Observations and immunohistochemical detection of Coronavirus, *Cryptosporidium parvum* and *Giardia intestinalis* in neonatal diarrhoea in lambs an kids

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Summary

In this study, clinical, parasitological, macroscopical, histopathological and immunohistochemical examinations were performed on 19 kids and 11 lambs (30 animals) with neonatal diarrhoea to detect the presence of Coronavirus, *Cryptosporidium parvum* and *Giardia intestinalis*. Clinically, severe dehydration, yellowish-green to brown coloured diarrhoea and death were observed. Mortality rates were 10–30% in the examined flocks. The most common agent was *C. parvum* diagnosed in 20 animals as a single causative agent, whereas *G. intestinalis* was found in 5 of 30 animals. These two protozoa were detected together in 4 animals upon faeces examination. Fifteen of 24 cases of *C. parvum* and 3 of 11 cases of *G. intestinalis* were also confirmed histopathologically. Following immunohistochemical examination, all cryptosporidiosis cases were confirmed by positive immunostaining of intestinal sections. Two additional Giardiosis cases with negative results upon parasitological and histopathological examinations were diagnosed by means of immunohistochemical examination. Coronavirus was detected immunohistochemically in one kid with neonatal enteritis. Following diagnosis, herds were treated with Trimethoprim + Sulfodoxine and multivitamin complexes. Intravenous and intramuscular administrations of these drugs were effective for both treatment and prevention of neonatal diarrhoea in lambs and kids.

Keywords: neonatal diarrhoea, Coronavirus, *Cryptosporidium parvum, Giardia intestinalis,* lamb, kid

Beobachtungen und immunhistochemischer Nachweis von Coronavirus, *Cryptosporidium parvum* und *Giardia intestinalis* bei Lamm und Zicklein mit neonatalem Durchfall

In dieser Studie wurden insgesamt 19 Lämmer und 11 Zicklein mit neonatalem Durchfall klinisch, parasitologisch, makroskopisch, histopathologisch und immunhistochemisch auf das Vorkommen von Cornonavirus, *Cryptosporidium parvum* und *Giardia intestinales* untersucht. Klinisch konnten starke Dehydrierung sowie gelblich-grün bis braun gefärbter Durchfall und Todesfälle beobachtet werden. Die Mortalität in den Herden schwanke zwischen 10–30%. Bei 20 Tieren konnte als alleinige Ursache *C. parvum* und bei 5 von 30 Tieren *G. intestinalis* diagnostiziert weren. Beide Protozoen zusammen wurden im Kot von 4 Tieren gefunden. 15 von 24 Fällen mit *C. parvum* und 3 von 11 Fällen mit *G. intestinalis* konnten histopathologisch nachgewiesen werden. Nach immunhistochemischer Untersuchung gelang es mittels positiver Immunfärbung von Darmschnitten alle Cryptosporidien-Fälle zu bestätigen. Zwei Giardia–Fälle mit negativen parasitologischen und immunhistologischen Befunden wurden immunhistochemisch diagnostiziert. Bei einem Zicklein mit neonataler Enteritis konnte Cornonavirus festgestellt werden. Nach gestellter Diagnose wurden die Herden mit Trimethoprim + Sulfodoxin und einem Multivitamin-Präparat behandelt. Dabei zeigte sich, dass die intravenöse wie auch intramuskuläre Verabreichung dieser Substanzen für die Behandlung wie auch die Prävention von Durchfall bei neugeborenene Lämmern und Zicklein wirksam waren.

Schlüsselwörter: Neonataler Durchfall, Cornonavirus, *Cryptosporidium parvum, Giardia intestinalis,* Lamm, Zicklein

Introduction

Diarrhoea in new-born farm animals under 21 days of age is one of the most common diseases the large animal clinician is faced in practice. It causes a significant economic loss in herds and may assume greater importance in the future as livestock production becomes more intensified. On a clinical basis it is not always possible to differentiate between the various known agents of diarrhoea in new-born animals (Blood and Radostits, 1989). The most commonly recognised causes of neonatal calf diarrhoea are rotavirus, coronavirus, cryptosporidia, and enterotoxigenic E.coli (Reinhardt et al., 1993; Munoz et al., 1996; Blood and Radostits, 1989; Naylor, 1990; Eisa and Mohamed, 2004).

