are trematode parasites which can infect several wild and domesticated ruminants. Their life-cycle shows great similarities with that of *Fasciola hepatica* and involves a snail as intermediate host (Taylor et al., 2007). Eggs are shed in the feces from infected animals and in these motile ciliated miracidia develop. After hatching these miracidia invade a suitable freshwater snail where asexual multiplication and development to cercariae takes place. When the pastures are flooding, the cercariae are shed on the herbage by the snail and they encyst to metacercariae. These infective stages are ingested by the final host while feeding on the pasture. In the final host the juvenile flukes exist in the small intestine where they attach to the mucosa and grow before they migrate to the rumen (De Waal, 2010). Adult flukes live on the wall of the rumen and reticulum and have a light to bright red color when fresh, are pear shaped and about 1.0 cm in length (Taylor et al., 2007). The prepatent period is between seven to ten weeks and the total cycle takes about three to four months to complete (De Waal, 2010; Taylor et al., 2007).

Traditionally, clinical disease caused by paramphistomes was considered to be confined to areas of the world with a warmer tropical and sub-tropical climate (Horak, 1971). However, recently renewed interest for this parasite has arisen in temperate regions. In several countries in Europe, veterinary laboratories noticed an increased incidence of paramphistome eggs in submitted fecal samples (Mage and Dorchies, 1998; Malrait et al., 2015; Murphy et al., 2008; Foster et al., 2008; Gordon et al., 2013).Techniques used for fecal examination on liver fluke eggs, may also be suitable for diagnosis of rumen fluke (Taylor et al., 2007)

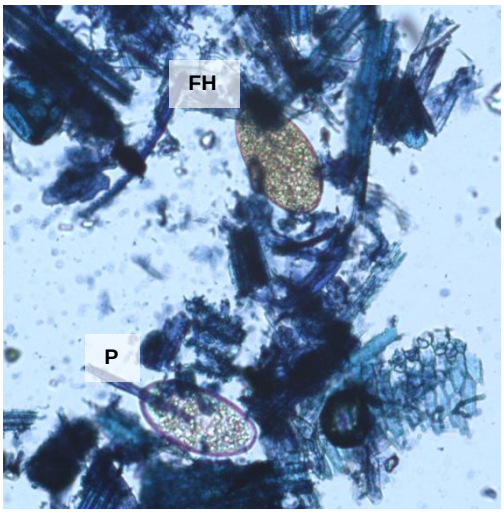


Figure 1. *Fasciola hepatica* (FH) egg and paramphistome (P) egg found with modified Dorsman technique at GD. Note the color difference of the content.
Photo: Coen Hegeman

Eggs of paramphistomes have a similar appearance to those of *Fasciola hepatica*; ovoid, large-sized, thin-shelled eggs with an operculum (Zajac and Conboy, 2006). However, the content of paramphistome eggs is pale grey and clear rather than yellow (Taylor et al., 2007) (Fig. 1). They are considered to be as large as or slightly larger than *Fasciola hepatica* eggs. Different sizes are mentioned in various sources, varying from 65-100 µm in width and 114-180 µm in length (Soulsby, 1965; Taylor et al., 2007; Zajac and Conboy, 2006; Thienpont, 1979).

Recently, cases were reported in which clinical disease in cattle and sheep was associated with paramphistome infection (Millar et al., 2012; Mason et al., 2012). Watery diarrhea with dehydration, severe condition loss, depression and mortality are the described clinical signs in these cases. At necropsy marked reddening of the proximal intestinal mucosa and hemorrhagic duodenitis was seen. Large numbers of immature rumen flukes (visible as nodules or bubbles on the mucosa) were found. In the sheep adult rumen flukes were present in the rumen, reticulum and omasum. Histolopathological findings confirmed lymphocytoplasmatic duodenitis and enteritis.

At the GD Animal Health Service (GD) questions were raised on the rumen fluke prevalence in livestock in The Netherlands. The objective of this study was to gain an impression of the prevalence and geographical distribution of rumen fluke infection in the Netherlands, to evaluate two fecal examination methods and to assess possible pathogenicity and risk factors of rumen fluke.

3. Materials and Methods

3.1. Data analysis

Results from routine parasitological examinations for liver fluke (*Fasciola hepatica*) in ruminants performed at GD Animal Health Service (GD) between May 2009 and September 2014 were used for estimation of the prevalence of rumen fluke in the Netherlands. The existence of regions with an increased risk of presence of a rumen fluke infection was evaluated. Also, the course of rumen fluke diagnosis throughout the year and the relation to a co-infection with liver fluke (*Fasciola hepatica*) was evaluated.

From 2009 until December 2012 a sedimentation technique was used to examine 10 gram feces per sample. Hereafter, a modified Dorsman technique (Dorsman, 1956) with a detection limit of 5 eggs per gram (EPG) was used (GD internal report). For this method, 10 gram sheep feces or 20 gram cattle feces was examined.

3.2. Slaughterhouse survey

Four abattoirs in the region of Utrecht and one in the region of Deventer were visited between November and December 2014 in order to collect data and samples of animals with and without rumen fluke. These data and samples were used to obtain information about the prevalence in addition to the above mentioned data analysis and to test two fecal examination methods for suitability in rumen fluke diagnosis. In addition, possible pathogenicity and risk factors were evaluated.

The abattoirs were selected based on their location and capacity. A selection was made of abattoirs in regions characterized by high groundwater levels and wet pastures with a lot of ditches. For practical reasons during sample taking, to be able to track the carcass and organs, relatively small (<100 cows slaughtered per day) were visited.

