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## **RESEARCH ARTICLE**

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# Parasitological and pathological findings of coccidiosis in an experimental infection caused by Eimeria ahsata in lambs

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#### ABSTRACT

This study was conducted to investigate the pathogenesis process of *E. ahsata* and its morphological, pathological, and distribution of lesions in the involved tissues during 42 days of infection. Twelve lambs were randomly divided into two groups including the control and the infected groups after confirmation of their health. The animals in the experiment group were orally infected with  $1 \times 10^5$  sporulated oocysts. From 7 days after inoculation (DAI), the feces were sampled and oocysts per gram of feces (OPG) were individually examined for each lamb. . At 7, 14, 21, 28, 35, and 42 DAI, one lamb from each group was necropsied and the abomasum, small and large intestine, mesenteric lymph nodes, spleens, and livers were grossly investigated. From 21 to 42 DAI, mild to severe clinical lesions such as congestion and edema were seen on the mucosal surface of the small intestine associated with white and small foci about 1-2 mm, especially jejunum and ileum. From 7 DAI to the end of the study various stages of the parasite life cycle, infiltration of inflammatory cells, epithelial hyperplasia of villi, and destruction of villi epithelium were seen. The results showed that E. ahsata was pathogenic in lambs and the macro and microscopic lesions were mostly seen in the jejunum.

Eimeria ahsata; Sheep; Pathology; OPG

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## Abbreviations

OPG: oocysts per gram DAI: days after inoculation FS: fecal score

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DPI: days post inoculation

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## Introduction

occidiosis is one of the most common parasitic diseases, caused by the genus Eimeria spp. in the alimentary system of sheep in the world [1,2]. The disease is more common in lambs aged 4-6 months [3]. The rearing stressor conditions such as weaning, transportation or transfer to a new pen, malnutrition, overcrowded population, and unsuitable weather play an important role in the incidence of the disease in sheep and goats [4]. The genus Eimeria causes the death of a large number of host intestinal cells and enterocytes leading to reduced absorbance of the critical electrolytes and nutrients [5]. The most common clinical signs of the disease are diarrhea, weight loss, anemia, rough hair coat, and weakness [5]. In addition, the disease reduces the production of meat and milk products and increases the mortality rate. The mechanism and grading of tissue damage depend on Eimeria species, the number of oocysts ingested, stress condition, age, physical condition, genetic sensitivity, and host immune system. Due to the sensitivity of young animals, the clinical form of the disease is reported at this age [6]. In the small ruminant, the induced hyperplasia by coccidia results in the thickness of the intestinal wall leading to poor absorbance of nutrients, diarrhea, and dehydration [4]. The mild to moderate histopathologic changes can be associated with the thickness of intestinal mucosa as well as the formation of the plague or the nodules with 1-2 mm in diameters in size [4]. In some Eimeria species, large schizonts are considerably seen. The most common clinical lesions of coccidiosis in young sheep and goats are non-pedunculated whitish nodules on the intestinal mucosa. These plaques are adhered to each other in advanced infection [4]. To date, 12 intestinal and 1 abomasal Eimeria species including E. crandallis, E. bakuensis, E. weybridgensis, E. ovinoidalis, E. intricate, E. gilruthi, E. pallid, E. faurei, E.parva, E. marsica, E.granulose, E. bakuensis, and E. ahsata have been identified in sheep [3].

Among the above species, *E. ovinoidalis*, *E. ahsata*, *E, crandallis*, and *E. bakuensis* are considered serious pathogens [3]. Regarding high prevalence of *E. ahsata* in sheep of different areas of Iran [7,8,9], this experimental study was conducted to study the pathogenesis of this parasite and to evaluate the morpho-pathology and distribution of lesions in lambs.

## Results

## **Clinical signs**

There were no clinical signs till 20 DAI in all animals. Diarrhea was the first clinical sign that was observed in two lambs at 21 DAI, which led to anorexia, weakness, dehydration, mucosal paleness and weight

Ahmadi Saleh Baberi et al., IJVST 2021; Vol.13, No.2 DOI:10.22067/ijvst.2021.71247.1057 loss. The fecal samples consistency of these lambs were semiliquid (Fecal score=2) at 21 DAI and watery diarrhea (fecal score= 3) at 35 DAI (Table 1). No clinical signs were seen in other lambs in infected and control groups and fecal samples consistency were normal till the end of experiment.

