Vet Res Commun (2012) 36:47–55
DOI 10.1007/s11259-011-9511-9

ORIGINAL ARTICLE

Experimental caprine coccidiosis caused by *Eimeria arloingi*: Morphopathologic and electron microscopic studies

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Accepted: 11 November 2011 / Published online: 30 November 2011

Abstract The progressive morphohistopathologic changes, distribution pattern of lesions and ultrastructural characteristics in *Eimeria arloingi* infection were investigated in experimentally infected kids. The 18 newborn animals allocated to 3 equal groups. Two of groups, A, B were inoculated with a single dose of 1×10^3 and1×10^5 sporulated oocysts of *E. arloingi*, respectively. At 7, 14, 21, 28, 35, and 42 days postinoculation (DPI), 1 kid from each group was necropsied for pathologic and ultrastructural studies. Progressive lesions were present at 21, 28, 35 and 42 DPI in the jejunum, ileum, cecum with fewer in the duodenum and proximal colon. The oocysts shedding begin between 16 to 18 DPI. Grossly, minimal changes were observed at 21 DPI as few whitish plaques or nodules and advanced lesions at 42 DPI as pseudoadenomatous pattern in the mucosa and a cerebriform pattern on the serosal surface of jejunum and ileum. Early histopathologic lesions due to schizogony phase were including presence of intracytoplasmic developmental stages of the parasite such as trophozoites, immature to mature schizonts and mild infiltration of inflammatory cells. In late lesions due to various stages of gametogony, the histological pattern was mainly remarkable hyperplasia of the villi and crypts epithelial cells, eventually developed into papillary projections of reactive epithelium. The mesenteric lymph nodes showed a few numbers of large schizonts in the cortical lacteals. This study showed *E. arloingi* as a highly pathogenic species for kids, the incubation period was 16–18 days and the main target organ was jejunum with characteristic morphohistopathologic lesions.

Keywords Experimental infection · *Eimeria arloingi* · Morphopathologic lesions · Coccidiosis · Kids

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Introduction

Coccidiosis is one of the most important parasitic diseases of sheep and goats worldwide (Cox 1998; Foreyt 1990; Valentine et al. 2007). This disease leads to economic loss from both clinical and subclinical or chronic infection, including mortality, low growth performance, decrease in productivity and treatment costs (Abo-Shehada and Abo-Farieha 2003; Jalila et al. 1998).

More than 15 *Eimeria* species were identified in goats but 4 of them including *E. ninakohlyakimovae*, *E. arloingi*, *E. christenseni* and *E. caprina* were considered to be the most pathogenic species or major concerns (Jalila et al. 1998; Koudela and Bokova 1998; Razavi and Hassanvand 2007; Sayin et al. 1980). Coccidial infection in goats resulting from complex interactions between parasites and host with many factors influencing the severity of disease. The development of lesions is considerably dependent on the species involved. *E. ninakohlyakimovae* and, *E. arloingi* were the predominant species in many countries including Netherlands (Borgsteede and Dercksen 1996), Czech Republic (Koudela and Bokova 1998), Malaysia (Jalila et al. 1998), Poland (Balicka-Ramisz 1999), South Africa (Harper and Penzhorn 1999), Sri Lanka (Faizal and Rajapakse 2001), Iraq (Al-Amery and Hasso 2002), Jordan

(Abo-Shehada and Abo-Farieha 2003), Turkey (Gul 2007), Iran (Razavi and Hassanvand 2007), Kenya (Githigia et al. 1992), Zimbabwe (Chhabra and Pandey 1991) and Tanzania (Kimbita et al. 2009).

In recent years, high mortality of goat kids (2 weeks to 6 months old) due to coccidiosis has been identified as one of the major constraints of goat production in Fars province of Iran and prevalence of different *Eimeria* species and pathologic lesions of naturally occurring disease have been described (Khodakaram-Tafti and Mansourian 2008; Razavi and Hassanvand 2007). Fecal sampling of affected kids showed *E. arloingi* as most prevalent species in this area similar to some other countries (Razavi and Hassanvand 2007).

To the best of our knowledge, there has been no experimental study of the histopathologic changes, distribution and, chronology of the development of the lesions of *E. arloingi* infection in goats kids. The present experiment was therefore designed to study the chronologically morphopathologic lesions and the ultrastructural characteristics of coccidiosis in kids experimentally infected by *E. arloingi*.

Materials and methods

Preparation of *E. arloingi* oocysts

A pure strain of *E. arloingi* was obtained from Department of Parasitology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. The oocysts were stored at 4°C in 2.5% potassium dichromate (K2Cr2O7) solution for about 3 weeks before their use in the experiment. Before inoculation, the suspension was freed of potassium dichromate solution by series washing and centrifuging at 2000 RPM for 5 min. The sediment mixed with 200 ml distilled water and the number of oocysts was calculated for each ml of suspension.

Experimental animals and design

Eighteen Iranian crossbred kids from a herd in a non infected area were separated from their dams immediately after birth, fed with a cow's milk replacer and were reared artificially under coccidia-free conditions in individual cages kept in closed rooms with restricted access.

12At 15 days of age, the kids were divided into 3 equal groups. The kids of groups A and B were infected orally with 1×10^3 and 1×10^5 sporulated oocysts of *E. arloingi* per each animal, respectively. The kids in group C obtained distilled water and remained uninfected as control.