Before slaughtering the type of cow (dairy, non-dairy), body condition score (BCS 1 “very poor” to BCS 5 “very excessive”, based on the dairy cattle scoring system) and a general impression of the coat (dull/glossy, rough/smooth, long/short) was noted. On the slaughter line, the liver was inspected for indications for a liver fluke infection (migration tracks, thickened bile ducts, adhesions and hemorrhages). After opening the rumen and removing the content, the rumen was inspected for the presence of adult rumen flukes. The number of flukes was estimated in a “fluke density score” from 0-4 (no flukes visible, 1-100 flukes, 100-500 flukes, 500-1000 flukes, >1000 flukes). The reticulum was opened to look for the presence of rumen flukes, however these were not scored. Specimens of the flukes from the rumen were collected and stored in ethanol 70% and in buffered formalin 10%. Approximately 30 centimeters of the first part of the duodenum and a fecal sample taken from the rectum were collected for further investigation. Also, consistency of the feces was scored (watery, thin, pasty, normal, firm).

A dairy farm in East Netherlands was visited to collect fecal samples intended as negative control samples. This farm was chosen because it didn’t have a liver fluke history based on slaughterhouse findings and the location, an area with sandy grounds, not being a typical liver fluke habitat.

The fecal samples of the animals with a fluke density score >0 were investigated at GD and at the Faculty of Veterinary Medicine, Utrecht University (FVM). In addition a random selection of samples from animals with no flukes visible in the rumen and samples from the dairy farm were used as a negative control. At GD the earlier mentioned modified Dorsman technique was used to determine the number of rumen fluke eggs and liver fluke eggs in the feces, expressed in eggs per gram feces (EPG). At FVM a centrifugation-sedimentation-flotation (CSF) technique with a 1,3 g/ml sucrose solution and a detection limit of 2 EPG was used for the same purpose.

At the pathology department of GD the collected duodenum pieces were cut open and inspected for abnormalities and signs of enteritis. The main focus was on thickening (washboard-aspect), reddening, hemorrhages and immature flukes (resemble bubbles) (Millar, 2012). Tissue samples of approximately 2x2 cm were taken and stored in formalin 10% for histopathological examination at a later time

3.3. Statistical analysis

Data analysis

In order to estimate the prevalence of rumen fluke in sheep herds and cattle herds an analysis of the results from routine parasitological examinations was carried out. Also, the course of rumen fluke positive samples over the year was analyzed with this data. The analyzed data set consisted of submissions presented for examination on liver fluke eggs. Submissions that were not examined for one reason or another (e.g. too little material) or submissions from foreign addresses were excluded. The finding of rumen fluke eggs during these examinations was noted per submission, rather than per sample. Besides, it was not always traceable whether a sample was a pooled or from a single animal. For these reasons, the results were analyzed per submission or herd. This means that the results of liver fluke were merged to a result per submission. So, if in one of the samples fluke eggs are found, the entire submission is considered “positive” for liver fluke of “positive” for rumen fluke.

Each unique farm number (abbreviation in Dutch: UBN) or the address in case of private submitters was considered as an individual herd. Herd prevalence has been defined as the number of positive tested herds divided by the number of submitting herds in a certain period of time. Significant difference in prevalence of rumen fluke in sheep and cattle was tested with a chi-square test. Statistical significance was defined at $P< 0.05$. To obtain an impression of co-infection with liver fluke as risk factor, the odds ratio (OR) with a confidence interval of 95% was calculated.

Slaughterhouse survey

In the entire analysis, the result of visual inspection of the rumen is considered as gold standard. Differences between the group cattle with visible flukes and the group with no visible flukes for the scored parameters (BCS, appearance of coat, consistency, and aspect mucosa of duodenum) were tested with the chi-square test. Statistical significance was defined at $P< 0,05$.For the chi-square test the scored coat impressions were combined to normal (“glossy-smooth-long” and “glossy-smooth-short”) and abnormal(“dull-smooth-short”, “dull-rough-long” and “dull-rough-short”). The same was done for consistency of the feces (normal = “firm”, “normal” and “pasty”, abnormal = “thin” and “watery”.
A mean BCS with confidence interval of 95% was calculated for dairy cattle, non-dairy cattle and the total group of animals.

The mean EPG in relation to the fluke density score as well as sensitivity and specificity with 95% confidence interval for both fecal examination methods were calculated. The control fecal samples from dairy farm were not used in these calculations, since there was no information of the rumens of these animals.

All calculations were done in either Microsoft Excel or Statistix.

4. Results

4.1. Data analysis

Prevalence of rumen fluke

In the selected period 3753 fecal samples, spread over 1984 submissions were sent to GD for examination of liver fluke eggs. The analyzed number of submissions after excluding irrelevant data and the number of related herds is outlined in Table 1.

Table 1. Analyzed number of submissions per year investigated at GD for liver fluke and the number of related herds.

Year	Cattle (#)			Sheep (#)		
	Submissions	Samples	Herds	Submissions	Samples	Herds
2009	29	59	26	32	65	24
2010	121	285	109	46	103	38
2011	74	156	72	57	118	48
2012	173	434	152	168	252	132
2013	403	908	323	345	583	246
2014	270	561	238	176	229	141
Total	**1070**	**2403**	**730**	**824**	**1350**	**489**

In total 15.8% of tested cattle herds and 8.0% of tested sheep herds were found positive for rumen fluke between 2009 and 2014. In the selected period, significantly more cattle herds were found positive for rumen fluke eggs than sheep herds (see Fig. 2).

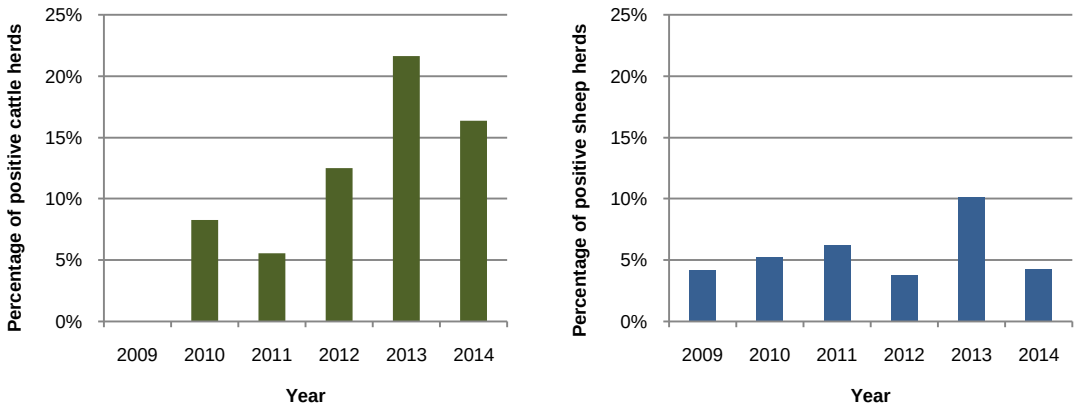


Figure 2. Percentage of cattle (left) and sheep (right) herds tested positive on rumen fluke at GD.