## **OPG** rate

The level of oocysts per gram of feces (OPG) of each lamb after the pre-patent period that was varying from 7 to 42 DAI are shown in Table 1. Two lambs with diarrhea had high OPG.

#### Body weight

Based on the results of the present study, the lambs in the case group had less weight gain than the control group and in the studied model, a significant difference was observed between the two groups (p < 0.05) (Table. 2).

#### Histopathological findings

**Gross lesions.** There was no considerable gross lesion in the sacrificed lambs at 7 and 14 DAI. Mild to severe lesions were seen in the jejunum and with less intensity in the ileum at 21 DAI. The congestion and edematous state of small intestine mucosa, especially, in the jejunum and ileum were identified. The small intestinal mucosal thickness associated with congestion and white nodules with 1-2 mm in diameter were detected on the internal surface of mucosal jejunum and ileum at 28 DAI. In addition to white nodules were creased on the small intestinal mucosal in particular jejunum at day 35 of infection (Figure 1).

At 42 DAI, the advance and diffuse glandular lesions associated with mucosal thickness and creasing from serosal surface of jejunum with less intensity in the ileum were noted (Figure 2). There was no gross lesion in the abomasum, liver, and spleen of all animals. The enlargement of mesenteric lymph nodes was the commonly detected lesion on all infected animals at 21, 28, 35, and 42 DAI. No gross lesions were observed in the gastrointestinal tract in lambs of the control group from the beginning to the end of the study.

**Microscopic lesions.** At 7 DAI, vascular congestion of mucosal and submucosal surfaces associated with different stages of the parasite life cycle including micro and macrogametes, and schizonts were seen in the jejunum and ileum. The epithelial and lymph tissues hyperplasia, infiltration of inflammatory cells such as eosinophilic cells in the lamina properia and villous tip as well as denuded villous tip and hyperplasia were seen in the ileum and jejunum.

At 14 DAI, the various stages of the parasite life

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#### Table 1.

The changes of OPG and fecal score in each lamb of infected group during experimental infection with *E. ahsata* at a dose of  $1 \times 10^5$ 

Lamb ·	7 DAI		14 DAI		21 DAI		28 DAI		35 DAI		42 DAI	
	OPG	FS	OPG	FS	OPG	FS	OPG	FS	OPG	FS	OPG	FS
1	0	1	-		-		-		-		-	
2	0	1	1200	1	-		-		-		-	
3	0	1	1000	1	38,000	2	-		-		-	
4	0	1	800	1	15,000	1	8400	1	-		-	
5	0	1	1000	1	33,000	2	30,000	3	45,000	3	-	
6	0	1	1000	1	10,000	1	6400	1	8000	1	3000	1

OPG: Oocyst per gram

DAI: Day(s) after infection

FS: Fecal score

cycle were seen in more parts of the small intestine. The vascular congestion of mucosal and submucosal, epithelial and lymph tissues hyperplasia, infiltration of inflammatory cells, and eosinophilic infiltration in the lamina properia and villous tip as well as denuded villous tip and hyperplasia was seen in the small intestine. The second-generation schizont and merozoites were detected within villi epithelial cells of the small intestine. Other stages of parasite life cycle such as progamonts, the developed micro and macrogametes, and a few oocysts were also detected within villi epithelial and crypts of duodenum, jejunum, and ileum. Most lesions were observed in the jejunum. A few first-generation schizonts were seen in the livers, spleens, and mesenteric lymph nodes.

#### Table 2.

Comparison of the body weight (Kg) of lambs in the infected and control groups

Weight (Kg)							
DPI	Control group	Test group					
0	$23.2\pm0.87$	23.60 ± 1.2					
7	$23.3\pm0.62$	$23.7\pm1.1$					
14	$23.2\pm0.53$	$22.32\pm0.93$					
21	$24.65 \pm 0.85^*$	21.62 ± 1.13*					
28	25.7 ± 1.04 *	21.66 ± 1.09*					
35	$27.2 \pm 1.30$	-					
42	$27.3 \pm 1.32$	-					

\*p < 0.05 was accepted as statistically significant.

