CHAPTER 11

COCCIDIOSIS IN RUMINANTS

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INTRODUCTION

The genus *Eimeria* (known as coccidia) contains a large number of species infecting vertebrate animals. To date is accepted that there is only a single host species for each species of *Eimeria* (Kreier and Baker 1987). *Eimeria* contains species that are important pathogens for domesticated animals (Kreier and Baker 1987). *Eimeria* species are usually parasites of the gastrointestinal tract, and most species of this parasitic genus are exclusively located in the intestine (Figure 1). They are not transmissible between different domestic ruminant species. *Eimeria* species conform a group of economically important parasites and a common cause of diarrhea in young ruminants. In this context, coccidia-related disease, also known as eimeriosis or coccidiosis (Keeton and Navarre 2017).

On the other hand, *Eimeria ovina, E. ahasta, E. ovinoidalis, E. faurei, E. crandalis,* and *E. intricati* are the most common species which infect sheep (Mohamaden et al. 2018). *Eimeria ninakohlyakimovae, E. hirci, E. aspheronica,* and *E. arloingi* are the most prevalent species in goats (Mohamaden et al. 2018); while *E. bovis*, *E. zuernii*, *E. canadensis*, *E. ellipsodalis*, *E. alabamensis*, *E. pellita*, *E. auburnensis* and *E. cylindrica* are the most common species in cattle (Heidari et al. 2014).

Etiological Agent

Eimeria spp. are protozoa that are part of the order Coccidia, which infect different vertebrate's species (Duszynski 2001; Trefancová et al. 2021). Currently, more than 1800 species of *Eimeria* are recognized; infection by these parasites is associated with the presence of enteritis, diarrhea, dehydration and weight loss in animals (Burrell et al. 2020).

Eimeria species belong to the phylum Apicomplexa, as do other protozoa of the order Coccidia (*Toxoplasma* spp.) that are also part of this phylum, possess a complex endomembrane system with specialized secretory organelles for invasion, residence and replication (Tenter et al. 2002; Tomavo et al. 2013; Marugan-Hernandez et al. 2021).

Eimeria spp. have an affinity for a specific host, even affecting the epithelial cells of a specific portion of the intestinal tract (Cowper et al. 2012). Unlike other species, humans are not affected by *Eimeria* spp. but by *Cyclospora* spp. a parasite similar in terms of pathogenicity and genetics (Liu et al. 2016). The production species in which *Eimeria* spp. infections have a significant impact are domestic fowl, swine and ruminants, although these parasites can infect other species such as fish and reptiles (Walker et al. 2013; Lucas et al. 2014). A wide variety of disease-causing *Eimeria* are recognized in ruminants.

Generalities

Eimeriosis or coccidiosis in small ruminants is considered one of the infectious-contagious diseases of worldwide distribution with great productive impact.

The presence of this disease is in a humid environment and another characteristic is overcrowding, although it is also observed in arid climates, under breeding systems where the population density is considered a predisposing factor. This parasitosis particularly affects young animals at the weaning stage, causing diarrhea, growth retardation and even death (Taubert et al. 2008). Currently, 12 different species of *Eimeria* with infective capacity are recognized in cattle, 11 in sheep and 9 in goats.

The disease is acquired when ruminants ingest the oocysts of the parasite through feed. The repeated exposure of the animal to *Eimeria* spp., and the constant replication of the parasite in the schizogony and gametogony stages in the intestine, cause damage and consequent clinical signs; the sporogony stage gives rise to the infective phase, the oocysts that are disseminated through the feces into the environment, contaminate the soil, pasture and feed, contributing to the transmission of the parasitosis in other animals (Burrell et al. 2020; Felici et al. 2021).

Biology

According to Tenter et al. (2002), coccidia have been found in almost all animals, including humans. During the 19th and early 20th centuries, classification of protozoa was based primarily on locomotion organelles, but with increasing knowledge about their morphology, biology, life cycle, and host specificity, a wide range of phenotypic characters were used to classify them into different taxonomic groups. In the 21st century, classification takes also into account phylogenetic studies and ultrastructural, biochemical, and molecular-biological data that have been generated from diverse studies of a wide range of protozoa.