Coronaviruses may be a problem in calves particularly at the age between 4 to 30 days. Pathology involves both the small and large intestine (Durham et al., 1979; Naylor, 1990). Clinically coronaviral enteritis is characterised by yellow fluid, sometimes bloody diarrhoea, depression, reluctance to nurse, dehydration and weakness. The course of infection, clinical signs and tissue damage are more prolonged in coronaviral enteritis than in rotavirus-induced disease, and death occurs after 2 to 4 days of diarrhoea. The gross lesions are indistinguishable from those of rotavirus enteritis or enterotoxigenic colibacillosis. The small and large intestines are moderately distended with yellow fluid. Microscopically, coronavirus infection is first apparent in villous epithelial cells of the proximal small intestine and colon. A haemorrhagic form of coronaviral enterocolitis is recognised if all colonic mucosa is damaged. It is not known if this form of the disease represents infection with a more virulent strain of virus or an interaction of the virus with other pathogens (Van Kruiningen, 1995).

Cryptosporidiosis, a disease of most domestic and wild animals, birds, fish, reptiles, and humans, is caused by members of the genus *Cryptosporidium* (Van Kuruiningen, 1995; Jones et al., 1997). Species identification, however, cannot be made from tissue section or simple examination of oocysts; therefore, the disease due to *Cryptosopridium* is usually designated only as cryptosporidiosis (Jones et al., 1997). *C. parvum* has a rapid, direct life cycle and infection occurs when viable oocysts in the environment are ingested by susceptible hosts, usually young stock under a month old (Blewitt and Angus, 1991). One major species, *C. parvum*, infects both farm animals and humans (Prichard and Fleetwood, 1995; Jones et al., 1997). The oocysts of *C. parvum* are so small that its association with acute diarrhoea was never suspected until the 1970s, when specific biological staining methods confirmed their presence in animals with diarrhoea. Cryptosporidiosis is widespread throughout the world. Lambs, as young as 3 days, can be affected by the disease and they are depressed and reluctant to suck. Very young lambs soon become dehydrated and die. In poor weather conditions lambs may die of hypothermia. The illness may last for up to 10 days and relapses after apparent recovery are common (Angus et al., 1982; Graaf et al., 1999).

Giardiasis occurs in many species, including human beings and domestic animals. The disease is frequently recognised in young animals. Giardiasis is caused by a unicellular flagellated protozoon. *Giardia intestinalis* inhabits the small intestine, particularly the duodenum, where the organisms attach to the microvillous border of epithelial cells producing craters in the surface. When *Giardia* are present in small numbers, they produce no clinical illness. However, when in great numbers or in an immunologically deficient individual, diarrhoea occurs. The parasites inhibit the absorption of simple sugars and disaccharides, which are then fermented by bacterial flora, creating intestinal gas. Clinically, animals with giardiosis have brown, fluid diarrhoea, signs of abdominal discomfort without fever, weight loss or melena. The diagnosis is made by demonstrating *Giardia* in preparations of fresh faeces. Histopathologically the diagnosis is made by examining the periphery of duodenal and jejunal villi, as the organisms are usually attached to villous epithelial cells or between villi. The parasites can be stained with Hematoxylin and Eosin (HE), but are more readily identified in Giemsa – stained sections (Buret and Olson, 1991; Taylor et al., 1993; Olson et al., 1995; Van Kruiningen, 1995; Olson et al., 1997).

Bacterial aetiology of neonatal diarrhoea is well known but knowledge about viral and parasitic aetiology is relatively low. The aim of this study was to investigate the presence of Coronavirus, *C. parvum* and *G. intestinalis* in neonatal lambs and kids with diarrhoea, and to examine clinicopathological findings of this illness and also investigate the efficacy of Trimethoprim + Sulfodoxine combination at treatment.