DPI: days post inoculation

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At 21 DAI, microscopic intestinal lesions were widely detected. Infiltration of lymphocytes and eosinophils in lamina properia with less intensity in small intestine submucosa. The various forms of the parasite including gamonts and a large number of oocysts were seen in different parts of the small intestine in particular the jejunum (Figure 3). The infiltration of lymphocytes was also seen in the ileum. The round micro containing a large number of basophilic and clear nuclei as well as the round macro-gametocytes containing a large number of eosinophilic granules were seen. There was no lesion in the cecum, colon, liver, spleen, and mesenteric lymph nodes.

At 35 DAI, The microscopic lesions were widely detected in the ileum and jejunum and some cases in the colon. The losses of epithelial surface, infiltration of eosinophils, micro and macrogametes and oocysts associated with lymphatic hyperplasia, mucosal thickness resulting from papillary hyperplasia, and infiltration of inflammatory cells especially eosinophils in the lamia properia were seen (Figure 4). Microscopically, nodular hyperplasia and noted white pulp were seen in the spleen. The liver and mesenteric lymph nodes were reported as normal and hyperplasic particularly in the cortex, respectively.

At 42 DAI, congested livers, spleen, and mesenteric lymph nodes were reported. In the jejunum, losses of the integrity of the lieberkühn gland, infiltration of inflammatory cells, micro and macrogametes, and the developed oocysts were seen. In the ileum, the presence of oocysts, villous epithelial hyperplasia, losses of integrity, infiltration of eosinophils, plasma cells, macrophages, micro, and macrogametes was seen (Figure 5). There was no lesion in the abomasum, rectum, and colon.

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**Figure 1.** The whitish nodules (arrows) on mucosal surface of the jejunum at 35 DAI



**Figure 2.** Cerebriform or gyrate pattern and depressions on the serosal surface of jejunum (arrows) at 42 DAI.



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# Figure 3

Histopatholoical section of jejunum at 21 DAI. There are a large number of gamonts (hollow arrows), and oocysts (soild arrows) within the epithelial tissue of intestinal glands. H&E, ×400

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#### Figure 4

Histopathological section of jejunum at 35 DAI. The presence of oocysts (arrows) associated with infiltration of eosinophils and other inflammatory cells, creasing of villi. H&E, ×400



#### Figure 5.

Histology of the jejunum at 42 DAI with the presence of oocysts, infiltration of eosinophils and other inflammatory cells, associated with partial epithelial hyperplasia and hyperemia and hemorrhage (arrow). H&E, ×100

# Discussion

Eimeria ahsata has been known as the most common Eimeria species in the sheep in Iran, Spain, and

China [8,15,5]. In the present study, the pathogenicity E. ahsata as one of the common species in lambswas experimentally investigated. The first detection of oocysts was at 14 DAI in fecal samples of the infected group which are consistent with the prepatent period of E. ahsata about 12 to 18 days in an experimental study [16]. Clinical signs appeared with diarrhea, anorexia, dehydration, and weakness in two infected lambs at 21 DAI. Few studies have been performed on the pathogenicity of E. ahsata. Smith et al

#### (1960)

showed that oral occultation with 1×105 E. ahsata oocysts caused diarrhea, loss of appetite, and listlessness at 15-16 DAI and death in some lambs at 23-32DAI[17]. Mahart and Sherrick (1965) showed the low pathogenicity of E. ahsata in feedlot lambs[18]. Cathpole et al (1976) compared the pathogenesis of four Eimeria species in lambs for 4 weeks. They reported no clinical signs in the lambs when E. ahsata oocysts were given 10 to 1000 oocysts per day in a week, whereas E. ovinoidalis caused diarrhea in lambs. The difference in clinical signs severity in experimental studies may be related to infective dose, age, sheep breed, infective dose, and concomitant in-

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Ahmadi Saleh Baberi et al., IJVST 2021; Vol.13, No.2 DOI:10.22067/ijvst.2021.71247.1057 fections[1,19]. The OPG of diarrheic lambs was higher than non-diarrheic lambs, indicating a positive relationship between oocyst excretion rate and diarrhea. Many experimental and field studies have demonstrated a positive correlation between the total OPG of Eimeria and diarrhea in dairy cattle [13,20,21].