Coccidia of the family Eimeriidae, such as *Eimeria* spp. and *Cystoisospora* spp. are parasites that fulfill their biological cycle in a single host; these parasites are known as monoxenes. Those belonging to the family Sarcocystidae, such as

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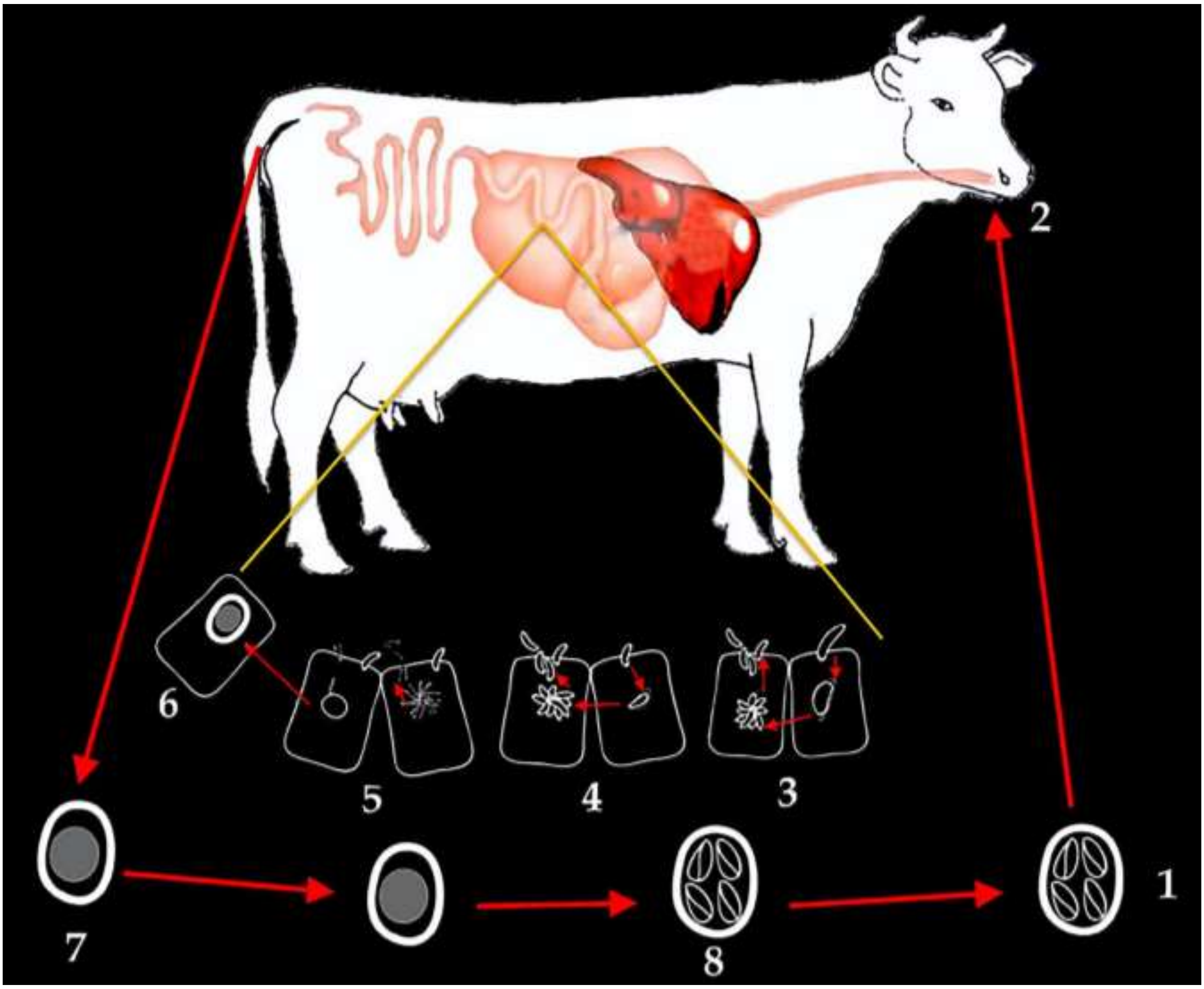