Animals, Material and Methods

In this study, a total of 30 animals (19 kids and 11 lambs) that had died due to neonatal diarrhoea and originating from ten different flocks were examined clinically, parasitologically and pathologically. Fresh faeces samples were taken from all animals at necropsy and then examined directly under the microscope. They were also stained with carbolfuchsin and Giemsa stain.

During necropsy, tissue samples were taken from all parts of the guts and fixed in 10% buffered formaline. Following the routine procedure, tissue samples were blocked in paraffin and cut at 5 μm thickness, and then stained with HE and examined by light micro-

scope. After histopathological examination, intestinal sections were immunostained using the streptoavidin-biotin technique, in accordance with the manufacturer's instructions, for detection of Coronavirus, *C. parvum* and *G. intestinalis*. For immunohistochemical observations, paraffin wax was sectioned at 4 μm and sections were attached to glass slides coated with poly-L-lysine. They were deparaffinized and rehydrated. To reduce non-specific background staining due to endogenous peroxidase, slides were incubated in 0.5% hydrogen peroxide/methanol for 10 minutes and boiled with 0.01 M citrate buffer solution (pH 6.0) for 20 minutes, than incubated in diluted normal horse serum for 10 minutes. Subsequently the primary antibody for Coronavirus [Bio-X Diagnostic (BIO 288) Mache-en-Famenne, Belgium], Cryptosporidium [Novo Castra (NCL-Crypto), Newcastle- UK] or Giardia intestinalis [Novo Castra (NCL-G$_1$) Newcastle-UK] was applied, and tissues were incubated for 60 minutes at 25°C temperature. Then tissues were incubated with biotynilated secondary antibody and streptoavidin/peroxidase each for 10 minutes at room temperature. Tissues were further incubated for 10 minutes at room temperature in a solution of DAB (3, 3' diaminobenzidine) Chromogen and counterstained with Mayer's Haematoxylin and cover slips were applied together with mounting media.

Following diagnosis, the herds were clinically examined and all animals under the age of 21 days included in the flocks were treated. The treatment consisting of Trimetoprim+ Sulfadoxine and multivitamin complex were started in all the kids and lambs in the affected flocks. For this purpose, 0.5-1 ml of Trimetoprim+ Sulfadoxine injectable solution was administered intravenously for two days, and subsequently by intramuscular route for 3 days to lambs and kids. In addition, multivitamin was administered intramuscularly at a dosage of 1 ml per 10 kg body weight. All of the new-born animals included in the infected flocks were also treated for five days with the same medicines. Furthermore, fluid therapy and nonsteroidal anti-inflammatory drugs were administered to severely infected animals.

Results

Owners stated that, lambs and kids were depressed and reluctant to suck throughout diarrhoea. The most common clinical findings were dehydration, depression, tenesmus, abdominal swelling, hypothermia and death. Watery and yellowish-green diarrhoea was the most prominent clinical finding in cryptosporidiosis positive cases. Brownish coloured diarrhoea and excessive gas formation in the guts were especially observed in giardiosis. In some cases, and particularly
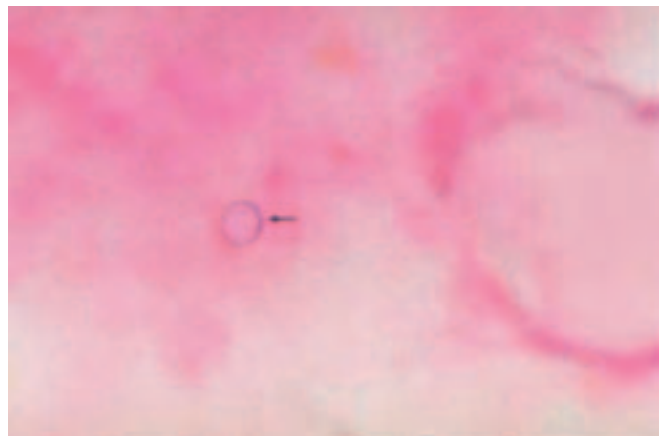

Fig. 1: Cryptosporidium parvum oocysts in a lamb's gut content (Ziehl-Neelsen).

