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Cryptosporidiosis in small ruminants☆

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ABSTRACT

Cryptosporidiosis is an infection caused by protozoan parasites belonging to the genus *Cryptosporidium* which is responsible for a potentially severe disease in new-born ruminants. This infection is highly prevalent in small ruminants throughout the world, especially in pre-weaned animals. The clinical expression is different between goat kids and lambs, the infection being generally more severe in the former. Molecular data demonstrate geographical variations in the species of *Cryptosporidium* infecting small ruminants. They also support the possibility of transmission of zoonotic species from these hosts to humans. Studies are still needed on molecular epidemiology, especially in goats, and on ways to control infection.

Cryptosporidiosis is an infection caused by protozoan parasites belonging to the genus *Cryptosporidium* which is responsible for a potentially severe disease in new-born ruminants. The zoonotic potential of cryptosporidiosis which varies according to the species or genotypes of *Cryptosporidium* involved and, in a more general manner, the transmission of causal agents through water and foodstuffs, are also of major public health concern.

Cryptosporidium includes many species which infect mammals but also birds, reptiles and fishes, of which 20 are currently considered as valid species and many others as cryptic species (Xiao, 2010; Fayer et al., 2010). Genetic characterization, but also morphology of the oocyst, biological and epidemiological data concerning the site of development of parasites and their natural host specificity with major and minor hosts are the main criteria taken into account to validate new *Cryptosporidium* species (Fayer, 2010).

For many years, *Cryptosporidium parvum* has been considered as a simple agent of co-infection, but it is now recognised as a major cause of neonatal diarrhoea in ruminants. Even if, currently, there is no effective treatment, it is possible to control the infection in young ruminants through hygienic measures and chemoprophylaxis during the first 7–12 days of their life. Most studies have been conducted in cattle. However, during the last years, a lot of work has been done in small ruminants especially on molecular epidemiology. The paper describes the main characteristics of cryptosporidiosis in sheep and goats.

1. Epidemiology

The current *Cryptosporidium* species identified in small ruminants are given in Table 1.

In sheep, two main species are identified: *C. parvum* and *C. ubiquitum* (previously identified as the *Cryptosporidium* cervine genotype (Fayer et al., 2010)), which are found in nearly all the surveys but with quite different prevalences depending to the location.

In Europe, several authors reported the predominance of *C. parvum* (Spain: Quílez et al., 2008 and Díaz et al., 2010a; UK: Mueller-Doblies et al., 2008 and Smith et al., 2010; Italy: Paoletti et al., 2009; France: Paraud et al., 2009) in diarrheic and non-diarrheic lambs. On the

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Table 1
Current *Cryptosporidium* species identified in small ruminants (from Xiao, 2010).

	Species/genotype
Sheep	*C. parvum*
	Cryptosporidium cervine genotype = *C. ubiquitum*
	C. bovis or *C. bovis*-like in sheep = *C. xiaoi*
	C. hominis
	C. andersoni
	C. fayeri
	C. suis
Goats	*C. parvum*
	C. xiaoi
	C. hominis

contrary, Geurden et al. (2008) and Robertson et al. (2010) in Belgium and Norway respectively reported the predominance of *C. ubiquitum*. Likewise, *C. ubiquitum* was the main species isolated in Australia, in the United States and in China (Ryan et al., 2005; Santín et al., 2007; Wang et al., 2010).

In Australia, Yang et al. (2009) first confirmed the low prevalence of *C. parvum* in lambs in this country but the use of a more sensitive and specific technique of qPCR demonstrated the presence of very frequent co-infection with *C. bovis* and *C. parvum* in lambs, showing the limits of generic PCR tools in detecting minor species.

The third important species in lambs is the recently described *C. xiaoi*, previously identified as the *C. bovis*-like genotype or as *C. bovis* in sheep (Fayer and Santín, 2009).

As described for cattle (Santín et al., 2008), 2 studies suggest a different distribution of parasite species across the age groups: *C. xiaoi* and *C. parvum* are mainly identified in lambs while *C. ubiquitum* is found in all age groups (Yang et al., 2009; Wang et al., 2010). However, this remains to be confirmed.

Subtypes of *C. parvum* in sheep, identified using the 60 kDa glycoprotein (gp60) subgenotyping site, belong to 2 families, IIa and IId (Geurden et al., 2008; Quílez et al., 2008; Díaz et al., 2010a; Smith et al., 2010).

Few studies have genotyped *Cryptosporidium* from goats. Two main species have been identified from goat kids: *C. parvum* (Ngouanesavanh et al., 2006; Geurden et al., 2008; Paraud et al., 2009) and recently *C. xiaoi* from 5 diarrheic animals (Díaz et al., 2010b). Subtypes of *C. parvum* were only identified by Geurden et al. (2008) and revealed 2 different subtypes belonging to families IIa and IId.