Necropsy and histopathologic examination

One kid in each group were euthanized and necropsied at each of 7, 14, 21, 28, 35 and 42 DPI. At necropsy, gross changes were noted carefully and wet smears or mucosal scrapings from mucosa of different parts of small and large intestines were prepared in view of existence of oocysts and other stages of the parasite. Appropriate tissue samples were taken from abomasum, small and large intestines, liver, spleen, pancreas and mesenteric lymph nodes. The sections of intestines were taken at 30 cm intervals from the pylorus to ileocecal valve including duodenum, jejunum, ileum, cecum, upper colon, middle colon, lower colon and rectum. All samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin for light microscopic examination.

Electron microscopy

For ultrastructural study, samples of the affected small intestines with dimensions about 1 mm were fixed in 4% cold glutaraldehyde solution, buffered and rinsed in 0.2 M sodium cacodylate, , postfixed in 1% osmium tetroxide. After further rinsing, the tissues were progressively dehydrated in graded acetone solutions, cleared in propylene oxide and embedded in epoxy resin. At least three Semithin sections (1 μm in thickness) from each sample were cut by ultramicrotome, stained with toluidine blue and were examined under a light microscope to confirm the preparation orientation. Ultrathin sections (60- to 90-nm) were cut from selected areas, mounted on copper grids, and double stained with uranyl acetate and lead citrate, then examined under a transmission electron microscope (Philips CM10, Philips, Eindhoven, Netherlands) and screened for the presence of developmental stages of the parasite.

Results

Clinical signs

No remarkable clinical signs were seen at 7 and 14 DPI. The beginning of the signs in group B was earlier and more severe than in group A. The advanced clinical signs of the infected kids had no prominent difference with the used inoculated doses but the beginning and the severity of symptoms were dose dependent. The change in the feces appearance was coincided with the first appearance of oocysts that was between 16 to 18 DPI in groups B and A. All infected kids continued to shed oocysts in the faeces during the experiment after prepatent period. In groups A

and B, diarrhea was started from 20 DPI, and 18 DPI, respectively. Clinical signs were from mild to severe degrees of depression, dehydration, paleness of conjunctiva and, listlessness and also semi-liquid to liquid diarrhea. During the period of diarrhea, the affected kids rapidly showed marked dehydration, emaciation, listlessness and diarrhea apparently without mucus or blood. Uninoculated kids in group C remained clinically normal and shed no oocysts during the experiment.

Gross lesions

No remarkable gross pathologic lesions were seen at 7 and 14 DPI in the intestinal mucosa except mild multifocal congestion or hyperemia in the distal small intestine, but progressive mild to advanced lesions were present at 21, 28, 35 and 42 DPI in the jejunum, ileum, cecum with fewer in the duodenum and proximal colon. At 21 DPI, in group A, necropsy revealed edema and redness of the mucosa of small intestines especially in the jejunum and ileum. In both groups but more prominent in group B, minimal to moderate changes were observed as thickening of the jejunal mucosa associated with a few scattered small whitish foci in the jejunum and ileum (Fig. 1).

At 28 DPI, in group A, white to grey, slightly raised pinpoint foci were observed from the proximal part of jejunum onwards to the ileum. In group B, thickening of the mucosa and presence of scattered whitish pin-head size (1–2 mm in diameter) nodules were observed mostly on the mucosa of the jejunum and ileum (Fig. 2).

At 35 and 42 DPI, the affected kids in groups A and B showed progressive thickening and folding or corrugating of the affected intestinal mucosa associated with numerous well-raised whitish nodules (Fig. 3). The lesions were multifocal to coalescent thickening of the mucosa of the duodenum, jejunum and ileum with numerous large whitish non-pedunculated nodules. At 42 DPI, in group B, in addition to advanced widespread lesions characterized by pseudoadenomatosis thickening of the mucosa, a cerebriform or gyrate pattern clearly were visible grossly on the serosal surface of the jejunum and with lesser degrees ileum (Fig. 4).

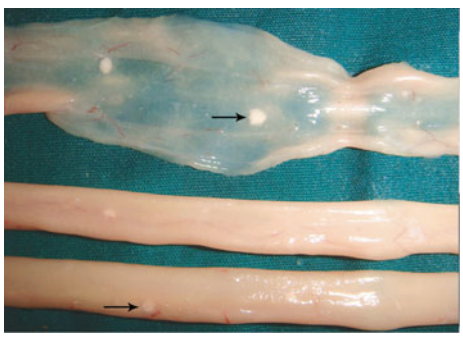

Fig. 1 Jejunum and ileum. Experimentally infected kid, 21 days postinoculation (DPI) . A few scattered small whitish foci in the jejunum and ileum (arrows)

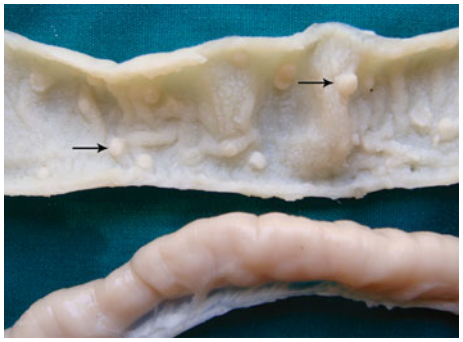

Fig. 2 Jejunum. Infected kid, 28 DPI (Formalin fixed). Thickening of the jejunal mucosa and presence of scattered whitish pin-head size (1–2 mm in diameter) nodules (arrows)

In all animals, no remarkable gross lesions were seen in the abomasum, distal colon, rectum, liver, spleen and pancreas. The mesenteric lymph nodes of the most of infected kids were observed enlarged and edematous.

