CHAPTER 10
Coccidiosis in Goat (*Capra hircus*)

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10.1 INTRODUCTION

During the last 2 decades, the number of goats has increased to more than 1000 million worlwide. Thus, goat production is currently not only the livelihood of small farmers in developing countries, but also an important economic opportunity in a world with a continuously growing population. Goat coccidiosis is a cosmopolitan disease, but with special relevance in semi-arid geographical areas that depend economically on goat production, such as the Mediterranean basin, North Africa, and certain regions of Asia or Latin America, where *Eimeria* infections may affect animal health and thus the profitability of goat production (Cavalcante et al. 2012 [41807] [188]). Coccidiosis can affect up to 100% of goats between 4-10 weeks of age, depending on the type of housing, the distribution and sanitary conditions of the farm, the immune status of the animals, and the climatic conditions, as has already been considered in some studies (Ruiz et al. 2006 [40863] [1459]). The mortality rate from this disease can be as high as 50% of kids and those who do not die suffer significant stunting, resulting in significant economic losses (Jalila et al. 1998 [44248] [777], Smith and Sherman, 2009 [46166] [1592]). These huge losses could be prevented with a correct diagnosis of the *Eimeria* species involved and their adequate prophylactic control discussed here.

10.2 ETIOLOGY

Based on cross-infection studies showing hight host-specificity in coccidian of small ruminants, goat *Eimeria* species are now accepted to be different from than those of sheep. Currently, 13 species of *Eimeria* in goats are recognized (Shah and Joshi 1963 [42074] [1536]; Lotze et al. 1961 [44258] [1093]). (Table 10.1). Of these, *E. ninakohlyakimovae* and *E. arloingi* are considered the most pathogenic and often the most prevalent (Ruiz et al. 2006 [40863] [1459], Silva et al. 2014b [41070] [1576]).

Morphological characteristics are summarized in Tables 101 and 10.2. Like *Eimeria* in other hosts, oocysts are excreted unsporulated and sporulation occurs in the environment 2 or more days later (Table 10.1).

Of the 13 species of goat *Eimeria*, more is known of the life cycle of *E. ninakohlyakimovae* than others. It has 2 generation of schizonts. The first generation schizonts develop 10-12 DPI and may have a diameter of up to 166 µm x 124 µm (Vieira et al. 1997b [41443] [1748]). They contain thousand of merozoites and are in endothelium of lymphatic vessels (Table 10.2). Presumably, the sporozoites initially enter enterocytes and are then carried to lymphatic endothelial cells by an unknown mechanism. The *in vivo* development of first generation schizonts was confirmed by *in vitro* studies using ruminant cell lines (Ruiz et al. 2010 [606] [1458]) (Figure 10.1). In contrast, second generation schizonts are smaller (average size 17 µm x 12 µm) and develop around 13 DPI in epithelial cells of the crypts of the cecum and colon. Gamonts also develop in the large intestine and oocysts are excreted 14-15 DPI (Vieira et al. 1997b [41443] [1748], Ruiz et al. 2013 [42059] [1461]). Ultrastructural studies of second generation schizonts revealed that development occurs in enterocytes above the host cell nucleus (Vieira

et al. 1997b [41443] [1748]). The merozoites are formed at the periphery leaving a prominent residual body.

The second most pathogenic *Eimeria* species, *E. arloingi* follows the same life cycle pattern as *E. ninakohlyakimovae*. It also has 2 generations of schizonts, the first one very large (300 µm) and the second generation small (10-20 µm) (Hashemnia et al. 2012 [41909] [681]). The gamonts are formed as early as 7 DPI and ultrastructurally gametogony is like other *Eimeria* species (Hashemnia et al. 2012 [41909] [681]).

E. christenseni and *E. alijevi* also have 2 asexual generations preceding gamonts but full details are unknown. Relatively little is known of the endogenous stages of other *Eimeria* in goats (Table 10.2). First generation schizogony in other *Eimeria* species generally occurs in epithelial cells of a different part of the small or large intestine. The location of the different asexual and sexual stages of most prevalent *Eimeria* species in goats has been summarized in Table 10.2; their prepatent periods vary from 7 to 23 days.

10.3 EPIDEMIOLOGY

10.3.1 Prevalence and Geographical Distribution

Coccidiosis is one of the most ubiquitous and widespread enteric diseases in goat production systems. It has been described in several regions and countries in Europe, Africa, Asia, and America (Table 10.3) (Penzhorn et al. 1994 [44264] [1349], Balicka-Ramisz 1999 [3974], Faizal and Rajapakse 2001 [44240] [513], Agyei et al. 2004 [44769] [15]). As an example, in a Brazilian study, 92.1% of the goats were positive; 8 species were identified, of which *E. alijevi*, *E. arloingi* and *E. hirci* were the most prevalent (Cavalcante et al. 2012 [41807] [188]). Also in North America (southwestern Montana, USA), *Eimeria* oocysts were observed in 97.2% of the total Cashmere goat feces; 9 *Eimeria* species were identified, of which *E. arloingi*, *E. ninakohlyakimovae* and *E. alijevi* accounted for 88.3% of the total oocysts identified (Penzhorn et al. 1994 [44264] [1349]). In Tanzania, a high prevalence (94.7%) was found in some climatic areas, but in general the infection rates were slightly lower than that described on the American continent around 80% (Kusiluka et al. 1998 [44253] [928]). In this study, the predominant species were *E. arloingi* (91.7%), *E. alijevi* (80.3%), *E. ninakohlyakimovae* (71.4%) and *E. christenseni* (45.2%). A high prevalence of *Eimeria* infections (97.3%) was also reported in Shaanxi province, northwest China, in Saanen and Guanzhong breeds (Zhao et al. 2012 [41723] [1900]). The authors mentioned that mixed infections by several species of *Eimeria* were common; in this case, the most frequent species were *E. jolchijevi*, *E. arloingi*, *E. alijevi*, *E. caprina*, *E. hirci,* and *E. christenseni*, with different prevalence per breed. Several studies have also shown a high prevalence of coccidiosis in Europe. For instance, in Central Europe (Ukraine and Poland) the prevalence of *Eimeria* infection in goats was estimated at 74%, particularly, *E. arloingi*, *E. christenseni*, *E. jolchijevi* and *E. ninakohlyakimovae* (Balicka-Ramisz et al. 2012 [44231] [61]).

The prevalence and intensity of *Eimeria* infections in goats also seem to be influenced by the age of the animals as reported in several geographical locations worldwide, such as Brazil (Cavalcante et al. 2012 [41807] [188]), Tanzania (Kimbita et al. 2009 [39549] [891]) or Europe (de la Fuente and Alunda, 1992 [38579], Balicka-Ramisz et al. 2012 [44231] [61]), with young having higher degree of infection than adults. Fecal oocyst counts range from about 1000 to 2000

OPG in adults, while in young animals counts as high as 10^6 OPG can be found (Hidalgo Argüello and Cordero del Campillo 1999 [43929] [713], Ruiz et al. 2006 [40863] [1459]). Age-related differences have even been found in relation to the frequency of presentation of the different *Eimeria* species. For example, in Brazil a study found that *E. ninakohlyakimovae* was the most prevalent in young animals (97%), while *E. alijevi* (77%) was the most frequently found in adult goats (Cavalcante et al. 2012 [41807] [188]).