in cryptosporidial and giardial enteritis, faeces contained undigested milk and mucus, also rarely blood. Mortality rates ranged between 10–30% and were high if more than one agent was present. Hygiene and management were concluded to be very important with regard to both morbidity and mortality. Cryptosporidia were noted from 2 days after birth. Viable *G. intestinalis* was easily detected due to its specific movements in native preparations of faeces. It was also possible to detect non-viable agents upon careful examination of native faecal preparations. *Cryptosporidium* oocysts were observed to be transparent and they were easily detected in Carbolfuchsin and Giemsa stained faecal samples (Fig. 1). *G. intestinalis* was also easily diagnosed in Giemsa stained samples (Fig. 2). Carbolfuchsin staining of native smears was judged as the best routine technique for the detection of cryptosporidiosis. At necropsy, both the small and large intestines were observed to be hyperaemic and usually swollen with gas. The lumens of the intestines contained yellowish-green to brown coloured watery faeces. In some cases, the content of the intestines was bloody. Invaginations were common necropsy findings because of the increased peristaltic movements of the guts. Haemorrhages were seen on the mucosa of the abomasum in 12 animals, whereas in one case
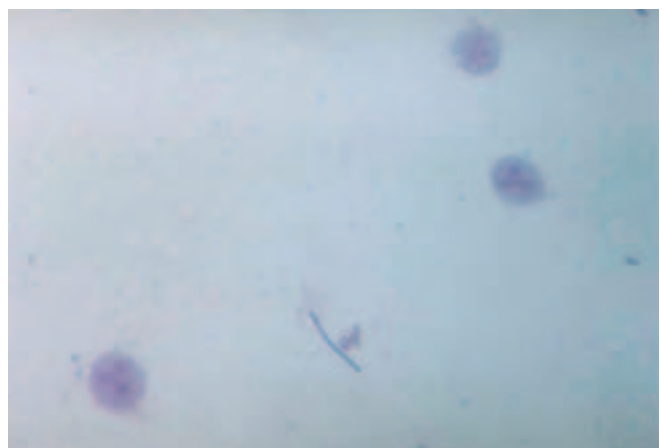

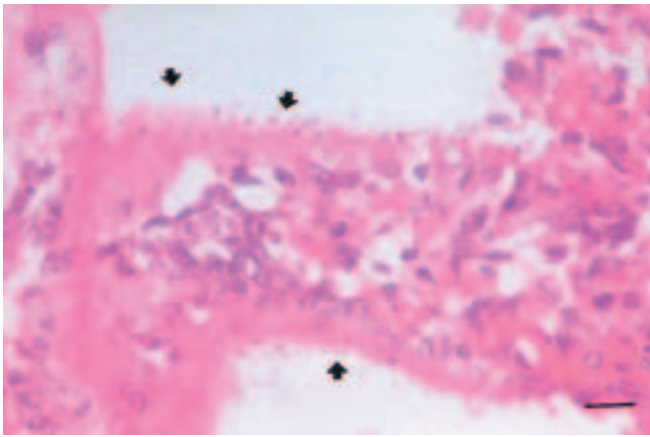*Fig. 2: Giardia intestinalis in a lamb's gut content (Giemsa).*

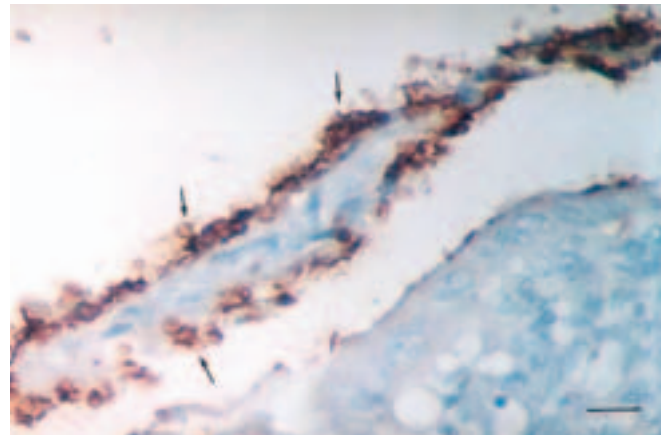*Fig. 3: Numerous C. parvum attached to the enterocytes in a kid, (HE, Bar = 50 μm).*

Fig. 4: Immunodetection of C. parvum in the gut of a kid (Bar = 50 μm).

abomasal perforation was observed. Hyperaemia and oedema in the abomasum were also among common findings. There were no prominent lesions observed out of the gastrointestinal system organs.