Incidence per quarter of a year

The incidence of rumen fluke positive submissions per quarter of a year showed no evident pattern. In Figures 3 and Figure 4 the course in percentage positive submissions and the total number of submissions is presented.

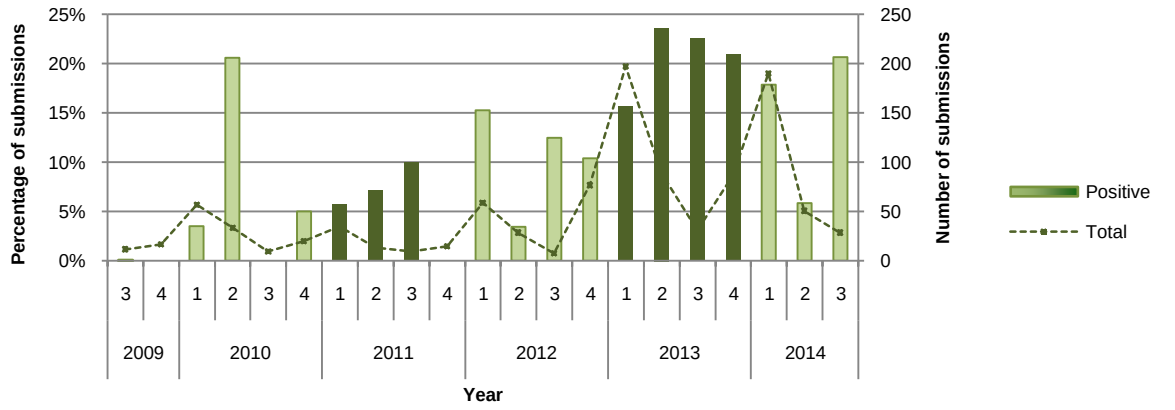


Figure 3. Distribution of percentage rumen fluke positive submissions and total number of submissions from cattle per quarter of a year

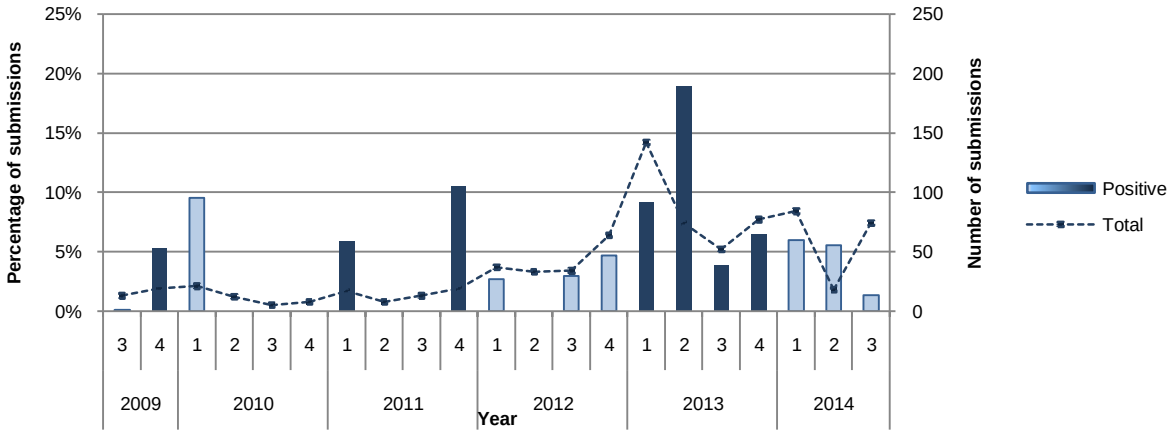


Figure 4. Distribution of percentage rumen fluke positive submissions and total number of submissions from sheep per quarter of a year

Geographical distribution of rumen fluke positive herds

Most herds in which rumen fluke was diagnosed, based on fecal examination, were situated in the Western part of the Netherlands. These regions are characterized by relatively wet farmlands and are areas with a higher risk of liver fluke infection (GD internal report). A lower incidence is seen in the Eastern and the Southern part of the country (Fig. 5).