10.3.2. Predisposing Factors and Transmision

Variation in prevalence and distribution of *Eimeria* species may be attributed to factors related to differences in management and hygienic conditions, temperature, agroecology, climate, weather conditions, the immune state of the host, sample size, breed, sex, sampling period, or breed susceptibility to coccidia (Khodakaram-Tafti and Hashemnia 2017 [43855] [880], Ruiz et al. 2006 [40863] [1459]; Harper and Penzhorn 1999 [44246] [678], Balicka-Ramisz 1999 [3974] [60], Hashemnia et al. 2011 [39168] [680], Zvinorova et al. 2016 [44272] [1924], Faber et al. 2002 [38797] [510], Ruiz et al. 2013 [44267] [1463]). Little is known of strain-dependent pathogenicity of *Eimeria* in all hosts, including goats. Even a high dose (10^6 sporulated oocysts) of *E. ninakohlyakimovae* induced only mild coccidiosis, and no deaths (Dai et al. 2006 [38535] [316]). In contrast, in experimental infections with *E. ninakohlyakimovae* GC strain using an infective dose 5 times lower animals developed such a severe clinical disease that an immediate emergency treatment was necessary to prevent death (Matos et al. 2017 [42002] [1142]).

The initial infection usually occurs in the first weeks of life, when goat kids ingest oocysts attached to the udders of their dams, but generally acquire low numbers to trigger clinical signs. From the 2nd-4th week onwards, kids start excreting oocysts in feces, which is the most important risk period for environmental contamination because they canexcrete millions of oocysts in a period when animals are very susceptibl disease (Kanyari 1993 [37376] [859]). Later on, infection may result from oocysts that survive from the previous breeding period or even from the previous year. The infection pressure can be high if goat kids are kept in pastures with their mothers and even higher if they are maintained in confined pens (Hidalgo Argüello and Cordero del Campillo 1999 [43929] [713]). The changes in diet and the stress suffered by the kids during weaning are the reason why this period is especially critical for the appearance of clinical coccidiosis in most goat production systems (Ruiz et al. 2006 [40863] [1459]).

10.4 IMMUNE RESPONSE (TABLE 10.5)

As mentioned before, previous exposure to *Eimeria* spp. in goats can lead to protective immunity against subsequent infections (Ruiz et al. 2013a [42059] [1461], Dai et al. 2006 [38535] [316]). Under field conditions, natural exposure to the parasite ensures continuous contact that allows the development of immunity, which would explain why adult goats have significantly lower OPG than kids (de la Fuente and Alunda 1992 [40863] [1459], Balicka-Ramisz et al. 2012 [44231] [61]). Accordingly, primary infections with the *E. ninakohlyakimovae* GC strain led to a significant reduction of OPG counts in challenged infected goat kids compared to infection controls as well as the amelioration of clinical disease. However, immunoprotection induced was only partial, as some clinical signs – although milder – still occurred after challenge-infections (Ruiz et al. 2013a [42059] [1461]). Comparable partial protection was also achieved when applying high infections and challenge doses (Dai et al. 2006 [38535] [316]). Challenge infections can be lethal if dose is high (around 10^6 oocysts), a circumstance that may occur in highly contaminated environments (Ruiz et al. 2006 [40863] [1459]).

As previously referred, some *Eimeria* species such as *E. arloingi*, *E. ninakohlyakimovae* and *E. christenseni* firstly parasitize endothelial cells of the lymphatic vessels of the intestine (Ruiz et al. 2010 [606] [1458], Silva et al. 2015 [42079] [1578]) and there is some evidence in literature that *Eimeria* first generation meronts represent a major target for protective immune reactions (Speer et al. 1985 [10461] [1611]). In agreement, it has been recently reported that protective immune responses during prepatency in goat kids experimentally infected with *E. ninakohlyakimovae,* which probably results from a complex framework of molecular mechanisms, effector cells and cytokines involving both innate and adaptative immune responses, can be addressed to first generation schizonts (Matos et al. 2017a [42002] [1142]).

Several immunocompetent cells have been found within the mucosa of goat kids experimentally infected with *E. ninakholyakimovae*, including polymorphonuclear cells (PMN), mast cells, eosinophils, globular leukocytes and lymphocytes (Ruiz et al, 2013b [44267] [1463], Ruiz et al. 2014 [40865] [1462], Matos et al. 2017a [42002] [1142], Matos et al. 2018 [40035] [1143]). In agreement to increased mean lymphocyte counts in the intestinal mucosa, goat kids challenge infected with *E. ninakohlyakimovae* had a higher number of TCD4+ and TCD8+ cells, and increased relative gene expression of IL-2, IL-4, IL-10 and INFγ, which suggests that both Th1- and Th2-mediated responses could be most probably generated against *E. ninakohlyakimovae* (Matos et al. 2017a [42002] [1142]). Besides, in relation to the involvement of the innate immune response in caprine coccidiosis, the circulatory levels of most systemic inflammatory markers, e. g. pro-inflammatory cytokines (IFN-γ, TNF-α, IL-4, IL-6), AGP (α1-acid-glycoprotein) and ADA (adenosine deaminase) increased significantly starting in goat kids infected with sporulated oocysts of three *Eimeria* species, including *E. caprina* (57%), *E. ninakohlyakimovae* (28%), and *E. arloingi* (15%) (Tadayon et al. 2016 [42096] [1660]). In addition, *E. ninakohlyakimovae* sporozoites and SOA (soluble oocyst antigen) significantly up-regulated pro-inflammatory cytokines and chemokines in a stimulated caprine PMNx ans induced caprine NETosis in a NOX-independent way and the same was found by in goats infected with *E. arloingi* (Silva et al. 2014 [41070] [1576]). Early innate reactions of monocytes against *E. ninakohlyakimovae* also induce NADPH oxidase-dependent monocyte extracellular trap and additionally upregulates gene transcription of critical immunoregulatory molecules including IL-12 and TNF-γ, in addition to IL-6 and CCL2 (Pérez et al. 2016 [40563] [1351]).

Humoral immunre reactions are also triggered in response to *Eimeria* infections in goats. So that, the detailed analyses of T cell subpopulations of *E. ninakohlyakimovae* infected goat kids showed that CD45+ T cells were increased in the ileum of challenge infected animals, and negative correlations to the number of inmature schizonts were recorded both in the ileum and colon, which could be related to the mild increase of specific IgA in mucus samples (Matos et al. 2017a [42002] [1142]). In agreement, the analysis of IgA levels in gut mucus samples of *E. ninakohlyakimovae* infected animals revealed significantly enhanced levels of this immunoglobulin both in challenge infected and challenge control goat kids in comparison to uninfected controls (Matos et al. 2017b [40034] [1141]). The authors also demonstrated that IgG and IgM levels in serum samples were significantly increased in infected animals, and a wide range of peptides of SOA was recognized by specific IgG as determined by immunoblotting (Matos et al. 2017b [40034] [1141]). However, no correlations were found between immunoglobulin levels and OPG counts after challenge infection, which agrees with controversial data on whether specific humoral responses against ruminant *Eimeria* species may convey immunoprotection or not.