Fig. 1: General *Eimeria* life cycle. 1. Sporulated oocyst. 2. Host ingests sporulated oocyst. 3. Sporocyst containing sporozoites are released in the small intestine and penetrate intestinal cells and transform into merozoites. 4. Merozoites multiply asexually within cells causing it to burst. These merozoites will then infect new cells and can repeat this cycle several times. 5. Some merozoites will infect new cells and transform into either macrogametocytes or microgametocytes. Microgametocytes will emerge from the host cell and fertilize a macrogametocyte, which will develop into an oocyst. 6. Unsporulated oocyst. 7. Unsporulated oocyst is passed through feces into the environment. 8. Oocyst become sporulated in the environment (Figure made by Carlos Ramón Bautista-Garfias).

Toxoplasma spp. and *Neospora* spp. are heteroxen, that is, they initiate their life cycle in a variety of intermediate hosts and conclude it in the intestine of a definitive host (Wohlfert et al. 2017). Infections by *Eimeria* spp., in ruminants and the severity of its clinical signs depend on different factors such as the ingested parasite load, the *Eimeria* species involved, the concomitance with other infections, the age and the state of immunocompetence of the host (Bangoura et al. 2012; Das et al. 2015; Carrau et al. 2018), as well as other factors like herd husbandry conditions (intensive, semi-intensive or extensive system), zootechnical purpose (milk or meat production), conditions that may generate animal stress and other environmental particularities such as soil type and pH, vegetation present in the region, solar radiation, temperature, humidity, rainfall and altitude (Sun et al. 2018; Chaiyos et al. 2018).

The range of survival of *Eimeria* spp. in climatic conditions is very wide, although the disease is more frequent in ruminants raised on moderate humidity and heat (Keeton and Navarre 2018), with a higher incidence in intensive production systems. In a study conducted in Mexico, Rodríguez-Vivas et al. (2017) reported the economic losses due to coccidiosis in small ruminants for US $23.7 million only for Mexico. In a retrospective study by Alcalá et al. (2020), it was determined that this disease is widely distributed in Mexico, regardless of the geographical area and climatic conditions in which ruminants develop.

The most frequently reported species in cattle were *E. bovis*, *E. zuernii* and *E. alabamensis*; in sheep is *E. crandallis*; and in goats, *E. ninakohlyakimovae* and *E. christenseni*, all of these are pathogenic. This study also describes that the presence of these and other *Eimeria* species in cattle and sheep is influenced by the time of year, the size of the herd or flock, the production system and the age of the animals. The months with higher humidity, farms with higher numbers of animals and under semi-intensive and intensive production systems, as well as calves under one-year-old and lambs up to six months old, were frequently reported with infection by *Eimeria* spp.; it is worth mentioning that for the goat species, the most relevant factor for the presence of coccidiosis was the size of the herd.