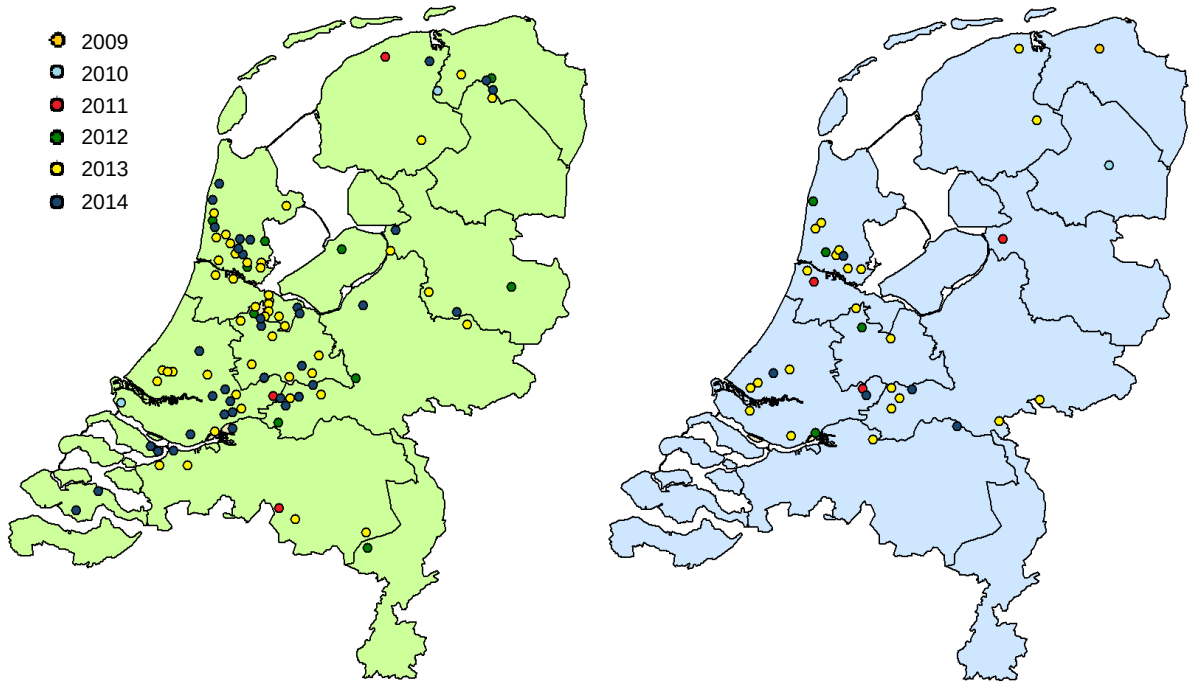


Figure 5. Geographical distribution based on cattle (left) and sheep (right) herds that tested positive for rumen fluke

Co-infection with liver fluke

A significant correlation was found between liver and rumen fluke infections. The estimated OR for cattle was 3.1 (2.02 – 4.63), meaning that herds with liver fluke infection have about three times more change of having a rumen fluke infection than herds without liver fluke infection. For the sheep herds, the estimated OR was 46.24 (6.29 – 339.7). In Table 3 and Table 4 the numbers of herds with the diagnosed flukes are presented for cattle and sheep, respectively.

Table 3. Diagnosed (based on fecal examination) fluke status of cattle herds.

Diagnosed with fecal examination	Number of herds	Percentage of herds
Only liver fluke	224	30.7%
Only rumen fluke	42	5.8%
Liver fluke and rumen fluke	73	10.0%
Neither	391	53.6%

Table 4. Diagnosed (based on fecal examination) fluke status of sheep herds

Diagnosed with fecal examination	Number of herds	Percentage of herds
Only liver fluke	203	41.5%
Only rumen fluke	1	0.2%
Liver fluke and rumen fluke	38	7.8%
Neither	247	50.5%

4.2. Slaughterhouse survey - cattle

Fluke burden

During eight slaughterhouse visits, in total 116 cattle were examined for the presence of flukes. Twenty-seven cows (23.3%) were found infected, with the majority of the flukes seen around the region where the esophagus opens into the rumen and around the passage between rumen and reticulum (Fig. 6). Fluke density score 1,2 and 3 were distributed equally as presented in Table 5. No rumen with score 4 was found. The impression was obtained that when there were more flukes in the rumen, it was more likely that they were also found in the reticulum.

Table 5. Distribution of fluke density scores of rumen from 116 cattle.

Fluke density score	Number of animals	Percentage of animals
0 (no flukes visible)	89	76.7%
1 (1-100 flukes)	9	7.8%
2 (100-500 flukes)	11	9.5%
3 (500-1000 flukes)	7	6.0%
4 (>1000 flukes)	0	0.0%

Rumen fluke was diagnosed significantly more often in non-dairy cattle than in dairy cattle (p= 0.02).Of the examined dairy cattle 17.8% (15/84 animals) was found positive, whereas 42.7% (9/21 animals) of non-dairy cattle was found positive. Eleven animals were not classified as dairy or non-dairy.

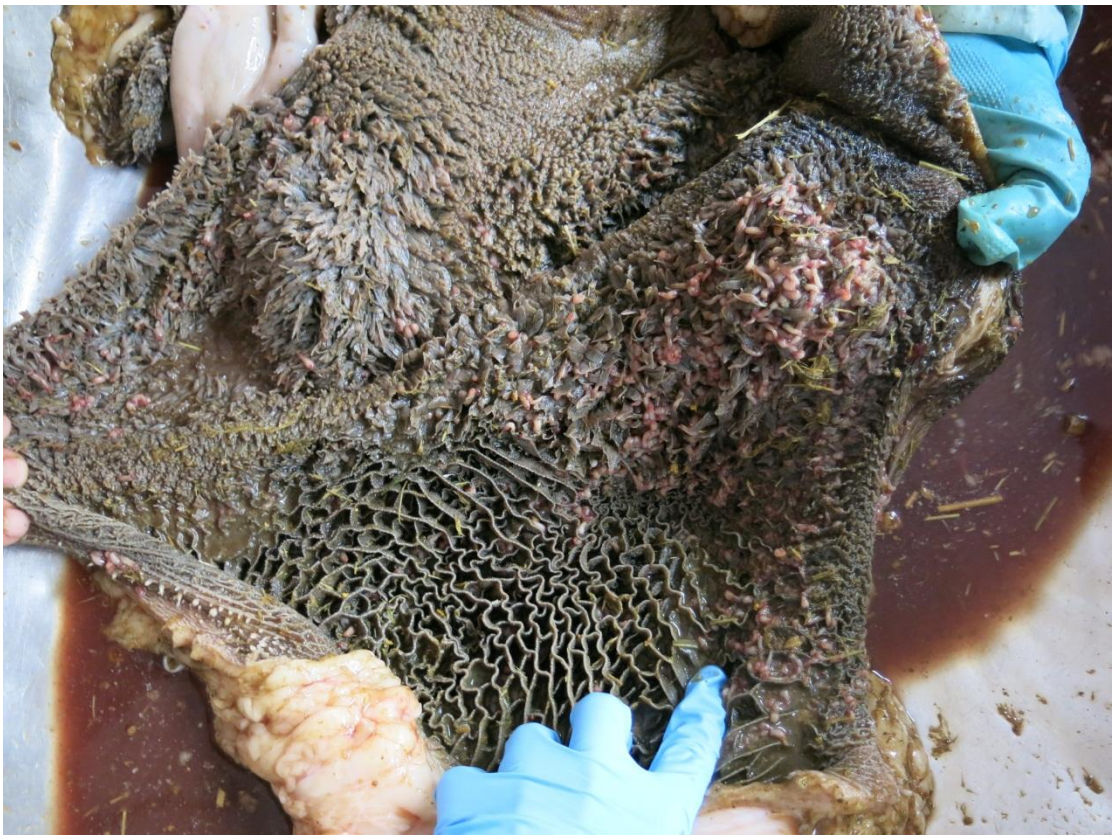


Figure 6. Bovine rumen and reticulum with fluke density score 3. Photo: Lianne Ankum

Body condition

The BCS was obtained from 102 animals whereby animals with rumen fluke infection had a higher mean BCS than animals without rumen fluke, as presented in Table 6 and Table 7 and in figure. 7.