Finally, some antimicrobial peptides that are produced by leukocytes and epithelial cells, known as defensins, have been recently involved in important innate immune responses against caprine eimeriosis (Ibarra-Velarde and Alcala-Canto 2007 [39311] [762]).

10.5 PATHOGENESIS, CLINICAL SIGNS AND LESIONS

10.5.1 Pathogenesis

The pathogenicity of different *Eimeria* species is particularly related to the location of replication of the parasite in the host and may influence the clinical outcome of the disease. Table 10.2 shows the locations of the main *Eimeria* species affecting goats. In general, the most pathogenic species in goats, i.e. *E. ninakohlyakimovae*, *E. arloingi* and *E. christenseni* must pass through the intestinal epithelium and invade the endothelial cells of the central lymphatic capillaries of the intestinal villi. Once in endothelial cells, they form macroschizonts, a process that requires a prolonged replication time and therefore, modulation at a larger scale of the host cell (Ruiz et al. 2010 [606] [1458]). Because of large size of schizonts, the cellular damage produced in the intestinal mucosa may be severe (Ruiz et al. 2010 [606] [1458], Ruiz et al. 2013a [42059] [1461], Ruiz et al. 2013b [44267] [1463]). Furthermore, the enormous numbers of first-generation merozoites resulting from the first schizogony consequently lead to an exponential destruction of the epithelium during the second schizogony and, finally, during the phase of sexual reproduction or gametogony. In general, the severity of coccidiosis is determined by the proliferation capacity of the pathogenic *Eimeria* species, which is defined as the number of merogonies and the number of merozoites produced by merogonia, and is closely related to the number of cells destroyed by each sporulated oocyst ingested. Therefore, the primoinfective dose (number of viable oocysts ingested) and the magnitude of reinfection may influence the development and course of the disease (Ruiz et al. 2013b [44267] [1463]).

The extensive damage to the intestinal mucosa during the development and proliferation of intracellular stages of the parasite clearly interferes with both digestion and homeostasis. Such events cause negative effects on animal health and production performance and thus substantial economic losses may occur, even when the typical clinical signs of the disease are absent (Ruiz et al. 2006 [40863] [1459]). The destruction of epithelial cells in different parts of the intestine, and endothelial cells in some cases, can affect large enteric sections, exposing the mucous membrane itself (Ruiz et al. 2013b [44267] [1463]). Because of fluid loss from diarrhea and malabsorption, animals often suffer a dehydration process, especially in long-term infections with pathogenic species such as *E. ninakohlyakimovae* (Dai et al. 2006 [38535] [316]). On the other hand, probably because mucosal damage in the ileum prevents reabsorption of bile in the small intestine, serum bile acids are decreased, as reported for other ruminant hosts. Increased serum bilirubin and decreased liver enzyme activity are more complex to explain, but may reflect anorexia and transient alteration of liver metabolism (Holst and Svensson 1994 [22364] [731]). Anorexia associated with dehydration can lead to a state of prostration and death of affected animals if urgent fluid therapy is not instituted (Ruiz et al. 2013b [44267] [1463]).

The pathogenic effect of *Eimeria* spp. can be complicated by concomitant infections with gastrointestinal nematodes affecting different parts of the digestive tract (Agyei et al. 2004 [44769] [15]; de la Fuente et al. 1993 [38580] [344]; Rahman 1994 [40656] [1383]). Goat *Eimeria* spp. may all coexist and exhacerbate the clinical course of other pathogens such as viruses or bacteria (Chartier and Paraud 2012 [42418] [247]). Thus, an outbreak of intestinal coccidiosis increased susceptibility to bronchopneumonia by *Pasteurella haemolytica* in experimentally vaccinated

goat kids against sarcocystosis (Dubey 1983 D0189 418).

10.5.2 Clinical Signs

When optimal conditions for the development of pathogenic species occur, common clinical signs of coccidiosis are usually observed: anemia, weakness, depression, abdominal pain, lethargy, anorexia, dehydration, coarse hair, poor weight gain, low conversion of food and pasty stools without blood streaks. In addition, in animals heavily infected with species that develop gamonts in the large intestine such as *E. ninakohlyakimovae,* accompanying all clinical signs previously mentioned, a severe liquid diarrhea can be observed, sometimes hemorrhagic, capable of dragging portions of intestinal mucosa (Dai et al. 2006 38535 316, Ruiz et al. 2013a 42059 1461). The acute phase may last 3-4 days, but diarrhea with elimination of liquid stools, with mucus, with or without blood, and color changes from brown to dark yellow or tarry may persist for several days or weeks, usually until turnover of the affected intestinal mucosa takes place (Dai et al. 2006 38535 316). Animals weaken, suffer ataxia, and may not be able to stand, although they usually recover, and mortality of more than 10% is not frequent, even with high infectious doses (Ruiz et al. 2013a 42059 1461, Ruiz et al. 2013b 44267 1463). The mortality rate can reach 30% in farms where *E. arloingi* and *E. ninakohlyakimovae* are the predominant species (Koudela and Boková 1998 39612 910). If the animals do not die within 7-10 days, they recover slowly but remain unthrifty (Matos et al. 2017a 42002 1142).

Under certain conditions, coccidiosis may be associated with sudden death with absence of clinical signs, especially in young animals, which has been related to the damage on the intestinal mucosa produced by some *Eimeria* species during the prepatent period due to first schizogony (Ruiz et al. 2013b 44267 1463, Matos et al. 2017a 42002 1142).

10.5.3 Biochemical and Hematological Finding

Despite the severity of the clinical signs, hematological alterations are not as striking as it would be expected in experimental infections with *E. ninakohlyakimovae* (Dai et al. 2006 38535 316, Ruiz et al. 2013a 42059 1461, Hashemnia et al., 2014 39169 682). Similarly, only slight increase of packed cell volume PCV values have been observed in *E. ninakohlyakimovae*-infected animals, which would be explained as a decrease of the total blood volume in circulation because of fluid losses during the phase of diarrhea (Dai et al. 2006 38535 316, Ruiz et al. 2013a 42059 1461). The same pattern was also demonstrated in experimental infections with *E. arloingi* (Hashemnia et al., 2014 39169 682).

Several serum enzymes and electrolytes have been also evaluated in *E. ninakohlyakimovae* experimentally infected goats, showing a slight fall in alkaline phosphatase (ALP) levels, while plasmatic concentrations of albumin, globulin, Na^+, K^+ and Cl^- exhibited no pattern and no significant differences were found between inoculated and non-inoculated groups (Dai et al. 2006 38535 316). In experimental infections with *E. arloingi*, the diarrhea was also associated with a reduction in ALP activity, while decreased levels of Na^+, K^+ and Cl^- was proved in this occasion (Hashemnia et al., 2014) 39169 682. On the other hand, although endogenous stages in livers and gallbladders of goats naturally and experimentally infected with *E. ninakholyakimovae* have been found, changes in levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), two enzymes that reflect liver damage, could not be demonstrated (Dai et al. 2006 38535 316).