Histopathological sections of the intestines of the animals with neonatal enteritis showed different histopathological changes. The most prominent lesions were hyperaemic vessels, submucosal oedema, desquamation erosions, ulcers and inflammatory cell infiltration to the intestinal mucosa. These lesions were observed in the intestinal sections belonging to all 30 animals. Increased mucus secretion in the intestinal mucosa was also diagnosed commonly and was observed in 25 intestinal sections. In severe cases, the entire layer of the mucosa appeared to be necrotic. In cases of cryptosporidial enteritis, sloughing of epithelial cells caused release of oocysts into the gut lumen in heavily infected animals. Segments of the ileum were the site of peak intestinal cryptosporidiosis (Fig. 3). Most of the *G. intestinalis* were attached to the intestinal mucosa, however free parasites in the lumen were also present. The site of peak for *G. intestinalis* included the duodenum and jejunum. After histopathological examination, tissue sections were immunostained with monoclonal antibody against Coronavirus, *C. parvum* and *G. intestinalis*. Upon

immunohistochemical observation, *C. parvum* was diagnosed in 24 of 30 animals (80%), *G. intestinalis* in 11 of 30 animals (36.6%) and Coronavirus in only one of 30 animals (3.3%). *G. intestinalis* was diagnosed in two animals which had given negative results in parasitological and histopathological examinations. Coronaviral enteritis was confirmed only by immunohistochemical examination. *Cryptosporidium* positive cases revealed the presence of numerous *C. parvum* agents on the mucosa (Fig. 4). Immunopositive *G. intestinalis* trophosoites were seen to be both attached to the villi and also free in the lumen (Fig. 5). Coronavirus positive reaction was detected at the crypt epithelium in the small intestines and also in macrophages located in the submucosa (Fig. 6).

After diagnosis, all of the young animals in the herds were treated with Trimethoprim + Sulfodoxine and multivitamin complexes. Fluid therapy and nonsteroidal anti-inflammatory drugs were also administered to severely ill animals. Despite failure of treatment in severely infected animals and those in a comatose status, drugs were found to be more effective in protection of susceptible animals from infection. Intravenous applications were considered to be more effective and obligatory in especially severely affected animals. Following administration of the
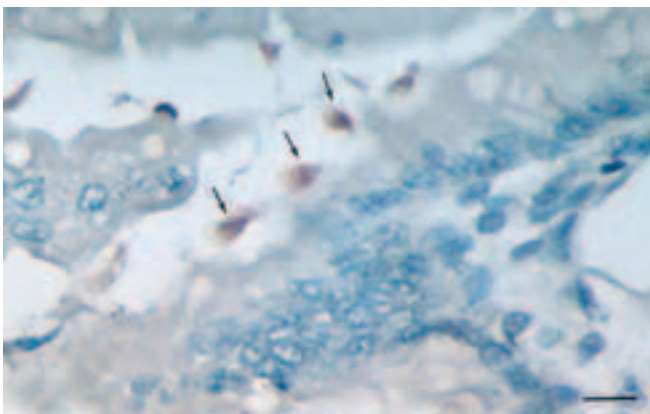

Fig. 5: Immunodetection of G. intestinalis in the gut of a lamb (Bar = 50 μm).
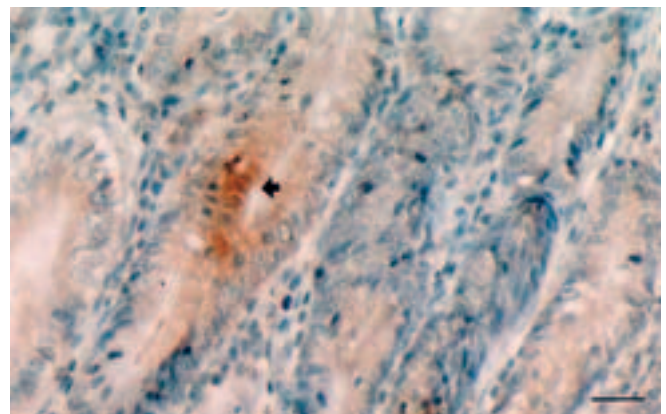