Table 6. Mean and most frequent BCS in relation to rumen fluke infection of dairy and non-dairy cattle.

Type of animal	Rumen fluke	Number of animals	Mean BCS (95% CI)	Most frequent BSC
Dairy				
	Infected	14	2.4 (2.2 - 2.7)	2
	Not infected	67	2.5 (2.4 - 2.6)	2
Non-dairy				
	Infected	9	4.0 (4.0 - 4.0)	4
	Not infected	12	3.8 (3.5 - 4.0)	2-3
Total				
	Infected	23	3.0 (2.8 - 3.3)	4
	Not infected	79	2.7 (2.5 - 2.8)	2

Table7. Mean and most frequent BCS in relation to fluke density score of rumen.

Fluke density score	Number of animals	Mean BCS (95% CI)	Most frequent BSC
0	79	2.7 (2.5 - 2.8)	2
1	7	3.0 (2.5 - 3.5)	4
2	10	2.7 (2.4 - 3.0)	2
3	6	3.7 (3.5 - 3.9)	4

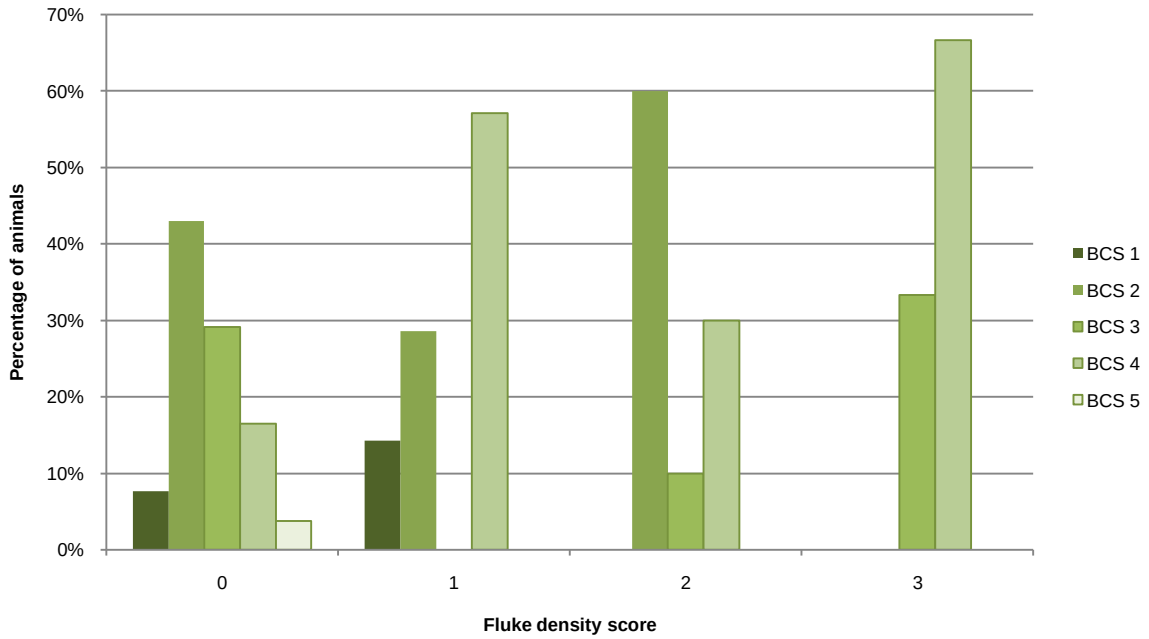
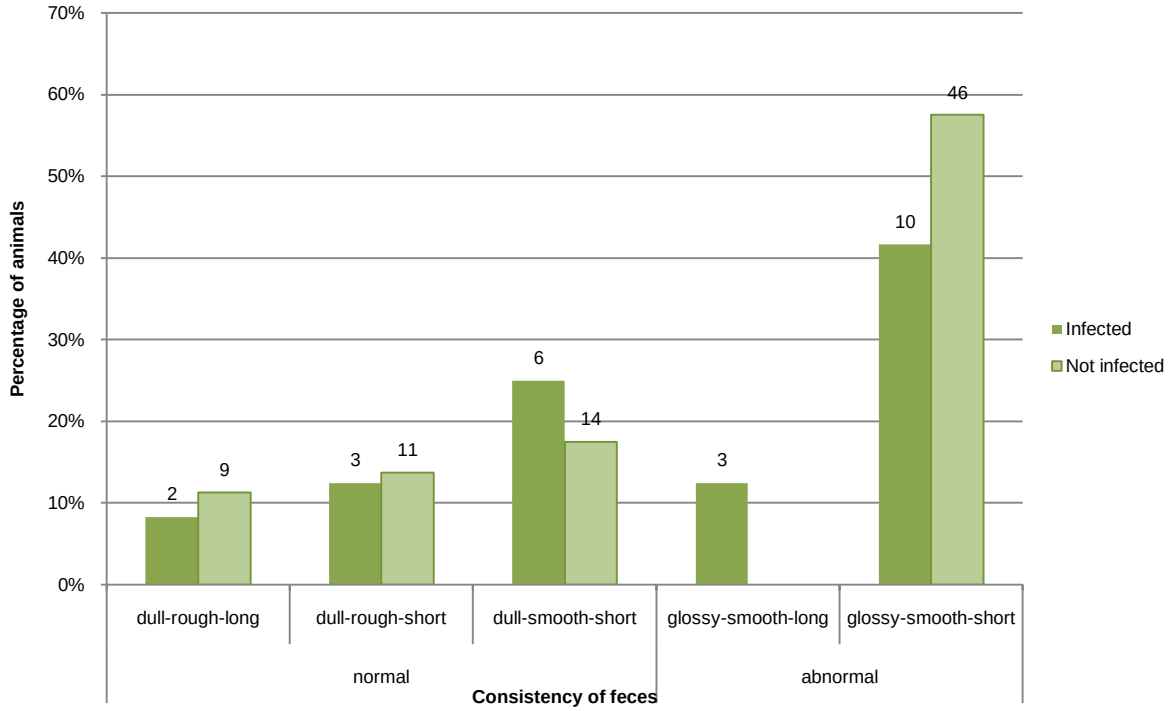