With respect to blood leukocyte counts, transient increase in eosinophil and monocytes counts were recorded for challenged animals in *E. ninakohlyakimovae* primary infected goat kids (Ruiz et al. 2013a 44267 1463), while lymphocytes ans neutrophils varied irregularly (Ruiz et al. 2013a 44267 1463; Ruiz et al. 2013b 42059 1461). Finally, responses of the enzymatic antioxidant systems during experimental coccidiosis indicate that infections with pathogenic species of *Eimeria* in goats can significantly reduce the major erythrocyte antioxidant mechanisms and enhance the blood levels of lipid peroxidation and total homocysteine (Rakhshandehroo et al. 2013 40661 1386).

10.5.4 Lesions

In animals that die due to experimental *E. ninakohlyakimovae* the perianal area is soiled by diarrheal feces, which may be liquid and of a variable colour, from yellow to reddish. Gross lesions differed per the period when the animal was necropsied and whether they were primary of challenge infected (Dai et al. 2006 38535 316, Ruiz et al. 2013a 42059 1461, Matos et al. 2017a 42002 1142). Thus, in challenge infected goat kids euthanized during prepatency (7 DPI) macroscopic lesions included various degrees of congestion and thickening of the intestinal mucosa, particularly affecting the ileum, colon, and cecum, while primary infected animals did not have gross lesions. No relevant macroscopic alterations were either observed in the intestines of *E. ninakohlyakimovae* experimentally infected animals when slaughtered during post-patency, when no oocysts were observed in feces or the counts were close to zero (Dai et al. 2006 38535 316, Ruiz et al. 2013a 42059 1461). On the other hand, when the animals are subjected to continuous infections under field conditions, macroscopic pathological changes in goat kids have been reported, including mucosal hemorrhages and whitish nodular polyps in the jejunum, most probably due to co-infection by *E. arloingi* and *E. ninakohlyakimovae* (Koudela and Boková 1998 39612 910).

Numerous developmental stages of *Eimeria* are microscopically found in enterocytes and lacteals of intestinal villi in naturally infected animals (Koudela and Boková 1998 39612 910; Ruiz et al. 2013a 42059 1461; Matos et al. 2017a 42002 1142). Histopathological changes found in goat coccidiosis are further characterized by local hypertrophy and hyperplasia of intestinal villi, villus blunting and inflammatory infiltration in the lamina propria (Koudela and Boková 1998 39612 910). Hypertrophy of the mesenteric lymph nodes and Peyer's patches have been also found in *E. ninakohlyakimovae* experimentally infected goat kids and, in some cases, mild villous atrophy in the lower small intestine (Dai et al. 2006 38535 316, Ruiz et al. 2013a 42059 1461). Futhermore, clear eosinophilic enteritis and a diffuse infiltration of mast cells, lymphocytes, and PMN have also been demonstrated (Ruiz et al. 2013a 42059 1461). A mild inflammatory cell infiltration involving different immune cells is already observed during prepatency of *E. ninakohlyakimovae* infected goat kids. Eosinophils and lymphocytes were significantly increased both in challenged infected and challenge control animals with respect to uninfected controls, while PMN were predominantly increased in challenge controls both in ileum and colon samples (Matos et al. 2017a 42002 1142). Interestingly, cellular immune responses and histopathological alterations in the gut mucosa of fatal *E. ninakohlyakimovae* challenge-infected goat kids showed severe eosinophilic enteritis in affected animals, with an extensive infiltration of intraepithelial lymphocytes and neutrophils (Ruiz et al. 2013b 44267 1463).

10.5.5 Billiary-Hepatic Coccidiosis and Other Atypical Locations

Isolated cases of billiary-hepatic coccidiosis associated with unknown *Eimeria*-like parasites have been reported in goats from different areas (Dubey 1986 [D0242] [419], Oruc 2007 [40452] [1290]). Schizonts, gamonts, and unsporulated oocysts were present in biliary epithelium. In the cases reported from China, oocysts from bile were morphologically like *E. ninakohlyakimovae* (Dai et al., 1991 [42354] [315]). In another case from the USA, the oocysts fit the size range of *E. pallida* (Table 10.1) (Dubey1986 [D0242] [419]).

Atypical location of *Eimeria* in goats has been described within epithelial cells of a Brunner's gland in duodenum (Main and Creeper 1999 [39982] [1119]). Light microscopic appearance of the oocysts in histological sections was considered compatible with *E. ninakohlyakimovae*, suggesting that this *Eimeria* species might be more ubiquitous than others.

10.6 DIAGNOSIS

10.6.1 Coproscopical Methods

Qualitative analysis using the flotation concentration method, e. g. with saturated NaCl solution, is probably the most common routinary method for the detection of goat *Eimeria* spp. However, a quantitative method is generally preferred to estimate the degree of infection goat kids and establish a more rational administration of anticoccidials, both for therapeutic and prophylactic purposes (Chartier and Paraud 2012 [42418] [247], Ruiz et al. 2012 [40864] [1460], Iqbal et al. 2013 [39323] [771]). McMaster's technique is problably the most commonly used method to quantify *Eimeria* oocyst countsWith different modifications, this technique has been employed in many epidemiological studies involving goat herds from diverse geographical areas (Faizal and Rajapakse 2001 [44240] [513], Ruiz et al. 2006 [40863] [1459], Kheirandish et al. 2014 [39519] [878]). Results are usually expressed as OPG and, when the parasitic load is very high, it is necessary sometimes to make fecal dilutions 1:10, 1:100, etc. to facilitate the counting (Ruiz et al. 2012 [40864] [1460]).

Most probably, different *Eimeria* spp. are present in all animals of the herd, but not all of them may be pathogenic species, so the simple presence of oocysts in feces is not a sufficient reason for the diagnosis of goat coccidiosis, even though very high OPG are found (Koudela and Boková 1998 [39612] [910]). Therefore, a specific diagnosis trying to determine whether the pathogenic *Eimeria* species are or not a major component of the samples taken in that farm is required. For this purpose, fecal oocysts are routinely incubated with 2% potassium dichromate to facilitate oocyst sporulation and then have more morphological and structural parameters available for characterization (Ruiz et al. 2006 [40863] [1459], Levine and Ivens 1986 [10712B] [979], Alyousif et al. 1992 [38150] [35], Soe and Pomroy 1992 [42083] [1594]). For illustration, Figure 10.2 depictes drawings of sporulated oocysts of the main *Eimeria* species in goats and example pictures of sporulated oocyts of the most prevalent species are showed in Figure 10.3.

10.6.2 Lesions

Post-mortem examination may include histopathological evaluation of the intestinal mucosa (Dai et al. 2006 [38535] [316], Ruiz et al. 2013a [42059] [1461], Ruiz et al. 2013b [44267] [1463]). Goat kids with high burdens of pathogenic *Eimeria* species may be ill before oocysts are excreted

in feces. In such cases, diagnosis is only possible by visualizing the endogenous stages of *Eimeria* by observation of intestinal tissues in necropsies of recently dead animals or in fragments of intestinal mucosa removed with feces (Ruiz et al. 2013b [44267] [1463]).

