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Original Research

Dynamics of Sporulation of Caprine Eimerian Species under Variable Temperature and Humidity in Semi-arid Region of India

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Abstract

The present investigation was carried out to understand the sporulation dynamics of prevalent nine Eimeria oocysts viz. E. alijevi, E. apsheronica, E. arloingi, E. caprina, E. christenseni, E. hirci, E. jolchijevi, E. ninakohlyakimovae and E. caprovina seen at Central Institute for Research on Goats, Makhdoom, Mathura was studied. Fresh faeces samples were collected from three different goat breed farms (Jamunapari, Jakhrana and Barbari) and examined for the presence of Eimerian oocysts. Eimerian oocysts were collected and kept under ordinary laboratory sporulation conditions in 2.5% potassium dichromate at different ambient temperature and humidity for sporulation. The proportion of sporulated oocysts was determined microscopically every 8-12 h. At the average temperature and relative humidity of 11.8℃ & 82.19, 16.9℃ & 64.73 and 30.5℃ & 48.33, the sporulation time was observed as 3-6 days, 2-5 days and 1-4 days, respectively. But 50% of oocysts completed sporulation at average temperature and humidity of 32.42 ℃ & 22.88, respectively.

Keywords: Caprine coccidiosis, CIRG, Sporulation Time, Temperature & Relative Humidity

Introduction

Caprine coccidiosis, an intestinal disease of goats, is caused by protozoan parasite of genus Eimeria, subfamily Eimeriinae, phylum Apicomplexa (Foreyt, 1990). The disease is distributed worldwide and is considered an important health hazard in goat production system. It is of significant economic importance because of losses caused by clinical disease and its sub clinical infection (Lefevre *et al.,* 2010). Clinical coccidiosis is caused by stress from overcrowding, dirty and/or wet pens, and unclean water. Finding of a few oocysts in the diarrhoea of lamb or kids does not necessarily justify the presence of clinical coccidiosis. It is always adviceable to depend on necropsy findings than faecal examination (Satish *et al.,* 2019). It is mainly suspected when there is diarrhoea, poor body growth, weight loss and abdominal pain (Verma *et al.,* 2016). The severity and pathogenesis of the disease is Eimerian species dependent and affected by host response to infection. Of nine *Eimeria* spp. affecting goats worldwide, two i.e., *E. arloingi,* and *E. ninakohlyakimovae* were considered to be the most pathogenic species (Razavi and Hassanv and, 2006; Verma *et al.,* 2017). It is often difficult to identify individual species of coccidia due to their similarity and overlapping in size and shape (Hendrix, 1998). Life cycle of *Eimeria* spp. consists of several asexual generations (merogony) followed by sexual generation ending in the development of oocysts which are passed in the faeces (Soulsby, 1982; Baker, 1997). The oocysts undergo a period of development (sporulation) after being passed and develop to infective stage. During sporulation four sporocysts, each containing two sporozoites is formed within the oocyst. The time range required for sporulation to an infective stage is specific feature of each species of coccidian and is used as a characteristic in identification. Sporulation of the oocyst depends mainly on three basic factors; temperature, humidity, and access to oxygen (Kheysin, 1972). After sporulation, the oocysts are very resistant to environmental conditions and ordinary disinfectants. Extremely dry weather and direct sunlight are the only environmental factors that are detrimental to sporulated oocysts. Keeping above points in consideration, the study was to conduct record the dynamics of sporulation of *Eimeria* oocysts viz. *E. alijevi, E. apsheronica, E. arloingi, E. caprina, E. christenseni, E. hirci, E. jolchijevi, E. ninakohlyakimovae* and *E. caprovina* seen at CIRG indifferent room temperatures and relative humidity.

Materials and Methods

Sample Collection

The study was conducted in three breeds of animals maintained in semi-intensive management system at the Central Institute for Research on Goats (CIRG), Makhdoom, Mathura, India. Faecal samples were collected directly from rectum of the host into labelled polythene bags and were stored at 4˚C until further examination. Meteorological data was collected from Livestock farm (Meteorological dept.) of CIRG in the months of December 2015 (Average temperature 11.8ºC and average RH 82.19), January (Average temperature 16.9ºC and average RH 64.73), February (Average temperature 30.5˚C and average RH 48.33) and April 2016 (Average temperature 32.42 ˚C and average RH 22.88).