Figure 7. Distribution of BCS over fluke density score of rumen.

Appearance of the animals

A general impression based on the appearance of the coat of 104 animals was obtained, of which 24 were infected with rumen fluke. The results are displayed in figure 8. No significant difference in appearance (normal vs. abnormal) was found between animals with and without rumen fluke infection.



Consistency of feces

The distribution of fecal consistency scores of 24 rumen fluke infected and 76 uninfected cattle is displayed in figure 9. There was no significant difference in fecal consistency (normal vs. abnormal) between infected and uninfected.

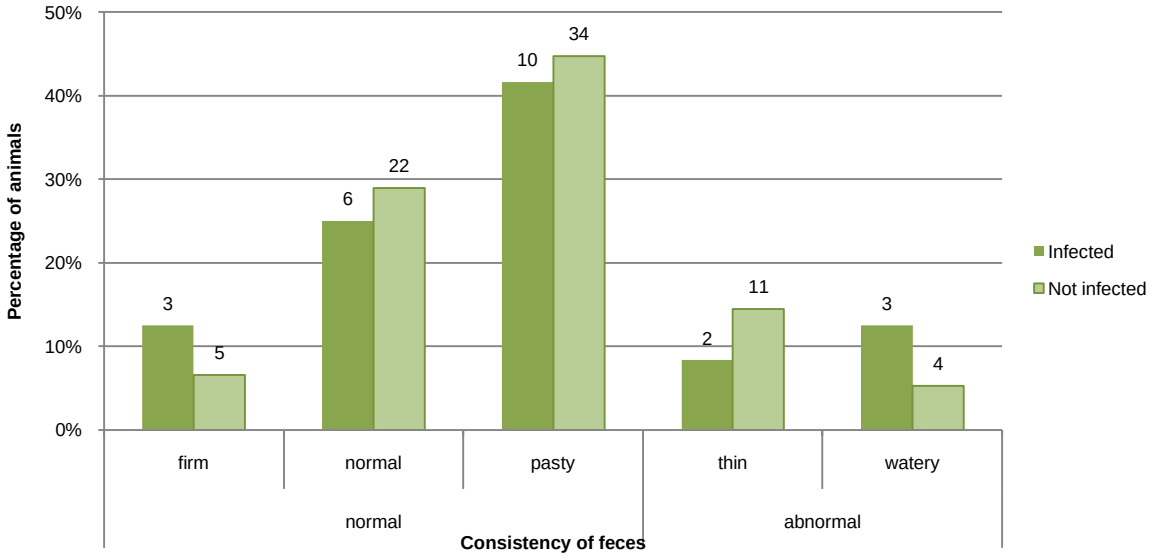


Figure 8. Consistency of feces in relation to rumen fluke infection. Numbers above the bars represent the number of animals.

Fecal egg count

At GD a total of 35 samples from slaughtered cows were examined and 31 of these were also examined at FVM. The EPG's of the positive samples at GD in comparison with the results from FVM are presented in Table 9. In both laboratories all 5 negative control samples from the dairy farm were reported as negative on eggs of rumen flukes. No further analysis was done on the FVM results.

Table 9. Comparison of rumen fluke EPG's in 20 samples between GD and FVM. All samples were positive at GD.

Laboratory	Egg per gram feces (EPG)																			
GD	5	5	5	5	5	5	15	20	35	40	75	80	85	90	95	95	175	300	715	>1000
FVM	0	0	0	0	0	0	0	0	8	0	0	20	0	0	0	0	0	0	124	16

When compared to the set gold standard of visual inspection of the rumen a sensitivity of 82.6% (74.3-90.9%) and specificity of 83.3% (77.5-89.1%) was calculated for the modified Dorsman technique. In 2 (out of 12) cattle with no flukes visible, an EPG of 5 was found at GD. An overview of the results found at GD is presented in Table 10 and figure 10.

Table10. Minimum EPG, maximum EPG and mean EPG with 95% confidence interval of 35 fecal samples examined at GD in relation to fluke density score.

Fluke density score	Number of samples	Minimum EPG	Maximum EPG	Mean EPG	95% CI
0	12	0	5	0.8	0.27 – 1.40
1	8	0	95	20.0	8.54 – 31.46
2	9	0	175	51.7	31.65 – 71.68
3	6	40	>1000	>371	208.31 – 533.35