Two subtypes of *C. parvum* isolated from small ruminants may potentially be zoonotic. Numerous studies have shown that *C. parvum* and *C. hominis* are responsible for most *Cryptosporidium* infections in humans (Xiao, 2010). The potential role of sheep and goats for transmission of oocysts to humans has been recently reviewed by Robertson (2009). This author concluded that in spite of large geographical variations, transmission of *Cryptosporidium* oocysts from small ruminants to humans is possible. Smith et al. (2010) investigated *Cryptosporidium* excretion in farms possibly linked to human patients with cryptosporidiosis in England and Wales and concluded that sheep may have been responsible for transmission in some of the studied cases.

2. Source and transmission of infection

Cryptosporidium infection is transmitted through the ingestion of oocysts, directly by licking a contaminated material or through the ingestion of contaminated foodstuffs or drinking water.

Young animals are the main source of oocysts for the environment. Prevalence in non-diarrheic pre-weaned animals is highly variable from 2 to 85% in lambs (Ryan et al., 2005; Santín et al., 2007; Geurden et al., 2008; Paraud et al., 2009; Yang et al., 2009) and from 5 to 30% in goat kids (Castro-Hermida et al., 2005b; Delafosse et al., 2006; Castro-Hermida et al., 2007b; Goma et al., 2007; Geurden et al., 2008; Paraud et al., 2009).

In a subclinical situation, Geurden et al. (2008) reported an average excretion of 6832 oocysts per gram of faeces (range: 0–300,000 opg) in lambs and a mean excretion of 231,929 opg in kids (range: 0–10,000,000 opg). In goat kids showing diarrhoea, the number of oocysts excreted is very high between 7 and 21 days of age, with individual figures reaching 2×10^8 oocysts per gram (Chartier et al., 2002).

The age of the animals has a great influence on the frequency of the isolation of *Cryptosporidium*: the highest prevalence of excretion is reported on lambs aged between 8 and 14 days old with 76% of them excreting oocysts (Causape et al., 2002). In naturally infected kids, excretion of *Cryptosporidium* oocysts begins at the age of 4 days with a peak at 7 days of age and a decline after 3 weeks (Paraud et al., 2010).

Adult ruminants also excrete *Cryptosporidium* oocysts. In adult healthy ewes, prevalences of excretion varied from 2.1 to 5.3% in Spain (446 and 575 ewes tested) and China (738 ewes tested) (Castro-Hermida et al., 2007a,b; Wang et al., 2010). In goats, Castro-Hermida et al. (2007a,b) reported a prevalence varying from 7.7 to 9% in Spain (148 and 116 goats tested). The level of excretion remains very low, from several tens to several thousands oocysts per gram of faeces on average without difference between ewes and goats (Castro-Hermida et al., 2007b).

A link has been established between parturition and an increase in oocyst excretion by parturient ewes and goats (Xiao et al., 1994). Out of 14 ewes, the level of excretion reached 20–440 opg in a period from one week before the birth to one week after (Ortega-Mora et al., 1999). Similarly, oocyst excretion in goats shows a ten-fold increase (from 8 to 80 opg) 3 weeks around parturition (Castro-Hermida et al., 2005a). Despite this rise, young infected animals are the most significant source of contamination of the environment, particularly due to the difference in level of excretion between neonates and adults.

The infectious dose is very low: Blewett et al. (1993) reported that in gnotobiotic lambs, the minimum infectious dose corresponds to a single oocyst and the average infectious dose is around five oocysts.

3. Clinical signs

C. parvum is one of the major agents of neonatal diarrhoea in lambs (Muñoz et al., 1996; Causape et al., 2002) and in goat kids (Muñoz et al., 1996).

The symptoms of infection with *C. parvum* are essentially acute diarrhoea, which occurs between the ages of 5 and 20 days.

In goat kids infected with *C. parvum*, there is a concomitance between a high number of excreted oocysts by goat kids and the severity of diarrhoea observed in the same animal (Paraud et al., 2010). In lambs, numerous infections are asymptomatic (Pritchard et al., 2008; Paraud et al., 2009). Ryan et al. (2005) did not show any relationship between excretion and diarrhoea whereas Causape et al. (2002) reported that probability of showing diarrhoea was significantly higher in lambs excreting *Cryptosporidium* oocysts than in the lambs not excreting oocysts.

Morbidity can often reach 80–100% and mortality rate can exceed 50% in goat kids (Thamsborg et al., 1990; Chartier et al., 1996, 1999). The consequences regarding the general condition of the animal are variable.

Studies of *C. parvum* infection in calves have shown that cryptosporidiosis mainly affects the jejunum and the ileum and demonstrated the diarrhoea to be caused by 2 mechanisms: an important secretory process, with the inhibition of the absorption of sodium and the high production of prostaglandins in the intestinal mucosa, or an increase in the permeability of this mucosa resulting from the increase of the γ interferon level (Foster and Smith, 2009). Abrasion of villosities leads to a poor absorption and poor digestion of food.