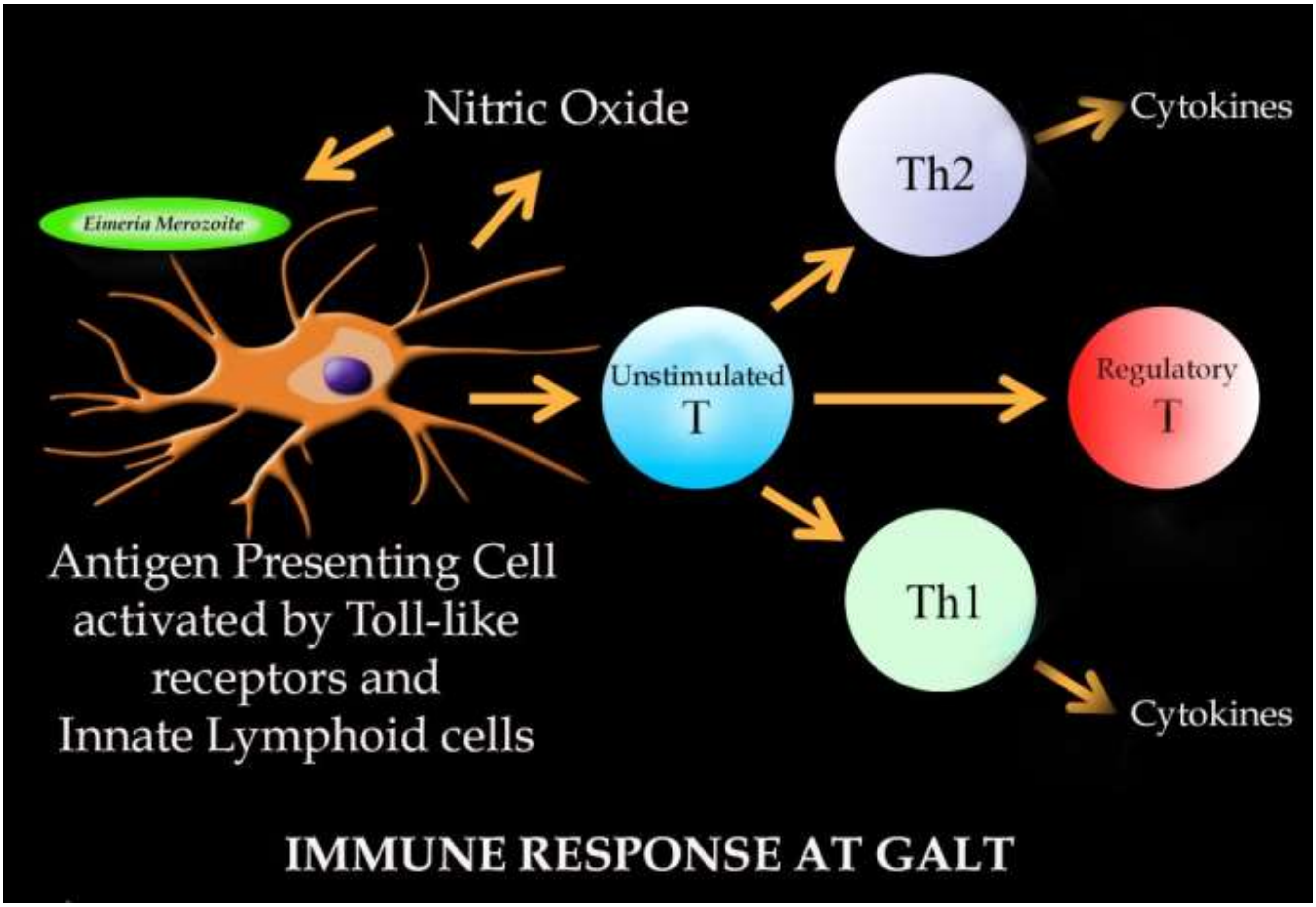

Fig. 2: The immune response at GALT (Gut Associated Lymphoid Tissue) against *Eimeria* spp. Th1: T helper 1; Th2: T helper 2. (Figure made by Carlos Ramón Bautista-Garfias based on Mirchandani et al. 2012; Min et al. 2013; Shivaramaiah et al. 2014; Iwasaki and Medzhitov 2015).

Life Cycle

The general life cycle of *Eimeria* has basically two stages: the exogenous phase (sporogony) and the endogenous phase (schizogony and gametogony) (Allen and Fetterer 2002). The initial infective unit of all *Eimeria* spp. is the sporozoite. The sporozoite of every apicomplexan parasite is characterized by a unique complex of structures specialized in the invasion of the host cells (Augustine 2001). The sporozoite is the beginning and the end of the life cycle of any coccidian (Bowman 2014). Sporozoites are the infective forms found in sporulated oocysts and are the result of protoplasm segmentation (Bowman 2014). Oocysts are ovoid and vary in size and shape according to the species.

The life cycle involves the following steps: The host ingests a sporulated oocyst (Figure 1: 1, 2). The life cycle process is described below: 1. Sporulated oocyst. 2. Host ingests sporulated oocyst. 3. Sporocyst containing sporozoites are released in the small intestine and penetrate intestinal cells and transform into merozoites. 4. Merozoites multiply asexually within cells causing it to burst. These merozoites will then infect new cells and can repeat this cycle several times. 5. Some merozoites will infect new cells and transform into either macrogametocytes or microgametocytes. Microgametocytes will emerge from the host cell and fertilize a macrogametocyte, which will develop into an oocyst. 6. Unsporulated oocyst. 7. Unsporulated oocyst is passed through feces into the environment. 8. Oocyst become sporulated in the environment.