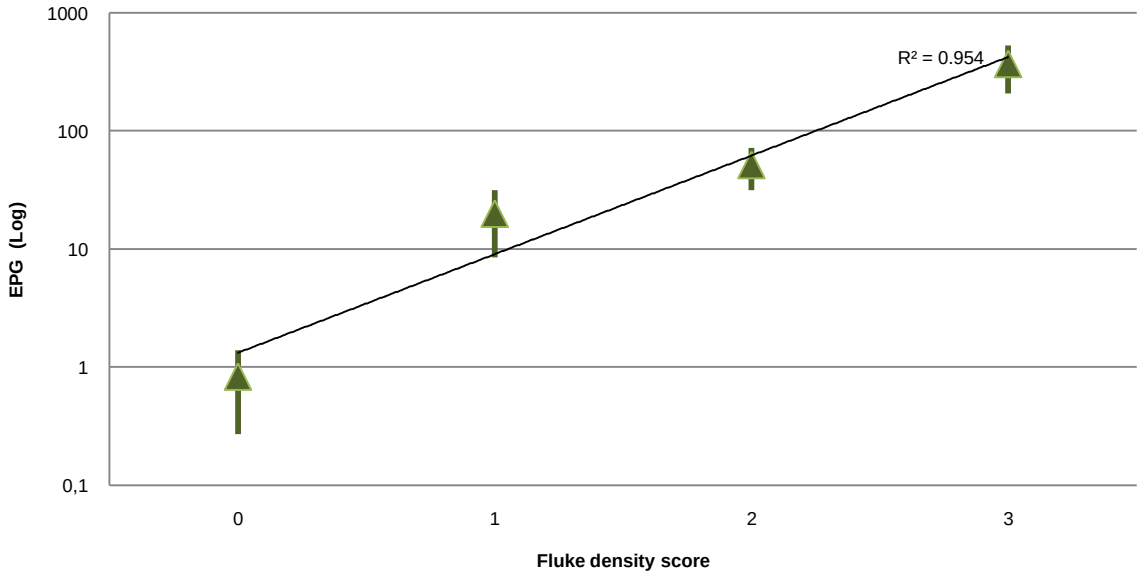


Figure 9. Mean EPG (with 95% confidence interval) as diagnosed at GD in relation to fluke density score.

Duodenum

Samples of the duodenum from 16 infected cattle and 38 cattle without rumen fluke were evaluated. No immature flukes were seen on the mucosal surface. The duodenum samples of animals infected with rumen flukes were significantly more often labeled as abnormal (p=0.007) (Table 11, Figure 11).

Table 11. Macroscopic aspect of the mucosal surface of duodenum samples in relation to rumen fluke infection.

Rumen fluke	Aspect mucosa	Number animals	Percentage of animals
Infected			
	Macroscopic abnormalities	11	68.8%
	No macroscopic abnormalities	5	31.3%
Not infected			
	Macroscopic abnormalities	11	28.9%
	No macroscopic abnormalities	27	71.1%

Figure 10. Abnormal mucosal aspect of a bovine duodenum labeled as "slightly thickened (washboard-effect) and slightly hemorrhagic". Photo: Lianne Ankum

Co-infection with liver fluke

The liver of 81 cattle, of which 13 animals with rumen fluke infection were examined. Nineteen livers showed signs of (previous) liver fluke infection (Table 12). No correlation was found between the presence of rumen fluke and liver fluke (both based on visual inspection).

Fecal samples of 24 animals with and 12 animals without rumen fluke infection were investigated on the presence of liver fluke eggs (Table 13). Again, no correlation was found between the presence of rumen fluke and liver fluke.

Table 12. Visual indications for liver fluke in relation to rumen fluke infection (based on visual inspection of rumen)

Rumen fluke	Indications for liver fluke	Number of animals
Infected		
	Not visible	9
	Yes	4
Not infected		
	Not visible	53
	Yes	15

Table 13. Finding of liver fluke eggs in feces in relation to rumen fluke infection (based on visual inspection of rumen)

Rumen fluke	Eggs of liver fluke	Number of samples
Infected		
	No	19
	Yes	4
Not infected		
	No	11
	Yes	1

4.3. Slaughterhouse survey - sheep

Two of the 41 examined sheep were considered infected and had a fluke density score of '1' and '2'. More (approximately 10) rumens with flukes were seen, but no records of originating animals were noted and these were therefore not included in an analysis. Based on these findings an estimated prevalence of >4,9% was calculated.

The flukes seen in the rumen of sheep were remarkably smaller (Fig. 12) and there seemed to be less indication for a preferred location compared with the findings in cattle. No eggs of rumen flukes (neither at GD nor at FVM) were found in the fecal samples of the two sheep with the presence of rumen fluke at visual examination at the slaughterhouse.

In 3 of the collected 14 duodenums brownish spots were seen under the mucosa (figure 13). Two of these animals had flukes in the rumen at visual inspection. Also, two samples showed some hemorrhages on the mucosal surface of the duodenum, but these were from sheep with no flukes in the rumen.

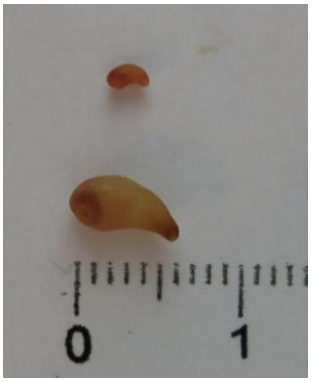


Figure 11. Rumen fluke from sheep rumen (above) and bovine rumen (below) after storage in formalin 10%. Photo: Lianne Ankum

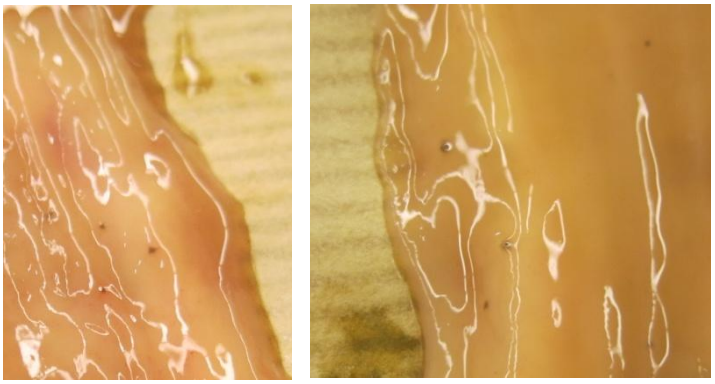


Figure 13. Mucosal surface of duodenum of sheep showing brown spots under the mucosa. Photo: Lianne Ankum

5. Discussion

Based on submissions of fecal samples in the years 2009 – 2014 (until September), for the probability diagnosis liver fluke, and presented as a result per herd instead of a result per individual cow, rumen fluke infection was found in 15.8% of the tested cow herds and in 8% of the sheep herds. To get a better insight in the real prevalence and to perform cluster analysis of rumen fluke infections these data are not suitable and could only give a rough indication, whereby most herds with rumen fluke seem to be in the same areas as liver fluke. The indication that the prevalence in cattle feces is increasing in the Netherlands is confirmed in our study. This is in line with the impression abroad (Murphy et al., 2008; Arias et al., 2011; Malrait et al., 2015). This increasing prevalence might be influenced by increased groundwater levels of the countryside in special areas of the Netherlands and by a relatively "new" diagnostic tool since 2012 in the laboratory of the GD to detect liver fluke infections in feces (the modified Dorsman technique, internal Report GD). Both in cattle and in sheep a positive correlation was found between the presence of rumen fluke and liver fluke (OR resp. 3.1 and 46.2) which is in line with the results of Arias et al. (2011).