Histopathologic findings

At 7 DPI, the main histopathologic findings in both groups A and B were hyperemia, edema and focal to multifocal involvement of the duodenum, jejunum and ileum. These small foci were scattered distant from each other and mostly a small part of a villous or multiple villi from bottom to tip were affected (Fig. 5). Adjacent villi were normal and unaffected. Endogenous stages of *E. arloingi* including trophozoites, first generation schizonts, numerous undeveloped macrogametocytes with red granules and a few undeveloped microgametocytes were observed in the epithelial cells of crypts (Fig. 5). A few numbers of trophozoites with a basophilic nucleus were seen within

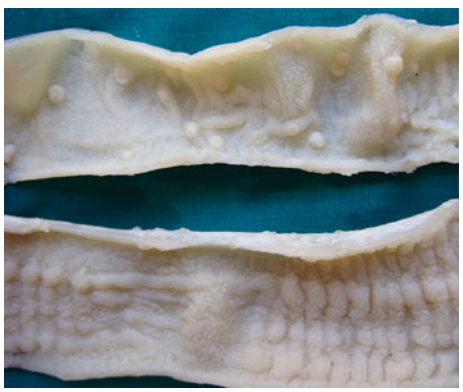

Fig. 3 Jejunum. Infected kid, 35 DPI (Formalin fixed). Affected mucosa of jejunum is thickened due to papillary hyperplasia of epithelium and pseudoadenomatous changes

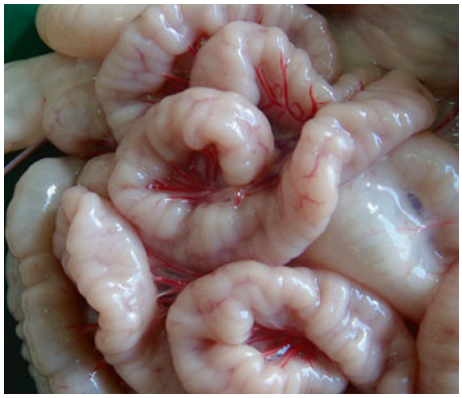

Fig. 4 Jejunum. Infected kid, 42 DPI. Cerebriform or gyrate pattern and depressions on the serosal surface of jejunum

the cytoplasm of enterocytes. The mature first generation schizonts, full of merozoites with regular or irregular arrangement, were observed in the lacteals of villi in the proximal part of the jejunum (Fig. 6). The affected enterocytes were enlarged and had flattened marginated nuclei. There was no any detectable necrosis in the villi or crypts epithelial cells. There were only mild inflammatory reaction with infiltration and aggregation of eosinophils and lymphocytes in the lamina propria around the lesion and on tip of villous. There were no remarkable hyperplasia of epithelial cells of affected crypts and villi. The most lesions were observed in the proximal part of jejunum.

At 14 DPI, the lesions in the kid of group A were milder than group B with some differences. The affected parts were more prominent than 7 DPI but in contrast to group B, hyperplasia of epithelial cells of crypts and villi and also hyperplasia of lymphoid aggregations in the lamina propria were more remarkable in the group A. Second generation schizonts and merozoites were seen in the epithelial cells of affected villi and crypts of small intestine (Fig. 7). The schizonts had several randomly or peripherally arranged

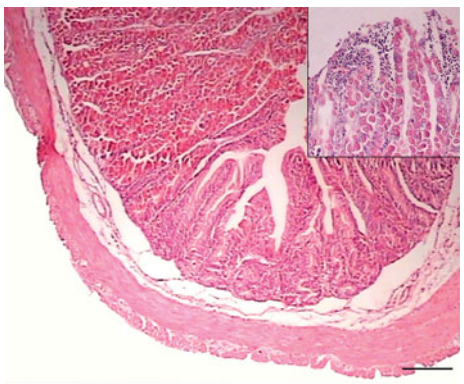

Fig. 5 Jejunum. Infected kid, 7 DPI. H/E. Mild inflammatory reaction in the lamina propria around the lesion and on tip of villous and endogenous stages of parasite in the epithelial cells of affected villi. Scale Bar=350 μm

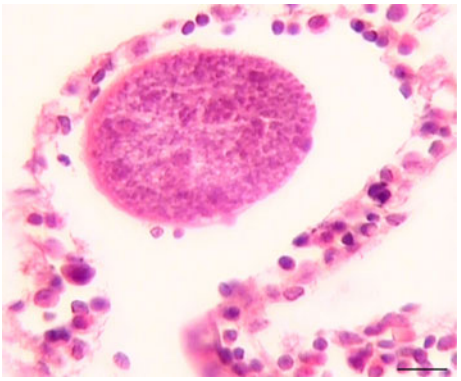

Fig. 6 Jejunum. Infected kid, 7 DPI. H/E. A large schizont containing numerous merozoites in the lacteal of a villus. Scale Bar=35 μm

nuclei. In the mature second generation schizonts, clusters of elongated or round shape of merozoites were seen in entrocytes. Also, other endogenous stages of the parasite including undifferentiated gamonts, undeveloped macrogametocytes and microgametocytes and also a few oocysts were present in the epithelial cells of crypts (Fig. 8) and associated villi of duodenum, jejunum, ileum and cecum. Mild, focal to multifocal hyperplasia of epithelial cells of crypts or villi either with or without developmental stages of *E. arloingi* were seen. The apparently normal epithelial cells of the crypts adjacent to affected crypts were hyperplastic as well. The most lesions were observed in the jejunum and ileum. A few numbers of large schizonts were seen in the lacteals of villi in the proximal part of the jejunum. The mean of fifty mature schizonts in lacteals measured 120×220 μm. Hyperemia of vessels, dilatation of lymphoid lacteals, edema and mild infiltration of lymphocytes and eosinophils (mostly in tips of affected villi) were observed in the lamia propria.