Immunological Aspects

Both innate and adaptive immunity are part of the whole immune responses against pathogens (Takeda K and Akira S, 2005; Bautista 2016; Mirchandani et al. 2012). In the case of *Eimeria* infections, the Gut Associated Lymphoid Tissue (GALT) has an important role in the first line defense against this parasite genera (Shivaramaiah et al. 2014). A larger group of tissues called mucosal-associated lymphoid tissue (MALT) is responsible for conferring immunity across mucosal surfaces in different areas of the body; GALT is a component of MALT (Lillehoj and Trout 1996).

Early investigations in both mammalian and avian species have revealed that the cellular immune responses through T cells and their associated cytokines play an important role in anticoccidial immunity (Rose and Hesketh 1979; Rose at al. 1979; Wakelin et al. 1993). Acquired immunity to murine coccidiosis is attributed more to T cells than B cells (Rose et al. 1979). Several immune cell types including NK cells, dendritic cells, and macrophages are involved in innate immune responses to avian coccidiosis (Min et al. 2013). It is important to mention that a subset of T cells (innate lymphoid cells - ILCs) participates in the overall immune response against parasites (Koyasu and Moro 2012). The information about ILCs indicates that they are vital regulators for gastrointestinal mucosal homeostasis through interactions with other structural and stromal cells in gut epithelial barriers (Fan et al. 2019). It has been indicated the innate immune response controls adaptive immune response against different pathogens (Iwasaki and

Medzhitov 2015). The general immune response against *Eimeria* spp. is shown in Figure 2. It is important to point out that the stimulation of the innate immune system by *Lactobacillus casei* in broiler chickens confers a protective immune response against a challenge with pathogenic *Eimeria* species (Bautista-Garfias et al. 2003), this finding represents a sustainable control measure of coccidiosis in ruminants that must be evaluated in the future.

Epidemiology

Coccidiosis is a globally distributed disease, but it is a very important animal health problem in developing countries (Squire et al. 2019), reported to be distributed in several countries in all continents (Macrelli et al. 2019; Alcala-Canto et al. 2020; Jansen et al. 2020; Olmos et al. 2020; Silva et al. 2020; Trejo-Huitrón et al. 2020; Gondipon and Malaka 2021; Thanasuwan et al. 2021; Mohammed et al. 2021; Yan et al. 2021). Pathogens that cause coccidiosis in ruminants are found in temperate, subtropical and tropical climates. (Alcala-Canto et al. 2020).

Although prevalence is usually 60% or higher among herds and flocks (Macrelli et al. 2019; Squire et al. 2019), coccidiosis is a disease that affects mainly young animals: calves between six and 1 year of age are most susceptible, as well as lambs from one to six months of age (Sudhakara-Reddy et al. 2015; Keeton and Navarre 2018; Silva et al. 2020). The chronic or subclinical presentation of the disease is most often seen in growing animals and occurs mainly in the wet seasons (Constable et al. 2016).

This disease is relevant to ruminant production; the economic impact was valued in 2012 as a 6-9% reduction in gross margin (Lassen and Østergaard 2012). The economic losses are a combination of costs, including prevention and treatment, and morbidity and mortality (Silva et al. 2017; Squire et al. 2019), resulting in adverse effects on feed intake, growth rate, fertility, wool growth and milk yield (Squire et al. 2019; Silva et al. 2020).

Transmission

Ruminants become infested with the pathogens when they ingest the oocysts with contaminated feed or water (Constable et al. 2016; Alcala-Canto et al. 2020). The main source of sporulated oocysts is contamination of the environment in which ruminants are found, since oocysts can survive for weeks or months in favorable conditions of heat and humidity (Silva et al. 2017; Keeton and Navarre 2018; Macrelli et al. 2019; Trejo-Huitrón et al. 2020; Gondipon and Malaka 2021). A large number of animals in a confined environment causes the environment to have a higher concentration of feces, which increases environmental contamination, although pathogens are also prolific in paddocks and feedlots, especially if water accumulates in nearby areas (Keeton and Navarre 2018; Macrelli et al. 2019). Healthy animals one-year-old or older also act as a reservoir for lambs or calves (Keeton and Navarre 2018; Bangoura and Bardsley 2020; Silva et al. 2020; Mohammed et al. 2021).