Fig. 6: Immunodetection of Coronavirus in crypt epithelium of a kid (Bar = 120 μm).

drugs by intravenous route for 3 days, death was observed to cease in the flocks. Treatment lasted 2 days when drug administration was performed by intramuscular route. Hygiene and management were also very significant with regard to the success of the therapy. Good hygiene practices decreased the severity of the disease and helped prevent re-infection. Desinfection of stables with 10% formalin solution was advised in intensive flocks.

Discussion

The infectious agent, *C. parvum*, causes diarrhoea in 1–4 week old calves, lambs and kids. These young animals are susceptible until they become functional ruminants. Diarrhoea is diffuse, watery and yellowish in colour. Faeces may contain undigested milk, blood, fibrin and mucus. Moderate dehydration, mild-to-moderate depression, tenesmus and low-grade fever are common signs. Chronically affected animals become emaciated and the disease causes high morbidity and low mortality. Most uncomplicated cases will recover in 6 to 10 days. Relapses are fairly common and can occur from auto-reexposure (Van Kruiningen, 1995; Jones et al., 1997). Similar clinical symptoms were seen in cryptosporidial enteritis cases in this study. Morbidity and mortality rates were much higher and in most cases the disease caused death in animals which exhibited clinically severe symptoms. The possible cause of this situation was attributed to the unhygienic conditions of stables and rearing of different aged animals within the same flock. The old animals were carriers of the disease and transmitted the illness to the young ones. For that reason, quarantine was advised for healthy young animals. Because of the inappetence possible immunosupression was seen in lambs and kids with diarrhoea. High mortality was observed in animals that couldn't suck colostrum.

The protozoon infects the brush border (microvilli) of the intestinal cells and it does not invade the cytoplasma. A membrane developed by the host surrounds the organism, thereby protecting it from antimicrobial agents (Van Kruiningen, 1995; Jones et al., 1997). Similar results were also obtained in this research. Most of the cryptosporidiums were attached to the intestinal epithelial cells and numerous organisms were seen to be free in the lumen. Treatment of heavy infections was difficult and usually impossible. However, Trimethoprim + Sulfodoxine were found to be effective in protection of animals against this illness. The probable cause of this effect was attributed to the efficacy of the medicine to prevent attachment of cryptosporidiums to the intestinal epithelium. Transmission occurs when an animal ingests the sporulated oocysts. The infection leads to villous

atrophy and crypt cell hyperplasia. Diarrhoea results from malabsorption and maldigestion and increased secretory activity (Van Kruiningen, 1995; Jones et al., 1997). Since the cases observed in this study were very severe and acute, chronic changes such as villous atrophy and crypt cell hyperplasia were not encountered. In severe cases of infection with *Cryptosporidium* and *Giardia*, all intestinal villi were observed to be covered with these organisms at histopathological and immunohistochemical examination. Diarrhoea caused death due to severe dehydration.

Cryptosporidiosis seems to be a problem mainly in neonatal ruminants. *C. parvum* is considered to be an important agent in the aetiology of the neonatal diarrhoea syndrome of calves, lambs and goat kids, causing considerable direct and indirect economic losses (Graaf et al., 1999). Although outbreaks in lambs are sporadic, mortality can be high. *C. parvum* is not host-specific, thus an environment contaminated with oocysts during an outbreak in calves can give rise to infection in lambs using the same premises or grazing area subsequently. Several studies have been carried out on neonatal diarrhoea in calves, but there are a few numbers of reports on neonatal diarrhoea in lambs and kids (Naylor, 1990; Graaf et al., 1999; O'Handley et al., 1999). Etiological factors have been very scantly examined. This study has revealed that neonatal enteritis may also be a big problem in lambs and kids. This disease can cause big economical losses in the sheep and goat industry. *C. parvum* is an important etiological agent found in neonatal diarrhoea in young small ruminants, especially in unhygienic stables and crowded flocks. *Cryptosporidium* was present in 80% of 30 suckling animals aged up to 21 days in examined flocks. Oocysts were exclusively recorded in lambs and kids that had manifested diarrhoea in our study.