At 21 DPI, In comparison to group B, the microscopic lesions were milder in group A. Hyperemia , edema of lamina propria, dilated lacteals, lymphocytes and eosinophils infiltration in the lamina propria and less in the

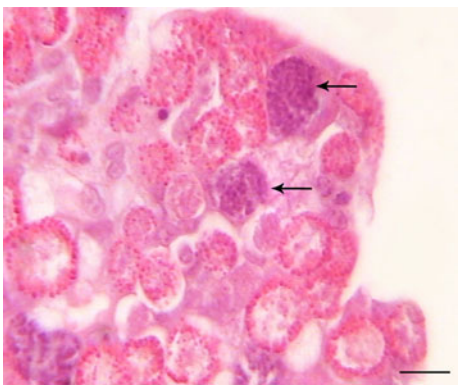

Fig. 7 Jejunum. Infected kid, 14 DPI. H/E. Second generation schizonts containing several merozoites within the cytoplasm of enterocytes (arrows). Scale Bar=35 μm

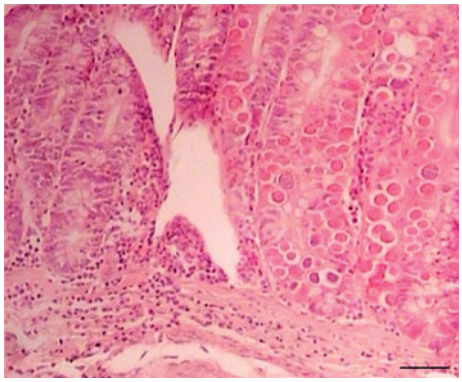

Fig. 8 Duodenum. Infected kid, 14 DPI. H/E. Undifferentiated gamonts, undeveloped macro- and microgametocytes in the epithelial cells of crypts and associated villi. Scale Bar=150 μm

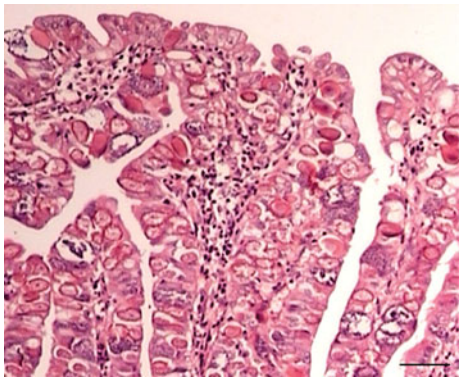

Fig. 9 Jejunum. Infected kid, 28 DPI. H/E. Remarkable hyperplasia of jejunal mucosa due to presence of intracellular developmental stages of the parasites including schizonts, progamonts, macro- and microgametocytes, immature and mature oocysts. Scale Bar=150 μm

submucosa, moderate hyperplasia of lymphoid aggregations, hyperplasia of epithelial cells lining of deep crypts and presence of different developmental stages of *E. arloingi* including schizonts, progamonts, macrogametocytes, microgametocytes, immature and mature oocysts, were seen in the cytoplasm of affected epithelial cells. Many epithelial cells contained macrogametes and microgametes in various stages of gametogony. The macrogametes were round with a central nucleus and brightly eosinophilic peripheral granules. The microgametes were round with peripheral brightly basophilic granules. Within the lumen of crypts there were a few oocysts, gametocytes and cellular debris. Moderate, multifocal hyperplasia of epithelial cells of crypts and villi either with or without developmental stages of *E. arloingi* were seen. Hyperplasia was more prominent in the epithelial cells of the crypts and villi which had extensive involvement with developmental stages of the parasite. The mean of fifty second generation schizons measured 30×22 μm and contained 10–22 merozoites. The lesions were not found in the colon and rectum.

At 28 DPI, the histopathologic lesions in a kid from group A were including moderate to severe hyperplasia of epithelial cells lining of the affected crypts in the duodenum to cecum. The most prominent lesions were seen in jejunum with remarkable thickening of the wall due to congestion and edema of submucosa, hyperplasia of epithelial cells of crypts, remarkable infiltration of lymphocytes and eosinophils as multifocal around the lesion and also presence of intracellular developmental stages of the parasites including schizonts, progamonts, macrogametocytes, microgametocytes, immature and mature oocysts (Fig. 9), In group B, the microscopic lesions were similar to group A but to some extent were more severe. The affected villi and crypts were disorganized due to the developmental sexual stages of the parasite. A lot of schizonts, merozoites and especially progamonts were observed in the cytoplasm of hyperplastic epithelium of the crypts. Rarely, the affected crypts were seen dilated with cellular debris and numerous neutrophils associated with degeneration and necrosis of the epithelium. The lesions were not found in the colon and rectum. The mesenteric lymph node showed lymphoid hyperplasia in the cortex associated with large schizonts in the capsule and cortical lymphatic vessels.

At 35 and 42 DPI, the microscopic lesions in the groups A and B were prominent and diffuse in the jejunum, ileum and to some extent in the cecum. Remarkable thickening of the mucosa due to papillary hyperplasia of the villi, hyperplasia of cells lining the crypts, and aggregation of different inflammatory cells especially lymphocytes in the lamia propria and less in submucosa were observed. The crypts and villi were disorganized due to developmental stages of the parasite. In some sections, necrosis of affected villi and presence of necrotic epithelial cells and scattered developmental stages of the parasites were observed within the lumen of intestines. No developmental stages of the parasite were observed in the submucosa of affected intestines. The histopathological lesions in the duodenum and proximal colon were similar but milder than jejunum. The lesions were not found in the abomasums, distal colon, rectum, liver, spleen and pancreas.