Aggravating factors are numerous: stress factors (weather, feeding, environmental conditions, etc.), intercurrent diseases with other enteropathogenic agents, the number of oocysts ingested and the quality of the colostrum (de Graaf et al., 1999).

The evolution towards healing or death happens in a few days. There is no progression to the chronic stage. Certain clinical cases have been observed in adult goats but that is quite exceptional.

The appearance of the diarrhoea is not sufficient to establish diagnostic. Macroscopic lesions (catarrhal enteritis) are not pathognonomic: the contents of the intestine are more or less liquid and distension of caecum and colon is often seen. Sometimes congestion and hemorrhagic inflammation in the last third of the ileum, associated with a hypertrophy of the mesenteric lymph nodes, can be noticed.

4. Diagnosis

4.1. Clinical and epidemiological diagnosis

Ruminant cryptosporidiosis has the following characteristics:

- diarrhoea which can be more or less serious, in animals between 5 and 21 days old
- more severe in goat kids than in lambs
- appears in the herd during the second half of the parturition period (Delafosse et al., 2006)
- usual anti-infectious treatments are inefficient.

However, no diagnosis can be made with certainty without the help of a laboratory. Several staining methods are commonly used (modified Ziehl–Nielsen staining, Heine staining) along with methods like indirect immunofluorescence or enzyme immunoassays (Casemore, 1991; Brook et al., 2008).

The differential diagnosis must include all the pathogenic agents of the complex "diarrhoea in neonate ruminants" that is to say viral infections (rotavirus, coronavirus), bacterial infections (colibacillosis, salmonellosis, *Clostridium perfringens*) and the dietary problems (Sargison, 2004). Moreover several enteropathogenic agents can exist together with *Cryptosporidium* infection in the same animal (Muñoz et al., 1996).

5. Treatment

No specific treatment for cryptosporidiosis exists because of the lack of really efficient drug. So far more than 140 molecules have been tested for activity against *Cryptosporidium* infection and none has given entirely satisfactory results (Stockdale et al., 2008). In small ruminants, only two products have given significant results when used in a preventive way: a coccidiostatic (halofuginone lactate) (Naciri and Yvoré, 1989; Causapé et al., 1999; Chartier et al., 1999; Giadinis et al., 2007, 2008) and an aminoside antibiotic (paromomycin sulphate or aminosidine) (Mancassola et al., 1995; Chartier et al., 1996; Johnson et al., 2000; Viu et al., 2000).

Several other potential anticryptosporidial drugs have been tested in sheep and goats without convincing results: β-cyclodextrin (Castro-Hermida et al., 2001), α-cyclodextrin (Castro-Hermida et al., 2004), decoquinate (Mancassola et al., 1997; Ferre et al., 2005), nitazoxanide (Viel et al., 2007). Recently, a product composed of activated charcoal from bark and of wood vinegar liquid, previously tested against cryptosporidiosis in calves in Japan (Watarai et al., 2008), gave promising results in controlling cryptosporidiosis in goat kids both in a preventive and in a curative way (Paraud et al., 2011).

Treatment of the symptoms is the same as that of other neonatal diarrhoea and consists in rehydration, replacement feed, intestinal adsorbents, etc.

In the absence of fully efficient treatment, the sanitary and breeding recommendations are essential (Angus, 1990). They are identical for the management of all the neonatal diarrhoea agents:

- sectioning of age groups
- checking sufficient colostrum supply and quality feed
- isolating sick animals from healthy animals
- avoiding over-crowding
- keeping a clean and dry environment
- disinfecting the premises
- daily cleaning of equipment

Cryptosporidium oocysts are highly resistant in the external environment, as long as they are not exposed to extreme temperatures or desiccation: the survival period is approximately 3 months at 15–20 °C, and over a year at 4–6 °C. Most chemical disinfectants are useless in their normal dosage. Only ammonia (between 5 and 50 p.100

concentration), hydrogen peroxide at 3% and formol at 10% are really effective (Fayer, 2008).

6. Conclusion

Cryptosporidium infection in small ruminant neonates presents different patterns according to the infected host species: while the infection is severe in goat kids and can lead to very high morbidity and mortality, the situation is less clear in lambs because of the high prevalence of suspected subclinical infections. No information is available on differences in immune response against *Cryptosporidium* among host species. Development of molecular and cell culture methods may give explanations on differences in infectivity, virulence among *C. parvum* isolates from different hosts. Management of cryptosporidiosis in small ruminants still needs an implementation of global knowledge on epidemiology but also more studies on prevention and treatment of the disease.

Conflict of interest

None.

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