10.6.3 Molecular and Other Diagnostic/Complementary Methods

Molecular techniques have been applied to *Eimeria* of goats for diagnosis and phylogenetic relationships. For this purpose, different loci have been employed, such as 18S rRNA, mitochondrial cytochrome oxidase gene (COI) and ITS-1 (Al-Habsi et al. 2017 [44247] [20]; Mohamaden et al. 2018 [44262] [1195]; Khodakaram Tafti et al. 2013 [41950] [881], Silva et al. 2017 [4656] [1577]).

Different isotypes of immunoglobulins (IgG, IgM and IgA) specifically increase after *E. ninakohlyakimovae* experimental infections when using antigens from sporulated oocysts, therefore serological diagnosis for the detection of *Eimeria* soluble antigens in feces or in tissue biopsies could also be used for the detection of *Eimeria* infections in goat kids (Matos et al. 2017a [42002] [1142], Matos et al. 2017b [40034] [1141], Matos et al. 2018 [40035] [1143]). However, these methods are not standardized currently for routinely identification of coccidiosis in this ruminant species.

On the other hand, some experimental studies have demonstrated changes in patterns of acute phase proteins and inflammatory mediators in serum from goat kids infected with *E. arloingi*. The magnitude and duration of the haptoglobin (Hp) and serum amyloid A (SAA) responses correlated well with the inoculation doses and the severity of the clinical signs and diarrhea in kids, and significant correlations were also observed with TNF-α and IFN-γ. Accordingly, Hp and SAA have been suggested as non-specific diagnostic indicators in caprine coccidiosis (Hashemnia et al. 2011 [39168] [680]).

Finally, a differential diagnosis must be made with respect to other gastrointestinal diseases including parasitosis, bacteriosis (colibacillosis, enterotoxemia, salmonel, osis), viral infections (viral enteritis), or even diarrhea produced because of inadequate diets (Smith and Sherman, 2009 [46166] [1592]).

10.7 TREATMENT

Most of the anticoccidials have been mainly registered for cattle and sheep but not for goats and, in certain parts of the world such as Europe, there are not anticoccidials specifically registered for caprine. In other countries, e. g. the United States, some compounds (ionophores and decoquinate) are approved for prevention and control of coccidia in goats when pre-mixed in feed, but there are no drugs approved for treatment. Table 10.4 summarizes anticoccidials described either for treatment or prevention, specifying which are referred as extralabel drug use (ELDU) or experimental tested used (EXTU). Some data on the effect of some anticoccidials experimentally tested in goats are described below. Anticoccidials used for treating coccidiosis in ruminants are discussed in **Chapter 6**.

10.8 PREVENTION

Currently, the coccidiosis prevention focuses on improving management practices in

combination with prophylactic/metaphylactic chemotherapy using specific anticoccidials. Other control alternative strategies are under investigation.

10.8.1 Pharmacologic Control

Coccidiosis prophylaxis by coccidiostats in drinking water or feed is commonly employed to control the disease, particularly in intensive goat production systems (Khodakaram-Tafti and Hashemnia 2017 [43855] [880]). Although there is no registered drug for pharmacological treatment of coccidiosis in goats in many areas, in certain countries (e. g. the United Stated) there are some compounds that can legally be used in prevention of the disease. For example, as depicted in Table 10.4, decoquinate (0.5 mg/kg) in feed mixtures for at least 28 days is safe and very effective coccidiostat in goats (Keeton and Navarre 2018 [39504] [868]). Besides, monensin at 20 mg per ton of feed controls shedding of oocysts and increases feed conversion in goats. Monensin could be considered the best choice for prevention of coccidiosis in goats, however, high levels of monensin render the feed unpalatable and toxic (Constable et al. 2012 [44236] [288]).

When a drug is administered either prophylactically of methaphylactically, a major concern is whether continuous or repeated treatments may interfere with immunity. In general, a primary infection exposure sufficient to trigger acquired immunity has to be ensured and, accordingly, in metaphylactic treatments with diclazuril (extralabel drug use) it is recommended that: (1) the timing of the first dose should be set at 4 weeks of life, once goat kids have already had contact with *Eimeria* species and thereby had the chance to develop immune reactions; (2) for the same reason, treatments at an interval of 3 weeks (instead of 2) would be a better option in case an additional dose is necessary to control goat coccidiosis in a particular herd (Ruiz et al. 2012 [40864] [1460]).

The continued use of coccidiostats reduces the number of oocysts passed in the feces over time, but it may also lead to selection for resistance, so that a regular monitoring of the treated animals is needed. There are no data currently available in literature on anticoccidial resistance in goats, but based on recently published results in sheep, it is not discarded that this should be an issue to investigate in detail in future (Odden et al. 2018 [43938] [1264]).

10.8.2 Management

General hygienic and management practices can be also applied to control goat coccidiosis. As an example, high density and the corresponding overcrowding has been correlated to an increased risk of clinical coccidiosis in large goat farms, something that can be overcome by regular prophylactic/metaphylactic treatments with anticoccidials (Ruiz et al. 2006 [40863] [1459]).

10.8.3 Alternative Control Methods

10.8.3.1 Phytotherapy

Several studies have been performed to evaluate the effect of different plant extracts against goat coccidiosis. For example, the exposure of *Melia azedarach* fruits to *Eimeria* lowers oocyst output in yearling Tswana goats (Madibela and Kelemogile 2008 [44259] [1110]) and

Aloe ferox and *Leonotis leonurus* caused significant reduction in *Eimeria* spp. oocysts (Maphosa and Masika 2012 44771 1123). Similarly, it has been found lower clinical signs in goats fed dried pelleted sericea lespedeza (*Lespedeza cuneata*) in comparison with the control group, in addition to a decrease in OPG counts (Kommuru et al. 2014 44251 905).

10.8.3.2 Strategic Nutrient Supplementation

In goat production systems with nutritional limitations of forages and other feed resources, supplementation with leguminous fodders, cactus during periods of severe drought, or energy sources such as molasses, cereal grains and byproducts, apart from minerals and vitamin A, are suggested as an alternative (Kawas et al. 2010 44249 865). Another nutrition-related control strategy in sustainable animal production has been the use of probiotics. Trying to investigate the effects of kefir on coccidial oocysts excretion and performance of dairy goat kids following weaning, reduced numbers of positive samples and lower OPG counts were recored, but the frequency of diarrhea, level of highest oocyst excretion, and performance of the kids remained unaffected (Das et al. 2012 44770 322).