Giardia can, however, be an important cause of diarrhoea, best established in humans. In animals, giardiasis is mostly observed in dogs, cats, laboratory animals and occasionally cattle (Jones et al., 1997). *G. intestinalis* has recently emerged as an important parasite of domestic livestock. Many reports indicate that *Giardia* infections occur in lambs and calves with a prevalence of up to 100% (Taylor et al., 1993; Olson et al., 1997; O'Handley et al. 1999). Infected calves and lambs may develop diarrhoea (O'Handley et al., 1999). The organisms localise in the small intestine on the surface of epithelial cells. Clinically the diarrhoea tends to be chronic, often lasting for several weeks and are sometimes recurrent. The stools tend to be bulky, malodorous and light coloured. The prolonged course can lead to malabsorption syndrome with weight loss. Morphologic lesions in the intestinal tract may be minimal or stunting of villi and an inflammatory infiltrate in the lamina propria can occur. Organisms may be difficult to discern in tissue section and are

better identified in faecal specimens where the typical trophozoites or cysts can be found (Jones et al., 1997). *G. intestinalis* infection results in a profound disturbance in mucosal structure and function. Alterations in small intestinal villus height, crypt depth, microvillus height and brush border surface area are associated with increased enterocytic proliferation and migration and reduced disaccharidase activity and absorption of electrolytes and nutrients (Buret et al., 1991). This study showed that giardiosis can cause severe diarrhoea and death in neonatal lambs and kids. If combined with other pathogens, *G. intestinalis* can cause higher mortality. In this study, clinical symptoms were similar as in previous reports. However, since the disease was acute, chronic microscopical findings were not detected.

Trimethoprim + Sulfodoxine treatment was applied in all neonatal enteritis cases. Because of the difficulty to detect the etiological agent under field conditions, only one drug combination was used. Compared to cryptosporidiosis, giardiosis caused less severe illness and the mortality rate was lower. However, in cases involving both agents, lesions were more prominent and the mortality rate was high. Diagnosis of coronavirus in formalin fixed tissue can be performed by means of immunuhistochemistry (Zhang et al., 1997; Daginakatte et al., 1999). While the coronavirus is an important etiological agent in calf neonatal diarrhoea, it has little importance in lambs' and kids' neonatal enteritis (Eisa and Mohamed, 2004; Munoz et al., 1996; Reinhardt et al., 1995; Durham et al., 1979). This study has also revealed that coronavirus is not an important etiological agent in small ruminant neonatal enteritis. Several reports are available on etiological agents of neonatal enteritis in lambs and kids, but most of them are based on the detection of the infectious agent in faeces whereas some on electron microscopy (Reinhardt et al., 1993; Olson et al., 1997; Eisa and Mohamed, 2004; Munoz et al., 1996). Immunohistochemical examination for the detection of the aetiology of neonatal enteritis has been rarely performed and this study involves all clinical, parasitological and pathological observations in neonatal enteritis in lambs and kids.

The results of this study indicate that while *C. parvum* and *G. intestinalis* are important agents of neonatal diarrhoea in lambs and kids, *Coronavirus* is not an important etiological agent in these species, but it is an important agent in calves. *Cryptosporidium* oocysts can be determined only by native examination of fresh faeces. However, examination of Carbol–fuchsin or Giemsa stained sections is usually adequate for rapid diagnosis. Histopathology and immunohisto-pathology can be performed to confirm diagnosis. *G. intestinalis* was found to be the second important agent in neonatal diarrhoea of small ruminants in this study. It can be diagnosed in fresh faeces and Giemsa stained specimens, but in case of presence in only a small number, it may be overlooked and cause misdiagnosis. Histopathology of the duodenum and jejunum may support diagnosis. However, immuno-histochemistry may be particularly required in cases that have yielded negative results upon parasitological and histopathological examination. Even in our study, two negative cases were diagnosed immunohisto-chemically. These examinations are required for the diagnosis of Coronavirus in neonatal enteritis in lambs and kids. Due to the difficulty of identification of infectious agents under field conditions, the same treatment was applied to all cases of cryptosporidial, giardial and coronaviral enteritis. A five day treatment with Trimethoprim + Sufodoxine and multivitamin complexes was found to be effective in the prevention and treatment of neonatal enteritis in lambs and kids. This treatment can be recommended in neonatal enteritis outbreaks in small ruminants.