The mesenteric lymph nodes showed marked lymphoid hyperplasia as secondary lymphoid follicles in the cortex associated with a few large schizonts in the lymphatic vessels (Fig. 10).

Ultrastructural studies

Ultrastructural characteristics of some developmental stages of *E. arloingi* in the epithelial cells of villi and crypts were observed. In the affected epithelial cells, early stages of schizigony accompanied by marked proliferation of endoplasmic reticulum and several granular dividing nuclei were diagnosed. The nuclei were enclosed by proliferated double membranes and distributed randomly, with no peripheral migration.

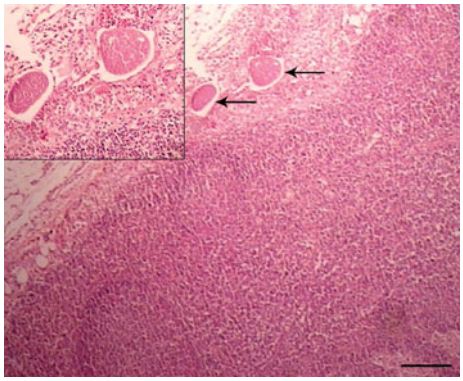

Fig. 10 Mesenteric lymph node. Infected kid, 42 DPI. H/E. Lymphoid hyperplasia as secondary lymphoid follicles in the cortex associated with a large schizont (arrows). Scale Bar=350 μm

Immature (Developing) macrogamete showed wall-forming bodies (WFB1 and WFB2), lipid and polysaccharide granules accumulations. In the immature macrogamete, the WFB2 were observed as a sponge-like texture and rough periphery. These structures were enclosed within the rough ER. In the mature macrogamete, WFB1 as some dense spherical granules with a smooth periphery were seen. The number and size of the WFB1 were increased in the fully mature macrogamete and were migrated to the periphery (Fig. 11).

In the immature oocyst, the early wall as an osmiophilic discontinuous layer due to fusion and degeranulation of marginated WFB1 were seen. This layer represents the outer layer of the mature oocyst wall. In the mature oocyst, in adition to the osmiophilic outer layer, the inner layer as a thinner electron lucent structure due to fusion of WFB2 was observed (Fig. 12).

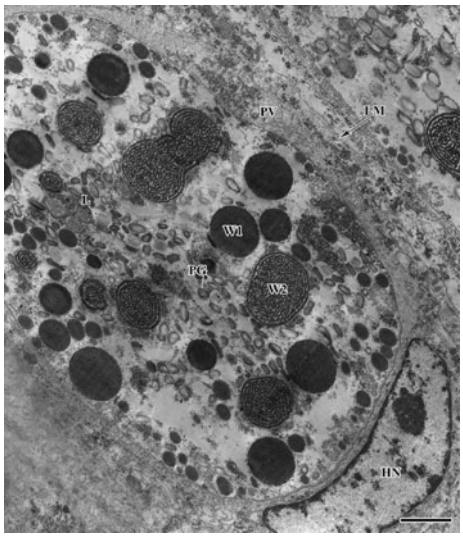

Fig. 11 Electron micrograph showing the ultrastructural appearances of the macrogametocytes. Sponge-like wall- forming bodies type 2 (W2) characterized by the presence of whorled cores enclosed by rough endoplasmic reticulum, some large peripherally located electron dense wall-forming bodies type 1 (W1), a large number of electron dense polysaccharide granules (PG), lipid droplets (L), limiting membrane (LM), parasitophorous vacuole (PV) and host nucleus (HN). Scale Bar=1500 nm

Discussion

In the present study, the chronological development lesions of *E. arloingi* infection in new born free coccidia goats kids, following inoculation of sporulated oocysts, confirms that *E. arloingi* is a highly pathogenic species for kids, the incubation period is at least 16 days, the main target organ is jeujenum and, the extent and severity of the lesions is dose dependent. In coccidiosis, the degree of tissue damage is dependent on the *Eimeria* species and infective dose of oocysts. Other factors including age, stress, genetic susceptibility, physical condition and the degree of immunity developed from previous low levels of infection are also important in pathogenesis (Dougschies and Najdrowski 2005; Jolley and Bardsley 2006; Jubb et al. 2007).

Most reported documents in caprine coccidiosis due to *E. arloingi* are parasitological studies that have shown a high prevalence of coccidial infections in many of countries. For the first time, *E. arloingi* was originally described from the goat by Marotel in 1905 under the name *Coccidium arloingi*. Then, Sayin et al. (1980) showed some evidences of the life cycle and pathogenesity of *E. arloingi* in 6 months Angora kids, but the morphohistopathological lesions and exact affected organs and tissues due to