Factors related to the pathogen species involved, the level of contamination or exposure and the immune status of the animals, determine the severity of the infection (Keeton and Navarre 2018; Macrelli et al. 2019; Alcala-Canto et al. 2020; Silva et al. 2020). An optimal immune response of affected animals is directly influenced by the general health status of the animals, which is related to age, physiological status and nutrition. External factors can be triggers because they can be a stressor, such as sudden changes in weather, the maintenance of hot and humid climates or the movement of livestock. The amount of vaccination and deworming status, as well as the presence of other concomitant diseases in the herd are also related to the occurrence of coccidiosis (Keeton and Navarre 2018; Alcala-Canto et al. 2020; Silva et al. 2020; Gondipon and Malaka 2021).

Diagnosis

To establish a diagnosis of coccidiosis, attention should be paid to the clinical signs of the affected animals and the herd/flock history (Keeton and Navarre 2018; Bangoura and Bardsley 2020). The most representative and relevant sign is diarrhea (which may be mucoid or bloody and some of the calves/lambs may have perineal areas stained with feces), followed by abdominal pain, straining to defecate and subsequent rectal prolapse, dehydration, anorexia, weakness, depression, pale mucous membranes and acute weight loss (Sudhakara-Reddy et al. 2015; Constable et al. 2016; Keeton and Navarre 2018; Gondipon and Malaka 2021). Nervous coccidiosis may occur as result of severe infection, producing among other nervous signs, muscle tremors, convulsions and nystagmus. Nervous coccidiosis is associated with a mortality rate of more than 80% (Sudhakara-Reddy et al. 2015; Keeton and Navarre 2018; Bangoura and Bardsley 2020).

Subclinical conditions often cause decreased appetite and reduced weight gains, due to intestinal damage, leading to poor growth rates and decreased feed efficiency, which in turn results in lost productivity and poor economic performance (Keeton and Navarre 2018; Squire et al. 2019).

One method of identification of the causal agent is the observation of oocysts by examination of fecal samples, however, the mere observation of oocysts does not ensure a definitive diagnosis, although it is ideal for identifying the species and obtaining the oocyst count (Constable et al. 2016; Keeton and Navarre 2018; Bangoura and Bardsley 2020). Some animals infected with other pathogens may present a high number of oocysts of non-pathogenic species (Keeton and Navarre 2018). Therefore, only the combination of history and clinical signs, as well as oocyst counts higher than 500 oocysts per gram will be suggestive of coccidiosis (Keeton and Navarre 2018; Bangoura and Bardsley 2020).

Common laboratory diagnosis is performed by direct smear, fecal flotation or McMaster's technique; quantitative fecal analysis is preferable to non-quantitative tests (Constable et al. 2016; Keeton and Navarre 2018; Alcala-Canto et al. 2020). Given that samples collected at the first days or days after the clinical phase of the disease may contain minimal numbers of oocysts (Constable et al. 2016), at least five individual rectal samples from a flock or herd must have significant oocyst counts to confirm coccidiosis (Constable et al. 2016).

When morphologic differentiation is difficult, is necessary to use molecular characterization to clarify species classification, although is not used routinely since it is much more expensive than fecal flotation (Silva et al. 2017; Bangoura and Bardsley 2020). Serologic diagnostic test is not available for routine use, although its potential to allow monitoring of herds or flocks; however, antibodies may persist after a self-limiting infection (Bangoura and Bardsley 2020).