In the present study, the white to gray nodules and pinpoint to large nodules on the mucosal surface of the small intestine in particular jejunum and some parts of ileum were noted from at 21 DAI to the end of the study. Nodular lesions in the intestinal mucosa indicate the accumulation of different stages of the parasite that induced enterocyte hyperplasia in the intestinal wall[1]. The morphology and distribution

pattern of them throughout the intestine depends on the infected Eimeria species [3]. Cerebriform or gyrate patterns and depressions were seen on the serosal surface of the jejunum. This gross lesion was reported in ovine and caprine coccidiosis which resulted in the projection of proliferative nodules in intestinal mucosa toward the serosa intestine [3,22,23]. The nodule formation and thickening of the intestinal wall can cause a reduction in food absorption, diarrhea, and dehydration. emaciation, serous atrophy of fat [3].

Histopathological examination showed that E.ahsata mainly invaded the jejunum and the ileum. The main lesions were mild to severe hyperplasia epithelial cells with the presence of intracellular developmental stages of E.ahsata with infiltration of lymphocytes and

eosinophils in lamina properia. It seems that the presence of intracellular stages of the coccidia especially pro gamont is caused by hyperplastic mucosa in lambs [12]. Recent in vitro findings, have shown that crypt

cell hyperplasia is initiated by T-cell activation[24]. Intracellular parasites would represent an appropriate stimulus to responsive lymphocytes. An experimental study showed an increased intraepithelial lymphocytes population due to activation of T cells in the distal jejunum and ileum of lambs 13 days after infection with coccidian[25]. The infiltration of eosinophils in the small intestinal mucosa may be associated.

The obtained clinical and histopathological findingsindicate the pathogenicity of E. ahsata in the lambs. The lesions caused by E. ahsata were mostly seen in the jejunum. The gross lesions were congestion and white nodules on the internal surface of the mucosal jejunum and ileum. The presence of various stages of the parasite life cycle within the villi and crypts associated with infiltration of inflammatory cells in particular eosinophils and lymphocytes were seen histologically. It seems that the gametogenic stage consisting gametocytes and oocysts had a major role in the destruction of villi and crypts. with the release of histamine from damaged intestinal cells in parasitic infection. Other studies have been reported an ileoileal intussusception associated with proliferative [26]. The mild to severe hyperplasia in the crypt and villi of the small and large intestine in naturally occurring coccidiosis in sheep [26]. In addition,

necrosis, denuding of villi and intestinal gland

epithelium, congestion, infiltration of inflammatory cells associated with various stages of Eimeria such as micro and macrogamete and oocysts in the small intestinal mucosa has been also reported in infected lambs [27]. For comparison, similar microscopic lesions such as hyperplasia of epithelial cells of villi and crypts of jejunum and ileum, remarkable infiltration of lymphocytes and eosinophils have been reported in kids that experimentally infected by E. arloingi [22].

It seems that the tissue damage intensity depends on the Eimeria species, the number of the inoculated oocysts, host immune system, age genetic, nutrition, and stress [28,29].

The gain weight in the present study was significantly decreased in the infected group compared to the control group. The loss of body weight associated with clinical coccidiosis is mainly due to loss of nutrients as a result of parasite-induced mucosal lesions and, to a lesser degree, to alterations of intestinal digestion and absorption of nutrients [30]. Subclinical coccidiosis may also lead to reduced growth, uneven *lamb size, and a higher food conversion ratio* [31].