Sample Processing and Oocyst Isolation

Each faecal sample was examined for the presence or absence of coccidian oocysts by a floatation technique using saturated salt solution. Coccidian oocysts per gram (OPG) of faeces were quantified using a modified McMaster technique. Oocysts rich positive faecal samples were diluted with distilled water and sieved to remove the large faecal debris. The washed faecal samples were then centrifuged at 3000 rpm for 10 minutes with saturated salt solution and oocysts were collected from centrifuge tube removing top layer gently. Collected oocysts were washed with water through centrifugation followed by transfer to a shallow petri dish with 2.5% solution of potassium dichromate. The proportion of sporulated oocysts was determined microscopically every 8-12 h until sporulation process completed.

Morphological Identification

To examine and identify oocysts, the sample collected and spread out in Petri dish was shaken and mixed well. A portion of it was put onto a glass slide through a pipette and covered by a cover slip. The sample was examined under 400X magnifications (10X ocular and 40X objective). To identify the species, the criteria of size and morphological characteristics (shape, colour, presence or absence of micropyle and its cap, presence or absence of residual, polar and stiedae bodies) of the oocysts along with sporulation time was followed (Pellérd, 1974; Levine,

1985; Soulsby, 1982). Approximately 20-30 sporulated oocysts from each species, tentatively identified were taken up for micrometry to assist identification of various Eimeria species (Norton, 1986).

Results and Discussion

In the present study, the sporulation time varied with various Eimeria species viz. *E. alijevi, E. apsheronica, E. arloingi, E. caprina, E. christenseni, E. hirci, E. jolchijevi, E. ninakohlyakimovae* and *E. caprovina* (Fig. 1) in different room temperatures and relative humidity.