Differential diagnoses include other intestinal parasites,

malnutrition, salmonellosis, toxins, or viral diseases (Constable et al. 2016). Because coccidiosis can occur as a co-infection, it is necessary to take a proper clinical history and identify clinical signs to eliminate other pathogens as additional causes of diarrhea (Constable et al. 2016; Macrelli et al. 2019).
When animals do not survive, necropsy may help confirm coccidiosis in the other ruminants affected (Wäsle et al. 2017; Bangoura and Bardsley 2020). Some of the changes observed during necropsy are intestinal hemorrhage as well as white/gray spots or lines in the mucosa; histopathological examination is necessary to confirm this (Wäsle et al. 2017; Keeton and Navarre 2018; Bangoura and Bardsley 2020).

Conventional Control

Coccidiosis is a self-limiting disease (Bangoura and Bardsley 2020). A controlled, limited or gradual exposure is necessary to develop a protective immune response (Keeton and Navarre 2018; Macrelli et al. 2019; Bangoura and Bardsley 2020; Silva et al. 2020).
Reduction of environmental contamination is important and reachable by limiting overcrowding; adequate sanitation of all areas and equipment, exposure to sunlight for desiccation are very efficient ways to reduce the count of oocysts in the environment (Constable et al. 2016; Keeton and Navarre 2018; Silva et al. 2020; Gondipon and Malaka 2021).
Control should include correction of management factors that contribute to development of clinical disease such as poor housing conditions and low or minimal ventilation (Gondipon and Malaka 2021). Other forms of control include the adoption of feeding practices that avoid fecal contamination of feed and water, grouping animals by size, and moving young animals from pen to pen (Constable et al. 2016; Silva et al. 2020).
Strengthening of the immune system is necessary to maintain a protective immune response (Bangoura and Bardsley 2020). To this end, it is highly recommended to reduce overcrowding, optimize feeding and minimize stress factors and other diseases through an adequate and effective health program (Bangoura and Bardsley 2020). Fecal examinations over time in the flock/herd may be helpful to maintain animal health and to control outbreaks (Keeton and Navarre 2018; Macrelli et al. 2019; Silva et al. 2020).
Preventive is preferable to corrective treatment due to the risk of subclinical infections that would lead to chronic disease and wide distribution within the herd or flock (Keeton and Navarre 2018; Silva et al. 2020).
There are several anticoccidial drugs available for treatment and prevention of coccidiosis in ruminants; these drugs work by impeding the growth and reproduction of coccidian parasites (Keeton and Navarre 2018). During an outbreak, affected animals should be isolated and given oral and parental fluid therapy; mass medication of water and feed may help to minimize the effects in the animals without clinical signs (Constable et al. 2016).
Sulfaquinoxaline (10-20 mg/kg/day for 3-5 days) and amprolium (10 mg/kg/day for 5 days) are drugs that can be used to treat clinically affected animals (Keeton and Navarre 2018). The first is particularly useful for weaned animals that develop bloody diarrhea (Constable et al. 2016).
Sulfonamides in the feed at 25–35 mg/kg for ≥15 days are effective to control coccidiosis in calves (Bangoura and Bardsley 2020), corticosteroids are contraindicated (Constable et al. 2016).
Amprolium (5 mg/kg/day for 21 days), decoquinate (0.5 mg/kg/day for 28 days) and lasalocid (1 mg/kg/day to a maximum of 360 mg/head/day), or monensin (100–360 mg/head/day) can be used for prevention (Constable et al. 2016; Keeton and Navarre 2018; Bangoura and Bardsley 2020).
Toltrazuril administered at 15 mg/kg as a single oral dose, 14 days after animals are moved into group housing, effectively prevents diarrhea due to coccidiosis (Constable et al. 2016).
The major benefits of coccidiostats are through improved feed efficiency and rate of gain, because allows immunity to develop (Constable et al. 2016).
To minimize risk of resistance to coccidiostats development its long-term preventive uses most be limited; once clinical signs appear, the apparent ineffectiveness of a treatment is due to intestinal damage that has already occurred (Keeton and Navarre 2018; Bangoura and Bardsley 2020).