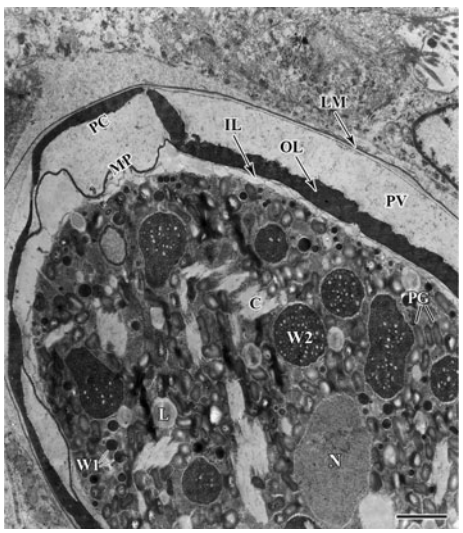

Fig. 12 Electron micrograph showing the longitudinal section of the oocyst. Nucleus (N), wall-forming bodies type 1 (W1), wall- forming bodies type 2 (W2), polysaccharide granules (PG), lipid droplets (L), canaliculi (C), parasitophorous vacuole (PV), inner layer of cyst wall (IL), outer layer of cyst wall (OL), limiting membrane (LM), Polar cap (PC), Micropyle (MP). Scale Bar=1500 nm

infection of this species still are not fully clear. For many years, researchers believed that the *E. arloingi* occurred in both sheep and goats. Later, the information on *E. arloingi* in sheep and goats reviewed and stated that this species in these two hosts are not the same and named *E. arloingi* in goats and *E. ovina* in sheep (Levine and Ivens 1970). The investigations failed to transmit these species to another host and concluded they are host specific (Sayin et al. 1980).

In the present study, the clinical signs and severity of infection were variable with the inoculating dose and DPI. In coccidian-induced kids, enterocyte hyperplasia and thickening of the intestinal wall caused malabsorption, emaciation, diarrhea, and dehydration. The early clinical signs of kids inoculated with 1×10^5 oocysts (group B) were more severe than group A. These findings were consistent with other reports (Dai et al. 2006; Sayin et al. 1980). The kids began oocyst sheding 16–18 days after inoculation and continued during the experiment. The prepatent period in this study was similar to which reported by Sayin et al. (1980).

Mostly, there have been parasitological studies on naturally occurred caprine coccidiosis in many countries mentioned before those have been found *eimerian* oocysts in the faeces. The reports and information about morphopathologic lesions of *E. arloingi* infection in kids are rare and are restricted to a brief description by Sayin et al. (1980). Previously, the life history, histopathology and pathogenic effects of *E. ninakohlyakimovae* were studied experimentally in goats (Dai et al. 1991, 2006). In present study, the most common gross lesion of *E. arloingi* in kids were consisted of white to grey, slightly to well-raised pinpoint foci to relatively large nodules on affected mucosa of the jejunum onwards to the ileum at 28–42 DPI. The distribution and severity of lesions were dose dependent, so that in low dose inoculation group, mild lesions including a few scattered whitish non-pedunculated plaques to nodules were observed in the mucosa of the jejunum and ileum. In comparison, high dose inoculation group showed multifocal to coalescent progressive thickening, folding or corrugating to pseudoadenomatosis of the intestinal mucosa associated with numerous well-raised whitish nodules in the jejunum and ileum. Also a cerebriform or gyrate pattern clearly was visible grossly from the serosal surface of the jejunum and ileum. Recently, for the first time, the term of cerebriform or gyrate pattern were used as a diagnostic gross lesion of advanced naturally occurring coccidiosis in kids and lambs (Khodakaram-Tafti and Mansourian 2008). On basis of present experiment, it seems *E. arloingi* is one of the most common causative agent of this lesion in kids. The term pseudoadenomatous has been used to describe the polypoid lesions and the oocyst patches or plaques in coccidiosis of small ruminants and may be the result of mitogenic stimuli from progamonts of the parasite (Jubb et al. 2007).

Necropsy and histopathologic examination as diagnostic tool is necessary in kids affected to early coccidiosis without remarkable gross lesions. In this experiment, early lesions due to pathogenic phase of schizogony were including the presence of intracytoplasmic developmental stages such as trophozoites, early and intermediate immature to mature schizonts. The mature first generation schizonts were full of numerous crescent or banana shaped merozoites. There was no any detectable necrosis or hyperplastic change in the villi or crypts epithelial cells. There were only mild inflammatory reaction with infiltration of a few eosinophils and lymphocytes in the lamina propria of tips of affected villi. In this stage, there were no remarkable hyperplasia of epithelial cells of affected crypts and villi. The most lesions were observed multifocally in the proximal part of jejunum.

In late lesions due to various stage of gametogony, the histological pattern was mainly remarkable hyperplasia of the epithelial cells with presence of intracellular developmental stages of the coccidia and infiltration of inflammatory cells. The apparently normal epithelial cells of the crypts adjacent to affected crypts were hyperplastic as well. Second generation schizonts and merozoites, and also gamonts, macrogametocytes, microgametocytes and oocysts were seen in the epithelial cells of affected villi and crypts. The most lesions were seen in the jejunum and ileum. Schizogony in intestinal epithelium induced necrosis and subsequent hyperplasia due to second generation schizonts and gamonts, eventually developed into papillary projections of reactive epithelium.

Cellular mechanisms of hyperplasia vary in details, depending on the cell affected and the cause. There are multiple controls as to whether or not a cell enters the replication cycle. In some circumstances hormones trigger cell replication, whereas in others growth factors, increase receptor for growth factors, and activation of cell signaling pathways may all have a role. In some circumstances, cytokines are important. Ultimately, transcription factors may influence the expression of a new cadre of gene leading to cell proliferation (Zachary and McGavin 2012).

Large (first-generation) schizonts, observed at 7 and 14 DPI in the villi lacteals of the middle small intestine, had a mean size of 220 μm×120 μm and thousands of merozoites. *E. ninakohlyakimovae* and *E. christenseni* in goats have giant schizonts up to 300 μm in diameter that develops in cells deep in the lamina propria of the terminal ileum and in the endothelium of the lacteals in villi of the middle small intestine, respectively (Jubb et al. 2007).