10.8.3.3 Vaccines

Immunization of kids with oocysts attenuated by X radiation developed protective immune responses against coccidiosis produced by *E. ninakohlyakimovae* (Ruiz et al. 2014 40865 1462). The immunization protocol was based on the use of oocysts attenuated by irradiation X, following a methodology previously described for the attenuation of oocysts in avian vaccines (**Chapter 4- Vaccines**). Immunized animals showed no apparent symptomatology during the primary infection and released fewer oocysts than animals infected with unattenuated oocysts. Furthermore, during challenge infection, the immunuprotection conferred on the vaccinated group (in terms of reduction of fecal oocysts counts and general improvement of the clinical picture of coccidiosis) was comparable to that obtained on kids challenged with non-irradiated oocysts (Ruiz et al. 2014 40865 1462). Similar results have been obtained when using a mixture of *Eimeria* spp. in the vaccination protocol, although the response to multi-species infection was more complex than when using the mono-specific *E. ninakohlyakimovae* strain (Guedes et al. 2017 44244 633).

Recombinant vaccines have not been tested yet against ruminant coccidiosis, but some attempts have been performed to identify target candidates. Interesting results were obtained by using a phage display library to identify surface proteins of caprine umbilical vein endothelial cells (CUVEC) (Ruiz et al. 2015 40866 1464). The authors could identify two peptides that specifically bind to the surface of CUVEC (PCEC2 and PCEC5) and selectively reduced the infection rate by *E. ninakohlyakimovae* sporozoites.

Recently, age-related studies on immune response to experimental infection with *E. ninakohlyakimovae* in goat kids have demonstrated that goat kids of either 3, 4 or 5 weeks of age can develop patent infections and immunoprotective responses against *E. ninakohlyakimovae*. Nevertheless, detailed analysis of immunological data showed some differences among the 3 age groups, related both to the *Eimeria* infection outcome and the resulting immune response, suggesting that youngest goat kids are not fully immunocompetent. These findings may be of interest for the design of immunoprophylactic approaches (Matos et al. 2018 40035 1143).

Table 10.1 Morphological Characteristics of the Oocysts of *Eimeria* Species of Goats (Alyousif et al. 1992 [38150] [35]; Soe and Pomroy, 1992 [42083] [1594])

***Eimeria* spp.**	**Shape**	**Size (µm)**	**Shape index**	**Wall colour**	**Micropyle**	**Micropylar cap**	Shape	Size (µm)	Shape index	**Stieda body**	**Sporocyst residuum**	**Sporulation time (h) at 25-28 °C**
E. alijevi. MUSAEV, 1970	Subspherical	16-23.7 x 14-22 (19.9 x 18.0)	1.10-1.44 (1.27)	Yellowish green	-	-	Broadly ovoid	7-13 x 4-9	1.1-2.00	−/+	+	48
E. apsheronica. MUSAEV, 1970	Ovoid	24-37 x 18-27 (30.5 x 22.5)	1.14-1.70 (1.31)	Yellowish pink	+	-	(10 x 6.5)	1.1-2.00	(1.55)	−/+	+	60
E. arloingi. MAROTEL 1905, MARTIN 1909	Ellipsoid	22-36 x 16.2-26 (29 x 21.1)	1.10-1.72 (1.41)	Yellowish brown	+	+	(1.55)		1.2-2.20	−	+	48
E. caprina. LIMA, 1979	Ellipsoid	27-40 x 20-26 (33.5 x 23)	1.2-2.1 (1.7)	Brownish yellow	+	-	Pear shape	11-17 x 7-11	(1.7)	+	+	68
E. caprovina. LIMA, 1980	Broadly ellipsoid	26-36 x 20-28	1.10-1.5	Light pink	+	-	(14 x 9)	1.2-2.20	1.2-2.3	+	+	72

		(31 x 24)	(1.3)									
E. christenseni. LEVINE, IVENS, and FRITZ 1962	Pear shape	34-43.8 x 23-28.5 (38.9 x 25.8)	1.4-1.70 (1.55)	Yellowish brown	+	+	(1.7)		(1.75)	−/v	+	104
E. hirci. CHEVALIER, 1966	Roundish oval	18-27 x 14-20 (22.5 x 17)	1.09-1.5 (1.3)	Greenish	+	+	Elongate ovoide	10.9-17 x 6-10	1.60-2.25	−/+	+	72
E. jolchijevi. MUSAEV, 1970	Ovoidal to ellipsoid	25-37 x 18-26 (31 x 22)	1.25-1.69 (1.47)	Brownish green	+	+	(14.0 x 8.0)	1.2-2.3	(1.9)	+	+	84
E. ninakholyakimovae. YAKIMOFF and RASTEGAIEFF 1939; LEVINE,196	Subspherical to ellipsoid	19-28 x 14-23 (23.5 x 18.5)	1.0-1.62 (1.31)	Greenish brown	+	-	Elongate ovoid	12.6-17 x 7-10	(1.80)	+	+	96

1

E. pallida. CHRISTENSEN, 1938	Ellipsoid to ovoid	13-18 x 10-14 (15.5 x 12)	1.2-1.6 (1.3)	NS	-	-	(15 x 8)	1.60-2.25	1.3-2.27	−	+	NS
E. punctata. LANDERS, 1955	Truncated ellipsoid	20-31 x 15-23 (25.5 x 19)	1.2-1.7 (1.45)	Yellowish brown	+	+	(1.9)		(1.79)	NS	NS	60

NS, not stated.

Table 10.2 Endogenous Stages, Site of Infection and Prepatent Period of *Eimeria* spp. in Goats

***Eimeria* spp.**	**Location**	**Prepatent period (days)**	**Schizont generations**	**Schizonts**	**Gamonts**
E. alijevi	Small and large intestine	7-12	2	1G: 260 x 180 µm 2G: 15-18 x 9-12 µm	Macrogamonts: 14-18 x 9-14 µm Microgamonts: 20-25 x 15-20 µm

E. apsheronica	NS	14-17	NS	NS	NS
E. arloingi	Small intestine	14-17	2	1G: 140-360 x 65-240 µm, thousands merozoites (9-12 x 1-2 µm) 2G: 11-44 x 9-20 µm, 8-24 merozoites (4-10 µm long)	Macrogamonts: 12-28 x 8-20 µm Microgamonts: 11-34 x 8-29 µm
E. caprina	Small and large intestine	17-20	NS	NS	NS
E. caprovina	NS	14-20	NS	NS	NS
E. christenseni	Small intestine	14-23	2	1G: 100-227 x 81-130 µm, thousands merozoites (6-8 x 1-2 µm) 2G: 9-20 x 8-12 µm, 8-24 merozoites	Macrogamonts: 19-35 x 13-25 µm Microgamonts: 19-50 x 12-40 µm
E. hirci	NS	13-16	NS	NS	NS

E. jolchijevi	NS	14-17	NS	NS	NS
E. ninakohlyakimovae	Small and large intestine	10-13	2	1G: 165.5 x 123.6 µm 2G: 16.8 X 11.6	Microgamonts: 16.1 x 13.0 µm, Macrogamonts: 14.7 x 12.5 µm

Data are compiled and adapted from Levine and Ivens, 1986 [10712B] [979]; Vieira et al., 1997a [41444] [1747]; Taylor et al., 2007 [44694] [1688]. 1G: first generation schizonts, 2G: second generation schizonts, NS: not stated.