Advantages and Disadvantages of Conventional Control

Conventional or chemical control for coccidiosis disease in bovines uses medications such as coccidiostats and coccidioicides; which reduce the parasitic loads of treated animals. It also reinforces natural defenses indirectly; however, they do not allow to eliminate definitively coccidia from a herd in the long term, since the coccidiosis disease persists due to the continuous reinfections of the treated animals and the infection of the healthy ones (Keeton and Navarre 2018).
Currently, the most commonly used sulfas for such effects are five and they are described below: 1) sulfaguanidine, 2) sulfaquinoxaline, 3) sulfamerazine, 4) sulfabromomethazine and 5) sulfamethazine. It is worth mentioning that in several studies it has been observed that if treatment with sulfa drugs is started 13 days after the start of the infection, the results are satisfactory; this is due to the fact that sulfas act on the merozoites, preventing the development of gametes that are the most harmful for cattle (Rodríguez-Vivas et al. 1996).
The advantages of using this type of chemical control is swiftness and dosage, as shown in the studies described below. In a study, by Mundt et al. (2005), carried out in Germany with cattle naturally infected with *E. bovis* and *E. zuernii*, it was found that treatment with toltrazuril (15 mg/kg b.w.) orally, applied in a single dose, controlled coccidiosis in calves housed in different conditions.
In another study, Mundt et al. (2007) evaluated the efficacy of toltrazuril (15 mg/kg b.w.) and diclazuril (1 mg/kg b.w.), in a single oral dose, observing that there was a greater efficacy of toltrazuril compared to diclazuril, since the animals treated with toltrazuril had a lower number of excreted oocysts and the duration of the excretion period was also significantly reduced compared to animals treated with diclazuril and the control group.
The disadvantages of the use of chemical products previously described for the control of coccidiosis in ruminants is the resistance to these products due to the use and abuse of these molecules (Stephan et al. 1997); Another very important factor is the residuality of these chemical products in the soil (Cruz et al. 2004) and damage to beneficial organisms (beetles and mites), which has been described in the use of antiparasitic agents, mainly anthelmintics (Quintero-elena et al., 2022).
In general, other recommendations to control coccidiosis infections are to carry out proper management, avoid mixing animals of different ages and thus control the infection in calves;

on the other hand, in the farms the feeders and drinkers must be placed on a base to avoid fecal contamination; During grazing, calves should be prevented from consuming dirty and stagnant water and, finally, overcrowding of animals in pens should be avoided.

Sustainable Alternatives to Control the Coccidiosis in Ruminants

Livestock provides the main source of protein for the human population throughout the world and is the most widespread form of land use worldwide and particularly in Mexico, for this reason this activity has great importance in several aspects: 1) economic, 2) social and 3) environmental (SIAP 2022). It should be noted that, in particular, Veracruz is one of the states in Mexico where livestock is one of the main economic activities, ranking first nationally in meat production and sixth in milk production. However, this apparent success of livestock activity throughout the State has had a great environmental impact and its expansion represents a complex ecological problem mainly due to the use of chemical products for different pests (Barrera-Bassols et al. 1993).

Derived from the frequent and indiscriminate use of chemical products for the control of coccidiosis in ruminants, sustainable alternatives with the environment have been sought. Among these alternatives are the use of ethno-veterinary medicine, plants with medicinal properties such as *Artemisia annua* (secondary metabolites), and the tannins obtained from “Quebracho” against *Eimeria* spp. and gastrointestinal nematodes in small ruminants. The clinical signs caused by coccidiosis decreased in animals that consumed the former plant and improved body condition and weight compared to animals that were not fed this plant (Acharya et al. 2018).

Another type of sustainable alternative is the use of edible mushrooms (*Lentinula edodes* and *Ganoderma lucidum*), secondary metabolites such as fatty acids (linoleic acid), antioxidants, an example is the secondary metabolite “Curcumin”, present in the medicinal plant *Curcuma longa*, could reduce the severity of an infection of the upper and middle part of the small intestine caused by *E. acervulina* and *E. maxima* pathogens) (Quiroz-Castañeda and Dantán-González 2015).

Another type of control is essential oils, particularly used for formulations or diets to control coccidiosis (Quiroz-Castañeda and Dantán-González 2015).

Conclusions and Perspectives

The objective of this chapter was to present updated information on the epidemiology, transmission mechanisms, immunological aspects, diagnosis and conventional control of coccidiosis in ruminants. As a perspective, the use of sustainable coccidiosis control measures that do not damage the environment must be considered.

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