It has been reported that the second generation schizonts of *E. arloingi* are quite different from those of *E. ninakohlyakimovae* in sheep (Sayin et al. 1980). Sayin et al. (1980) observed small schizonts, 11 to 21 μm in diameter and contained 8–24 merozoites in the epithelial

cells of crypts and villi of the lower jejunum. The second generation schizonts in our kids were in the distal part of jejunum and contained about 8–22 merozoites. The presence of developmental stages of *E. arloingi* including mature schizonts, undifferentiated gamonts, developing macro- and microgametocytes and immature oocysts at 21 DPI was coincided with prepatent period. The structure and location of the sexual forms of parasite in our kids were in consistent with other report (Sayin et al. 1980). Although it is reported that oocysts and gametocytes may develop in submucosa and lymphoid aggregates of intestines in caprine coccidiosis (Jubb et al. 2007), it was not observed in our study. Infiltration of inflammatory cells especially lymphocytes and eosinophils were observed prominently in advanced lesions. Aggregations of macrophages and giant cells in the base of infected crypts in the centers of polyps have been reported as a part of immune response in caprine coccidiosis (Jubb et al. 2007). In present experiment, we found no such lesions even in advanced cases.

In the present study, large schizonts were observed in the lymphatic vessels of capsule and cortex of mesenteric lymph nodes. The presence of schizonts or other stages in lymph nodes are result from establishment of sporozoites or merozoites migrating from the lacteals into the lymphatic drainage in early infection (Dai et al. 2006; Jubb et al. 2007). Although, it has been reported various number of oocysts in bile and endogenous stages in liver and gallbladders of goats experimentally infected with *E. ninakohlyakimovae* (Dai et al. 1991), no similar lesions were found in the present study.

There have been no previous ultrastructural studies on *Eimeria* species in goats; whereas some researchers have been described structural characteristics of *Eimera* species in poultry (Abdel-Ghaffar 1990; An et al. 2001; Mouafo et al. 2000). Present experiment showed mature macrogametes of *E. arloingi* has two types of WFB1 and WFB2. It is reported that macrogametes of some *Sarcocystis* species and *E. truncate* had only one type of WFB (Entzeroth et al. 1981; Zaman and Colley 1975). Mehlhorn (2001) stated that the number of wall-forming bodies is a genus-dependent character. However, the reports about the types and formation of WFB are controversial in literature. Some of the reports regarded that the electron-denser WFB2 appeared earlier than WFB1 (An et al. 2001; Zhou et al. 2006). Some investigators believed that both of them appeared at the same time (Liu et al. 1993; Zhou et al. 2006). In our experiment, we believed that, in the early period of macrogametocyte development, the WFB2 appears first (immature) with a sponge-like consistency, then will be matured as a electron dense WFB1. About the function of WFBs, previous reports have revealed an equal contribution of both WFB1 and WFB2 in oocyst wall formation. In this relation, WFB1, which appeared after WFB 2, produce the outer layer, whereas WFB2 form the inner layer (An et al. 2001; Mouafo et al. 2002). However, on basis of our TEM observations, it seems that the role of WFB1 is more prominent in oocyst wall formation. About the wall of oocysts of *E. arloingi* in kids, we found two distinct layers; an outer electron-dense and an inner electron-lucent layer that is in agreement with other observations that reported in many other *Eimeria* species (Al-Hoot 1997; Mehlhorn 2001; Zhou et al. 2006).

Conclusion

In summary, our results showed some differences in the clinical signs, gross, and histopathologic lesions based on inoculating doses. The most common lesions were in the jejunum and ileum, grossly as nonpedunculated to polyp-like whitish nodules and microscopically, as proliferative enteritis with the presence of endogenous stages of *E. arloingi* in the hyperplastic enterocytes. The gross and histopathological lesions in group B were more severe than group A. Although the developmental stages of *E. arloingi* were observed in the duodenum and proximal part of the jejunum at 7 and 14 DPI, they localized in the distal part of the jejunum, ileum and cecum at 21, 28, 35 and 42 DPI. Our results showed *E. arloingi* as a highly pathogenic species for kids, the incubation period was 16–18 days, and the main target organ was jejunum with characteristic morphohistopathologic lesions. Various stages of schizogony and gametogony in intestinal epithelium induced necrosis and subsequent remarkable hyperplasia due to second generation of developmental stages of the parasite, eventually developed into papillary projections of reactive epithelium.

Acknowledgment We thank the authorities of Veterinary School, Shiraz University for their financial support and cooperation. We are grateful to Mr. L. Shirvani, Mr. G. Yousefi, Mr. H. Yazdanpour and Mr. A. safavi from the Departments of Pathobiology and Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, for their technical assistance.