Table 10.3 *Eimeria* spp. Prevalence in Goats Using Fecal Examinations (Last 20 Years)

Country	Nº goats tested	% positive	Most prevalent *Eimeria* spp.	References
Brazil	202	(77.2% animals)	*E. ninakohlyakimovae (28.7%), E. alijevi (25.2%), E. jolchijevi (11.4%), E. caprovina (10.4%)*	Coelho et al. 2012 [44235] [282]
Brazil (Northeast)	215	(88.1% adults) (100% kids)	*E. alijevi (26.7%), E. arloingi (20.6%), E. hirci (18%),*	Cavalcante et al. 2012 [41807] [188]
Brazil (Western Santa Catarina)	217	(68.2% animals)	Not determined	Radavelli et al. 2014 [44265] [1381]
China (Northeast)	199	(87.9% animals)	*E. christenseni (78.3%), E. alijevi (73.7%), E. caprina (62.3%), E. arloingi (44.6%)*	Wang et al. 2010 [42114] [1776]
China (Shaanxi province)	584	(97.3% animals)	*E. arloingi (83.9%), E. alijevi (73.8%), E. jolchijevi (63%), E. caprina (48.5%)*	Zhao et al. 2012 [41723] [1900]

Egypt (Suez Governorate)	135	(60% animals)	*E. ninakohlyakimovae, E. hirci, E. caprina, E. christenseni, E. jolchijevi, E. apsheronica, E. arloingi*	Mohamaden et al. 2018 44262 **1195**
India (Meghalaya)	834	(23.0% animals)	*E. christenseni, E. hirci, E. caprina, E. jolchijevi, E. ninakohlyakimovae, E. arloingi, E. kocharii*	Das et al. 2017 44765 **323**
Italy (Lombardy)	2554	(8.3-100% farms)	Not determined	Di Cerbo et al. 2010 44238 **366**
Iran (Southeast)	208	(89.91% animals)	*E. arloingi (68.26 %), E. christenseni (50.9 %), E. ninakohlyakimovae (41.8 %), E. caprina (31.7 %)*	Kheirandish et al. 2014 39519 **878**
Papua New Guinea	55	(17.3% animals)	Not determined	Koinari et al. 2013 41965 **904**
Poland	110	(81% adults) (100% kids)	*E. arloingi (80%), E. christenseni (39.3%), E. ninakohlyakimovae (40%), E. caprina (20%)*	Balicka-Ramisz 1999 3974 **60**
Poland (Western Pomerania) and Ukraine (West region)	311	(87% adults) (100% kids)	*E. arloingi (36.4%), E. christenseni (46.8%), E. ninakohlyakimovae (33.5%), E. alijevi (30.6%)*	Balicka-Ramisz 1999 3974 **60**
Portugal (South)	144	(98.61% animals)	*E. ninakohlyakimovae (88%), E. arloingi (85%), E. alijevi (63%), E. caprovina (63%)*	Silva et al. 2014 41070 **1576**
Spain (Gran Canaria)	2646	(96.1% animals)	*E. ninakohlyakimovae (30.0%), E. arloingi (28.6%), E. alijevi (20.5%), E. caprina (9.1%)*	Ruiz et al. 2006 40863 **1459**
Sri Lanka	203	(87% adults) (80% kids)	*E. ninakohlyakimovae (31%), E. alijevi (29%), E. arloingi (21%), E. christenseni (7%)*	Faizal and Rajapakse 2001 44240 **513**

South Africa	824	(88.7-100% animals)	*E. arloingi (97.47%), E. hirci (84.34%), E. caprovina (61.11%), E. ninakohlyakimovae (45.95%)*	Harper and Penzhorn 1999 44246 **678**
Switzerland	148	(100% animals)	Not determined	Marreros et al. 2012 44261 **1131**
Tanzania	81	(64.2% animals)	Not determined	Kimbita et al. 2009 39549 **891**
Turkey (Igdir province)	212	(82.55% animals)	*E. arloingi (47.43%), E. christenseni (45.14%), E. ninakohlyakimovae (36%), E. alijevi (26.85%)*	Gül 2007 44245 **640**
Turkey (Van province)	242	(73.6% animals)	*E. arloingi (40.9%), E. christensini (34.3%), E. alijevi (32.6%), E. pallida (31.0%)*	Deger et al. 2003 44237 **353**
Zimbabwe	580	(43% animals)	Not determined	Zvinorova et al. 2016 44272 **1924**

Table 10.4 Anticoccidial Agents for Use in Treatment and Prevention of *Eimeria* Infection in Goats*

Agent	Treatment	Prevention	Comments
Amprolium[a] (Corid)	25-40 mg/kg BW for 5 days (ELDU)		Available in multiple forms: • 9.6% oral solution • 20% soluble

			powder • 1.25% or 2.5% crumbles/pellets
Decoquinate (Deccox)		5 mg/kg BW for at least 28 days	Feed additive For prepartum use in sheep and goats • I kg fo 13% premix in 22 kg of trace mineralized salt
Lasanocid (Bovatec)		1 mg7kg BW continuously (ELDU)	Feed additive For prepartum use in sheep and goats: • 1 kg of 6% premix in 22 kg of trace mineralized salt
Nomensine (Rumensin)		20 g/ton of feed	Feed additive May be best choice for goats
Sulfaquinoxaline	10-20 mg/kg BW for 3-7 days (SLDU)		As a 0.015% solution in water

Toltrazuril		20, 30, 40 mg/kg BW oral, single dose (EXTU)	No adverse effects were found (Chartier et al. 1992 [44233] [248])
Ponazuril	10 mg/kg BW oral, single dose		Well absorbed (Gibbons et al 2016 [44243] [593], Love et al. 2015 [44256] [1095])
Diclazuril		1-2 mg/kg BW oral, single or double treatment (EXTU)	Adapt treatment to risk of clinical disease (Ruiz et al. 2012 [40864] [1460])

* Adapted from (Keeton & Navarre 2018 [39504] [868])
Abreviations: BW, body weight; ELDU, extralabel drug use; EXTU, experimental tested use
[a] Amprolium is a thiamine analog can cause polyencephalomalacia, especially at high doses

Table 10.5 Clinical, Pathological and Immunological Studies on Experimental Infections with *Eimeria* spp. in Goats

Eimeria spp.	Age range	Infection dose (SP)	Samples	Method/analysis	Main findings	Reference
E.	20	1×10^4,	Blood	Biochemistry	• No significant	(Dai et al.