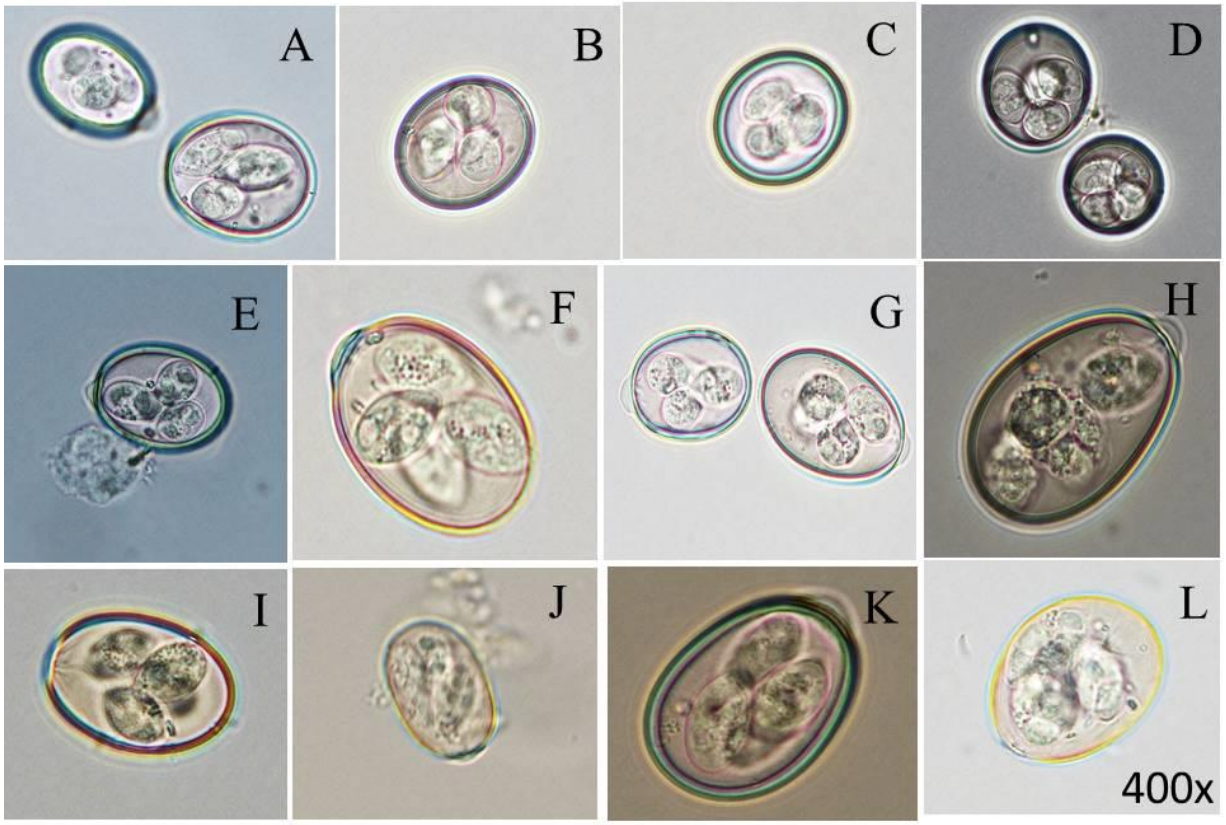

Figure 1: Oocysts of *Eimeria* species of goats as identified microscopically (400X) after sporulation, **A.** *Eimeria hirci* & *Eimeria ninakohlyakimovae,* **B.** *Eimeria ninakohlyakimovae,* **C.** *Eimeria alijevi,* **D.** *Eimeria ninakohlyakimovae & Eimeria alijevi,* **E.** *Eimeria hirci,* **F.** *Eimeria arloingi,* **G.** *Eimeria arloingi & Eimeria christenseni,* **H.** *Eimeria christenseni,* **I.** *Eimeria caprovina,* **J.** *Eimeria caprina* **K.** *Eimeria jolchijevi,* **L.** *Eimeria apsheronica*

Such studies in available literature were scanty. However, a similar type of investigation was reported in poultry by Rao *et al.* (2015). The number of sporulated oocysts and course of sporulation are important factors determine the level of challenge and thus influencing the epidemiology of Eimeria species. In the present investigation, sporulation time was delayed (4-6 days) when the average temperature and relative humidity was 11.8˚C - 16.9˚C & 64.73 - 82.19, respectively (Table 1). The finding supports Faizal *et al.* (2001) and Rupesh *et al.* (2018) who reported that the prevalence of coccidiosis in winter season is low as compared to hot and humid season. Soulsby (1982) also reported that the low temperature during winter is unfavorable for sporulation of oocysts. Lower temperatures delay sporulation. Once sporulated, some species of ovine coccidia can remain viable for up to a year at 4°C (Foreyt, 1990). Moreover, due to very cold weather, animals continue to remain under sheds where concentration of sporulated oocysts increases and heavy doses of infection are available for severe diseases (Bhatia, 2000). In the present investigation, sporulation of oocyst is fast (1-4 days) when average temperature and relative humidity was 30.5˚C & 48.33, respectively. When average temperature and humidity was 32.42 ˚C & 22.88, respectively only 50% of oocysts to complete sporulation process and rest remained unchanged till 12 days or showed vacuoles formation.

Table 1: Average sporulation time of caprine *Eimerian* species under variable temperature and humidity

Name of Species	Average Temperature = 11.8˚C	Average Temperature = 16.9˚C	Average Temperature = 30.5˚C	Average Temperature =32.42 ˚C
	Average RH=82.19	Average RH=64.73	Average RH =48.33	Average RH =22.88
E.alijevi	3-4	3-5	1-2	3-12
E.apsheronica	5-6	3-5	1-2	2-12
E.arloingi	5-6	2-3	1-2	2-12
E.caprina	5-6	4-5	1-2	2-12
E. christenseni	4-5	2-4	2-3	2-12
E.hirci	5-6	3-4	2-4	3-12
E.jolchijevi	5-6	3-4	2-4	2-12
E.ninakohlyakimovae	4-5	2-4	1-3	2-12
E.caprovina	5-6	2-4	2-3	3-12

Normally sporulation time of caprine coccidia species ranged from 1 to 5 days (Verma *et al.,* 2017), but in our study was 1 to 12 days depending on the ambient temperature and humidity. Our finding however was in contrast to optimum temperature for the sporulation of most *Eimeria* spp. oocysts of sheep and goats seen as 28-31°C (Foreyt, 1990). As more sporulated oocysts are resistant to heat and desiccation and at 0-5°C oocysts may remain viable for up to 10 months in faecal sediments and moist pellets. Unsporulated oocysts are more susceptible to extreme changes in climatic conditions than sporulated oocysts. Oocysts can withstand freezing at -5°C to-8°C for several months (Schineider, 1992). Sunlight and low oxygen tension are detrimental to the oocyst (Soulsby,1982). The climatic conditions of the humid tropics are favorable for the survival and development of coccidian throughout the year (Kusiluka and Kambarage, 1996). Oxygen is necessary for sporulation. Suboptimal concentrations of oxygen could be a limiting factor in the moist samples, for example, due to microbial activity. Microbial activity could also affect sporulation through liberation of ammonia in concentrations that may be lethal to unsporulated and sporulated oocysts (Williams, 1995). However, this factor was not limiting in the drier samples, because sporulation reached the same level (close to 100%) as oocysts kept in potassium dichromate.

Conclusion

Based on this study concluded that too hot & dry and cold weather conditions delay the sporulation time and average temperature 30.5˚C & RH 48.33 is the ideal condition for rapid sporulation.

Conflict of Interests

There is no conflict of interest.

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