A slaughterhouse survey was performed during visits in areas which were familiar with rumen fluke infections and an impression was obtained of the density of the score (see Table 5) and the location of the flukes (around the esophagus and the reticulum), which is completely in line with the results of a Spanish study, who found the most flukes in the frontal part of the rumen (Ferreras et al., 2014). The prevalence found (23.3%) in this study is in between found prevalence of other studies. In Portugal and the North-Western an estimated prevalence of 12% (Arias et al., 2011), a later Spanish study estimated a prevalence of 6% during a slaughterhouse study (Ferraras et al., 2014). A recent report from Belgium found a prevalence of 28% during a slaughterhouse survey (Malrait et al., 2015). Rumen flukes were more often found in non-dairy cattle which may be explained by differences in used pastures. Non-dairy cattle are normally pastured on fields with a higher ground-water level and in the flood plains of rivers. These areas usually form a more suitable habitat for the intermediate hosts of Paramphistomidae. The higher body condition score of animals with rumen flukes, can be explained by the higher body condition score of non-dairy cattle in relation to dairy cattle. The number of animals was too low to test significance.

In this study a good sensitivity and specificity of resp. 82.6% and 83.3% was found for the modified Dorsman technique, whereby the presence of rumen fluke parasites at the moment of slaughtering in the abattoir were taken as the gold standard. In this study the assumption was made that all flukes visible in the rumen were mature and shedding eggs. However, it might be that the seen flukes were not yet reproductive. In this case eggs cannot be found in the feces, lowering the sensitivity of the modified Dorsman. This laboratorial technique scored better than the CSF method with and is in line with the used mini-Flotac technique in the Belgium study (Malrait et al., 2015).

Since the CSF is a valid method for detecting liver fluke eggs and rumen fluke eggs have similar characteristics, more questions were raised on the execution of the test rather than the suitability to explain the low amount of found eggs. The low sensitivity of the CSF might be caused by the use of a sucrose solution with a density <1.3 g/ml. Using sugar of different brands with the same recipe for the sucrose solution resulted in solutions with densities varying from 1.25-1.3. Rumen fluke eggs will not float in a solution with a density that is too low and thus are not detected. Comparison of both methods in a new study might be preferable to evaluate both methods with the same data.

Based on the modified Dorsman technique, a linear correlation (R^2= 0.95) was found between the flukes density in the rumen and the estimated amount of rumen fluke eggs in the feces. Neither a correlation between the visual presence of both rumen fluke and liver fluke, nor between the visual presences of liver fluke and *Fasciola hepatica* eggs at microscopic fecal examination was found in the

slaughterhouse part of the study. In this case, liver fluke infection doesn't seem to be a risk factor for rumen fluke infection.

Cattle with rumen fluke infection had significantly more often an abnormal aspect of the duodenal mucosa, however no immature flukes were seen. More severe changes of the intestinal mucosa are described by Millar et al. (2012) and Mason et al. (2012), however these were in clinically diseased animals. The slaughtered animals in this study were not clinically diseased and this might explain the less severe abnormalities in the duodenum.

The found rumen flukes in sheep were smaller than the ones in cattle, it might be that these were not yet fully grown, supported by not finding eggs in the feces, or are different species. Not much recent information is known about which rumen fluke species are present in the Netherlands. The Fauna Europaea (2014) mentions *Paramphistomum cervi, P. hiberniae, P. leydeni* as present. *Paramphistomum* species are also mentioned in reports from Ireland (Murphy et al., 2008), Scotland (Mason et al. 2012) and Great Britain (Foster et al., 2008; Millar et al., 2012). No data is provided in the Fauna Euroaea (2014) about *Calicophoron daubneyi* in the Netherlands. This species is found and molecular confirmed in Belgium (Malrait et al., 2015), Spain (Ferrerras et al., 2013), Great Britain and Scotland (Gordon et al., 2013) and North Portugal and North-West Spain (Arais et al., 2011)

6. Conclusions and recommendations

In this study an indication of the status of rumen fluke infection of ruminants in The Netherlands was obtained. A rough indication of the prevalence of rumen fluke and area's with a higher prevalence were found, but these were partially biased because a large part of the samples were sent to the GD laboratory because of liver fluke problems. To get a better idea of the prevalence among herds and ruminants, fecal samples from random livestock holdings and animals need to be taken and examined. The results from routine parasitological examinations might be of more value when the finding of rumen fluke eggs is noted per sample rather than per submission.

Also, it can be concluded that the modified Dorsman technique is suitable for diagnosing rumen fluke and the severity of infection. However, pathogenicity and importance of rumen fluke for Dutch livestock holdings is still unknown. Based on the findings of both parts of this study in combination with recently published articles it can be concluded that more research is needed. Approaching comparable livestock holdings with and without rumen fluke infection to question farmers about production, grazing, state of pastures, liver fluke treatment and (diarrhea) problems in grazing youngstock is advised to gain more information about the relevance of and possible risk factors for rumen fluke.

Since not much information is known about which species within the Paramphistomidae family are present in the Netherlands, collecting flukes found in animals submitted for pathology at GD, store them in ethanol 70% for later DNA examination as described by Gordon et al. (2013) is recommended.

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