ninakohlyakimovae	days	1×10^5, 1×10^6			differences in: serum AST, ALT, total protein, albumin, globulin, Na^+, K^+, Cl^- • No serum indication of liver damage	2006 [38535316])
			Intestinal mucosa, liver	Pathology (post-patency)	• Mild subacute to chronic proliferative enteritis in small and large intestine	
				Clinical inspection	• Clinical signs more severe at higher doses	
E. arloingi	15 days	1×10^3, 1×10^5	Blood	Biochemistry: haptoglobin (Hp), serum amyloid A (SAA), TNF-α, and IFN-γ	• Hp ↑, SAA ↑ • Significant correlations for TNF-α and IFN-γ with SAA and Hp, respectively	(Hashemnia et al. 2011 [39168680])
			Small and large	Pathology (post-patency)	• Thickened mucosa due to mucosal hyperplasia and	

			intes-tines, liver, spleen, pancreas, and mesenteric lymph nodes		adenomatous-like changes. • Proliferative enteritis with the develop-mental stages of parasites • Mild lymphoid hyperplasia of the Peyer's patches.	
				Clinical inspection	• Clinical and pathological changes more severe at higher doses	
E. caprina (65%), *E. ninakohlyakimovae* (33%), *E. arloingi* (2%)	14 days	2×10^3	Blood	Biochemistry	• ↓ activities of the main erythrocyte antioxidant enzymes • ↓ total antioxidant capacity • ↑ serum levels of malondialdehyde • ↑ total	(Rakhshandehroo et al. 2013 40661 **1386**)

					homocysteine	
E. ninakohlyakimovae	4 weeks	2×10^5	Blood	Haematology	• ↓ Total protein, ↑ PCV, ↑ eosinophils • Differences not statistically significant	(Ruiz et al. 2013a 42059 **1461**)
			Intestinal mucosa	Pathology (post-patency)	• Moderate hyperplasia of the intestinal epithelium • Hypertrophy of the mesenteric lymph nodes and Peyer's patches • Eosinophilic enteritis + diffuse infiltration of mast cells, lymphocytes and neutrophils	
				Clinical inspection	• Moderate to severe clinical disease • Important immunoprotection after challenge	

E. ninakohlyakimovae	4 weeks	2×10^5, 1×10^6	Blood	Haematology	• Decrease in PCV and Hb • Leucocytosis with neutrophilia and eosinophilia • Differences not statistically significant	(Ruiz et al. 2013b 44267 **1463**)
			Intestinal mucosa, mesenteric lymph nodes, spleen	Pathology (patency and post-patency)	• Inflammatory cellular infiltration • Hyperplasia of globet cells • Hyperplasia of Peyer's patches • Infiltration of eosinophils and reactive hiperplasia in mesenteric lymph nodes and spleen	
					• ↑ Eosinophil, lymphocyte and neutrophil counts	
				Clinical inspection	• Severe to fatal clinical disease,	

					particularly in goat kids challenged with the higher dose	
E. arloingi	1×10^3, 1×10^5	14 days	Blood	Haematology and Biochemistry	• ↓ ALP • ↑PCV and Hb • ↓Na^+, Cl^- and K^+ • No significant differences in AST, ALT, GGT, albumin and total proteins • There was no hepatic damage	Hashemnia et al., 2014 [39169 682]
E. ninakohlyakimovae (vaccination with attenuated oocysts)	5 weeks	2×10^5	Blood	Haematology	• Slight ↑PCV and Hb • Non-significant changes in leukocyte subpopulations	(Ruiz et al. 2014 [40865 1462])
			Intestinal mucosa, mesente	Pathology (post-patency)	• Moderate hyperplasia and hypertrophy of the mesenteric lymph nodes	

			ric lymph nodes		and Peyer's patches • Moderate to severe enteritis • ↑Eosinophils, ↑ lymphocytes, ↑ globular leukocytes, ↑ mast cells, ↑ neutrophils • In association to ↓ OPG, animals vaccinated with attenuated oocysts had ↓ clinical signs	
				Clinical inspection		
E. ninakohlyakimovae	5 weeks	2×10^5	Intestinal mucosa, mesenteric lymph nodes	Pathology (pre-patency)	• Moderate hyperplasia and hypertrophy of the mesenteric lymph nodes and Peyer's patches • Eosinophilic enteritis with diffuse infiltration of mast cells, lymphocytes, neutrophils and	(Matos et al. 2017a 42002 **1142**)

		globular leukocytes • Less immature schizonts in challenged animals
Ileal mucus	ELISA	• ↑IgA
Ileal and colonic mucosa	IHC	• ↑$CD4^+$, ↑$CD8^+$, ↑$CD45^+$, mainly in challenged animals • ↑ MCHII and myeloid/histiocyte markers, mainly in primary infected goat kids
Ileal and colonic mucosa	Real time PCR	• ↑IL2, ↑IL4, ↑ IL10 • INFγ without changes
	Clinical inspection	• Clinical signs only during the patency of primary

					infection	
E. ninakohlyakimovae	5 weeks	2×10^5	Blood	ELISA	• ↑IgG, ↑IgM	(Matos et al. 2017b 40034 **1141**)
				Immunoblotting	• Most proteins appeared in the range of 54–108 kDa • Polypeptides of smaller mw (16–38 kDa) were also detected • Most prominent bands: 74, 54, 23 and 20 kDa	
			Ileal mucus (post-patency)	ELISA	• ↑IgA	
Eimeria spp. (vaccination with attenuated oocysts)	5 weeks	2×10^5		Clinical inspection	• In association to ↓ OPG, animals vaccinated with attenuated oocysts had ↓ clinical signs	(Guedes et al. 2017 44244 **633**)

E. ninakohlyakimovae	3, 4, 5 weeks	2×10^5	Blood	Haematology	• Hematologic changes were very mild in all groups: moderate increase in the total number of leukocytes, neutrophilia and temporary monocytosis • Moderate eosinophilia	(Matos et al. 2018 40035 **1143**)
			Intestinal mucosa, mesenteric lymph nodes	Pathology (post-patency)	• Moderate hyperplasia and hypertrophy of the mesenteric lymph nodes and Peyer's patches • Moderate to severe enteritis • ↑Eosinophils, ↑ lymphocytes, ↑ globular leukocytes, ↑ mast cells, ↑ neutrophils • The youngest age group	

		was the only in which no statistical differences were observed on lymphocytes counts in challenge infection
	Clinical inspection	• The three age groups develop patent infections • Slightly longer prepatent periods in goat kids primary infected at younger age • The severity of the disease was milder in challenged animals of all age groups

SP: sporulated oocysts; IHC: immunohistochemistry; ELISA: enzyme linked immunosorbent assay; ↑: increased in comparison to uninfected control; ↓: decreased in comparison to uninfected control.

FIGURE LEGENDS

Fig. 10.1 Infection and development of *E. ninakohlyakimovae* in caprine umbilical vein endothelial cells (CUVEC). CUVEC were grown to confluency and infected with freshly isolated *E. ninakohlyakimovae* sporozoites. Infection and development was monitored daily for up to 22 days. (A) 4 DPI, intracellular sporozoites are indicated by an arrow; (B) 7 DPI, immature schizont; (C) 16 DPI, mature schizont; (D) 19 DPI, rosette-type schizont with merozoites; (E) 20 DPI, periphery-type schizont with merozoites; (F) 22 DPI, merozoite I release.

Fig. 10.2 Sporulated oocysts of *Eimeria* spp. in goats (Eckert et al., 1995 41864 **459**).

Fig. 10.3 Sporulated oocysts compatible with some of the most frequent *Eimeria* species in goats: (A) *E. christenseni;* (B) *E. caprina;* (C) *E. arloingi;* (D) *E. ninakohlyakimovae;* (E) *E. hirci;* (F) *E. alijevi.*

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