Acknowledgements

Observation et mise en évidence immuno-histochimique de coronavirus, *Cryptosporidium parvum* et *Giardia intestinalis* chez les agneaux et chevreaux souffrant de diarrhée néo-natale

Dans cette étude 19 agneaux et 11 chevreaux souffrant de diarrhée néo-natale ont été examinés cliniquement, parasitologiquement, macroscopiquement, histopathologiquement, et immunohistochimiquement quant à la présence de coronavirus, *Cryptosporidium parvum* et *Giardia intestinalis*. Cliniquement on a observé une déshydratation importante ainsi que des selles jaunes-vertes à brunes et des décès. La mortalité dans les troupeaux variait entre 10 et 30%. Chez 20 animaux, *C. parvum* a été diagnostiqué comme cause unique et chez 5 des 30 animaux on a trouvé *G. intestinalis*. Les deux protozoaires ont été trouvés ensemble dans les selles de 4 animaux. 15 des 24 cas de *C. parvum* et 3 des 11 de *G. intestinalis* ont pu être mis en évidence par histopathologie. Il a été possible de confirmer tous les cas de cryptosporidies après examen immunohistochimique au moyen d'une coloration de coupes d'intestin. Deux cas de Giardia, négatifs parasytologiquement et immuno-histologiquement ont été diagnostiqués par immuno-histochimie. Chez un chevreau avec une entérite néonatale, un coronavirus a pu être décelé. Une fois le diagnostique posé, les troupeaux ont été traités au moyen de trimethoprime + sulfodoxine et d'une préparation polyvitaminée. Dans ce contexte, il est apparu que l'application intraveineuse ou intramusculaire de ces substances est efficace pour le traitement comme pour la prévention des diarrhées chez les agneaux et chevreaux nouveaux-nés.

Osservazioni e dimostrazione immunoistochimica dei Coronavirus, *Cryptosporidium parvum* e *Giardia intestinalis* negli agnelli e capretti che presentano diarrea neonatale

In questo studio è stata esaminata la presenza dei Coronavirus, *Cryptosporidium parvum* e *Giardia intestinales*, in 19 agnelli e 11 capretti affetti da diarrea neonatale sotto l'aspetto clinico, parassitologico, macroscopico, istopatologico e immunoistochimico. Clinicamente si è osservato una forte disidratazione, diarrea di colore gialla-verdastra fino a marrone e decessi. La mortalità nei greggi oscillava tra il 10 e il 30%. Sono stati diagnosticati come unica causa in 20 animali *C. parvum* e in 5 di 30 animali *G. intestinalis*. In 4 animali sono stati trovati negli escrementi entrambi i protozoi. Dimostrati istopatologicamente sono stati 15 di 24 casi con *C. parvum* e 3 di 11 casi con *G. intestinalis*. L'esame immunoistochimico ha confermato grazie alla immunocolorazione delle sezioni di intestino tutti i casi di Cryptosporidium. Sono stati diagnosticati con analisi immunoistochimiche due casi di Giardia con risultati parassitologici e immunoistoogici negativi. In un capretto che presentava enterite neonatale è stato trovato un Coronavirus. Effettuate le diagnosi i greggi sono stati trattati con trimethoprim + sulfadossina e un preparato multivitaminico. Abbiamo in questo modo riscontrato l'efficacia della somministrazione di queste sostanze sia per via intravenosa che intramuscolare per la cura e la prevenzione della diarrea negli agnelli e capretti neonati.

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