# **Materials & Methods**

## Isolation of E. ahsata

Before the start of the experiment, The fecal samples of 70 refereed lambs with diarrhea from the School of Veterinary Medicine were examined by the Mac-Master method [10]. A portion of each positive sample (3 gram) was mixed in 42 ml of phosphate buffer solution (PBS) and filtered through the sieve (Azman co. Iran) to omit the large particles. The filtered suspension was centrifuged at 2000 rpm for 5 min and the sediment was mixed with 2.5% (w/v) aqueous potassium dichromate solution (1:5) in Petri dishes and kept in a climate chamber. It was aerated continuously for twenty days at 27 °C. The rate of sporulated oocysts was determined by microscopic examination when more than 90% of oocysts were sporulated, and they were stored at 4°C until used. The frequency of the different Eimeria species with special regard to the pathogenic species was determined based on the morphological characteristics of the oocysts and related to the OPG counts[10,11]. Fifty-three samples were positive for Eimeria spp. The E.ahsata was the most prevalent (79%) species and other species included E. ovinoidalis (1%), E. bakuensis (1%), E. granulosa (2%), E. crandalis (7%), E. faurei (18%), and E. intricata (25%) less prevalent in the fecal samples of diarrheic sheep. The fecal samples containing 95 -100 % E.ahsata and more than 500 OPG were chosen for the experiment [11]. E.ahsata oocyst was ovoid with non-round polar cap, yellowish-brown, no residual body, and large steady body with sporocyst residuum,  $33.4 \times 22.6 \ \mu m$ 

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#### Figure 6.

A. Sporulated E. ahsata oocysts, ×100. B. Sporulated E. ahsata oocyst, ×400

#### (Figure 6).

#### **Experimental examination**

Twelve female lambs (Ovis aires), 2 months old were obtained from a non-infected herd raised under hygienic conditions. The lambs were transferred to the Research Center in the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. After clinical examination and confirmation of their health, the lambs were placed in individual pens in a protective environment for three weeks, to adapt to the diet and new environment. Lambs were fed daily with a standard diet consisting of alfalfa hay and concentrate during the study period. In addition, the fecal samples of lambs were examined three times a week to ensure not to have coccidia. Thereafter, the coccidia-free lambs were randomly divided into two equal infected and control groups. Before inoculation of the lambs, the oocytes suspension was washed with PBS solution by repeated centrifugation at 2000 rpm for 5 min until removal of potassium dichromate. Finally, the volume of the sediment was increased to 300 ml by adding distilled water, and the number of oocysts was calculated for each ml of suspension by Mac- Master methods. A single inoculum of an aqueous suspension  $1 \times 10^5$ sporulated oocysts (50 mL) was given to each lamb with a stomach tube [12]. The lambs of the control group received distilled water (50 mL per animal). During the study, clinical signs and parasitological findings including anemia, diarrhea, body condition, and OPG of each animal were evaluated. The fecal sample of each lamb was collected directly from the rectum in the morning at 7, 14, 21, 28, 35, and 42 DAI. The score of consistency of feces was assessed as follows: Normal to pasty (1), semiliquid to liquid (2), watery (3), hemorrhagic, and/or with tissue (4) [13]. In addition, the number of oocysts per gram (OPG) was counted using the Mac-Master method. Body weights were assessed by weighting each lamb on a scale of kilograms at 7, 14, 21, 28, 35, and 42 DAI before euthanasia.

## Necropsy and histopathology

At 7, 14, 21, 28, 35, and 42 DAI, a lamb from each group randomly was euthanized by intravenous sodium pentobarbitone solution and underwent necropsy for clinical evaluation of elementary system[14]. For microscopic examination, the tissue samples from the duodenum, jejunum, ileum, cecum, colon, abomasum, mesenteric lymph nodes, liver, and spleen were harvested and fixed in 10 % formalin buffer (Merck, Germany). The samples from small and large intestines were chosen with 10 cm intervals from the beginning of the duodenum to the end of the colon for microscopic examination. The fixed samples were embedded in paraffin, sectioned at 5 $\mu$ m, and routinely stained using hematoxylin and eosin (H&E).

## Statistical analysis

The SPSS software, version 22 (SPSS Inc., Chicago, USA) was used for data analysis. The *student's t*-test was used for investigating the effects of sampling time on the O.P.G and weight in two groups. *p*-values less than 0.05 were considered significant.

#### Ethics approval and consent to participate

The experiment on animals in the present study was approved by the Ethics Committee of Ferdowsi University of Mashhad (Approval ID: IR.UM.REC.1399.072).

# **Authors' Contributions**

NASB: Methodology, Software, Formal analysis, investigation, Writing-Original draft preparation. IK: Supervision, Methodology, investigation, Resources, Writing- Reviewing and Editing. HN: Validation. HAA: Validation, Resources. GR: Supervision, Conceptualization, Visualization, Resources, Writing- Reviewing and Editing.

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# **Competing Interests**

The authors declare that they have no competing interests

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