References

Abdel-Ghaffar FA (1990) Electron microscope studies on *Eimeria arvicanthi*: macrogametogenesis and oocyst formation. Proc Zool Soc AR Egypt 18:334–343

Abo-Shehada MN, Abo-Farieha HA (2003) Prevalence of *Eimeria* species among goats in northern Jordan. Small Rum Res 49:109–113

Al-Amery MAY, Hasso SA (2002) Laboratory diagnosis of novel species of *Theileria hirci*, *Eimeria caprovina* and *Eimeria pallida* in goats in Iraq. Small Rum Res 44:163–166

Al-Hoot AA (1997) Ultrastructural study of micro- and macro-gametogenesis of *Eimeria mehlhorni* infecting the field rat

Arvicanthis niloticus niloticus in Egypt. J Egypt Ger Soc Zool 23 (D):93–119
An J, Wang M, Kong F, Yin P (2001) Ultrastructral studies on the macrogametogenesis and oocyst forming of *Eimeria tenella*. Chinese J Vet Sci 21:559–561
Balicka-Ramisz A (1999) Studies on coccidiosis in goats in Poland. Vet Parasitol 81:347–349
Borgsteede FHM, Dercksen D (1996) Coccidial and helminth infections in goats kept indoors in the Netherlands. Vet Parasitol 61:321–326
Chhabra RC, Pandey VS (1991) Coccidia of goats in Zimbabwe. Vet Parasitol 39:199–205
Cox FEG (1998) Control of coccidiosis: lessons from other sporozoa. Int J Parasitol 28(1):165–179
Dai YB, Lin MC, Zhang SX, Fu AQ (1991) Hepatic coccidiosis in the goat. Int J Parasitol 21:381–382
Dai YB, Liu XY, Liu M, Tao JP (2006) Pathogenic effects of the coccidium *Eimeria ninakohlyakimovae* in goats. Vet Res Commun 30:149–160
Dougschies A, Najdrowski M (2005) Eimeriosis in cattle: current understanding. J Vet Med 52:417–427
Entzeroth R, Scholtyseck E, Sezen IY (1981) Fine structural study of *Eimeria truncata* from domestic goose (Anser anser dom.). Z Parasitenkd 66:1–7
Faizal ACM, Rajapakse RPVJ (2001) Prevalence of coccidia and gastrointestinal nematode infections in crossbred goats in the dry areas of Sri Lanka. Small Rum Res 40:233–238
Foreyt WJ (1990) Coccidiosis and cryptosporidiosis in sheep and goats. Vet Clin North Am Food Anim Pract 6:655–669
Githigia SM, Munyua WK, Kanyari PW (1992) Prevalence of *Eimeria* species in goats from parts of Central Kenya. Bull Anim Prod Afr 40:283–285
Gul A (2007) The prevalence of *Eimeria* species in goats in Igdir. Turk J Vet Anim Sci 31:411–414
Harper CK, Penzhorn BL (1999) Occurrence and diversity of coccidia in indigenous, Saanen and crossbred goats in South Africa. Vet Parasitol 82:1–9
Jalila A, Dorny P, Sani R, Salim NB, Vercruysse J (1998) Coccidiosis infections of goats in Selangor, peninsular Malaysia. Vet Parasitol 74:165–172
Jolley WR, Bardsley KD (2006) Ruminant coccidiosis. Vet Clin Food Anim 22:613–621
Jubb KVF, Kennedy PC, Palmer N (2007) Pathology of domestic animals, Vol. 2, 5th edn. Academic Press Inc, pp 264–267
Khodakaram-Tafti A, Mansourian M (2008) Pathologic lesions of naturally occurring coccidiosis in sheep and goats. Comp Clin Pathol 17:87–91
Kimbita EN, Silayo RS, Mwega ED, Mtau AT, Mroso JB (2009) Studies on the *Eimeria* of Goats at Magadu Dairy Farm SUA, Morogoro, Tanzania. Trop Anim Health Prod 41:1263–1265
Koudela B, Bokova A (1998) Coccidiosis in goats in the Czech Republic. Vet Parasitol 76:261–267
Levine ND, Ivens V (1970) The coccidian parasite (protozoa, sporozoa) of ruminants. Illinois Biological Monograph 44, University of Illinois press, p 278
Liu Q, Kong F, Ying P (1993) Ultrastructural studies on the endogeneous II. The forming of Macrogamete and the wall of oocyst. Acta Zoologica Sinica 39:343–347
Mehlhorn H (2001) Encyclopedic reference of parasitology, 2nd edn. Springer, Berlin
Mouafo AN, Richard F, Entzeroth R (2000) Observation of sutures in the oocyst wall of *Eimeria tenalla* (Apicomplexa). Parasitol Res 86:1015–1017
Mouafo AN, Weck-Heimann A, Dubremetz JF, Entzeroth R (2002) Monoclonal antibodies specie for the two types of wall-forming bodies of *Eimeria tenella* macrogametes (Coccidia, Apicomplexa). Parasitol Res 88:217–224
Razavi SM, Hassanvand A (2007) A survey on prevalence of different *Eimeria* species in goats in Shiraz suburbs. J Fac Vet Med Univ Tehran 61:373–376
Sayin F, Dincer S, Milli U (1980) The life cycle and pathogenicity of *Eimeria arloingi* (Marotel, 1905) Martin, 1909, in Angora kids and an attempt at its transmission to lambs. Zentrabl Vet Med (B) 27:382–397
Valentine BA, Cebra CK, Taylor GH (2007) Fatal gastrointestinal parasitism in goats: 31 cases 2001–2006. J Am Vet Med Assoc 231:1098–1103
Zachary JF, McGavin MD (2012) Pathologic basis of veterinary disease, 5th edn. Elsevier Mosby, London, pp 28–29
Zaman V, Colley FC (1975) Light and electron microscopic observations of the life cycle of *Sarcocystis orientalis* sp. in the rat (Rattus norvegicus) and the Malaysian reticulated python (Python reticulates). Z Parasitenkd 47:169–185
Zhou K, Wang Y, Chen M et al (2006) *Eimeria tenella*: further studies on the development of the oocyst. Exp Parasitol 113:174–178