รายงานสั้น

EIMERIA ZUERNII INFECTION IN THAI NATIVE CALVES

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Abstract

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A 3-month-old, female calf in the Dontan district of Mukdaharn province, showed clinical signs of bloody diarrhoea over a period of 3 days and finally died on April 4, 2001. After necropsy, severe, diffuse, haemorrhagic caeco-colitis and proctitis were markedly evident. Histopathologically, various stages of coccidial development, including schizogony and gametogony, were found in the villous epithelium and the epithelial crypts. Infiltration of lymphocytes, plasma cells, neutrophils and macrophages into the lamina propria, as well as into the submucosa was observed. Another 6-month-old, female calf from a nearby area also showed bloody diarrhoea with mild anorexia and numerous oocysts of coccidia were detected in the faeces. The oocysts of both native calves were examined microscopically and were seen to be morphologically similar to oocysts of *Eimeria zuernii*.

Keywords : coccidiosis, *Eimeria zuernii*, calves, Thailand

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***ผู้รับผิดชอบบทความ**

บทคัดย่อ

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โรคบิดจากเชื้อ *Eimeria zuernii* ในลูกโคพื้นเมืองไทย

ลูกโคพื้นเมืองเพศเมีย อายุ 3 เดือน ที่ อ.ดอนตาล จ.มุกดาหาร มีอาการถ่ายเหลวมีเลือดปน แสดงอาการอยู่ 3 วันและตาย ในวันที่ 4 เมษายน 2544 ภายหลังการชันสูตรซากพบ การอักเสบรุนแรงที่ลำไส้ใหญ่ มีการหนาตัวของผนังลำไส้ มีเลือดออก เป็นจุดเล็กๆ กระจายอยู่ทั่วไปบนผิวเยื่อบุของ caecum colon และกระจายไปตลอดจนถึงส่วนปลายของทวารหนัก การตรวจทาง จุลพยาธิวิทยาพบการหนาตัวของชั้นเยื่อบุโดยพบระยะต่างๆของเชื้อบิด เช่น schizogony และ gametogony ในเซลล์เยื่อบุผิวของ ลำไส้ใหญ่เป็นจำนวนมาก เชื้อบิดได้แทรกตัวลึกลงไปจนถึงชั้นล่างของเยื่อบุ (intestinal crypt) โดยเจริญอยู่ในเซลล์เยื่อบุผิว (crypt epithelium) และยังตรวจพบโอโอซีต์ ในช่องของ crypt อีกด้วยนอกจากนี้ยังพบการเข้าแทรกของเซลล์อักเสบชนิดต่างๆ เช่น ลิมโฟไซต์ พลาสม่าเซลล์ นิวโทรฟิว และ มาโครฟาจ ในชั้นใต้เซลล์เยื่อบุผิว และในชั้นใต้เยื่อบุ นอกจากนี้ยังพบลูกโคป่วย เพศเมียอายุ 6 เดือน ในพื้นที่ใกล้เคียงแสดงอาการถ่ายเหลวมีเลือดปนและตรวจพบโอโอซีสต์ ของเชื้อบิดเป็นจำนวนมาก จากการ ตรวจและวัดขนาดของโอโอซีสต์ ที่ได้จากโคทั้งสองตัวด้วยกล้องจุลทรรศน์ พบว่ามีรูปร่างเหมือนกับโอโอซีสต์ ของเชื้อบิด *Eimeria zuernii*

คำสำคัญ : โรคบิด *Eimeria zuernii* ลูกโค ประเทศไทย

Introduction

Coccidiosis in cattle is characterized by diarrhoea or dysentery and usually occurs in calves less than 1 year of age. The disease can be caused by several species of *Eimeria: Eimeria bovis, E. zuernii, E. ellipsoidalis, and E. aburnensis* (Barker and Van Dreumel, 1985). Among these species, *E. zuernii and E. bovis* are highly pathogenic but their oocysts can sometimes be seen in the faeces of calves showing no clinical signs (Ernst et al., 1984; Hasbullah et al., 1990). Experimental infection by *E. zuernii* in calves shows haemorrhagic enteritis of the ileum, caecum and colon and the second generation of gametogony are highly pathogenic (Stockdale, 1977). Many studies from various countries have reported the prevalence of *E. zuernii*, both in domestic cattle (Hasbullah et al., 1990; Munyua and Ngotho, 1990; Svensson et al., 1993) and wildlife animals (Penzhorn et al., 1994). In Thailand, only one report on *Eimeria beilleyi* infection in buffaloes has been made (Upatum et al., 1989) and very little information about Eimeria infections in cattle can be found.

The present study is the first official report of clinical cases of *E. zuernii* infection in calves in Thailand, and provides information about the clinical signs, gross lesions and histopathological findings.

Materials and methods

Case histories

Case 1. A 3-month-old, 35-kg, female, native calf in the Dontan district of Mukdaharn province had clinical signs of anorexia and bloody diarrhoea for 3 days. There were 13 cattle on this farm, kept in the pen beside the owner's house. The calf was kept in the same pen with some variably aged cattle and they grazed together in the area shared by the community. The calf developed dysentery, anorexia and dehydration and died on April 4, 2001. The owner observed some signs of seizures and

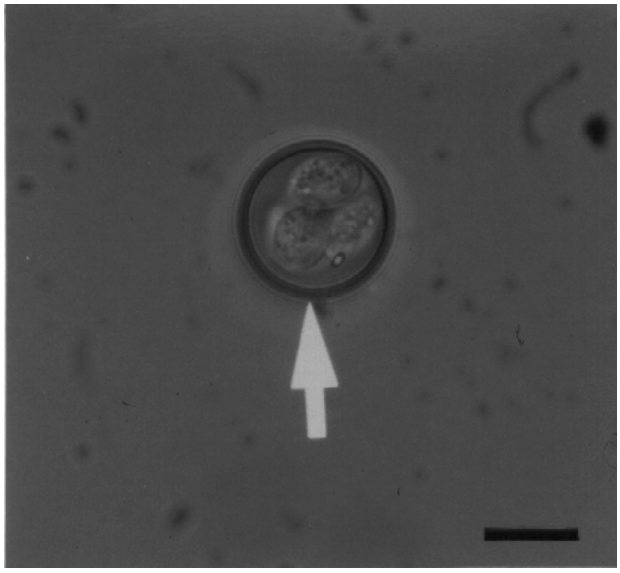

Figure 1. Sporulated oocyst of *E. zuernii* from the faeces of case 2 (bar = 10 μm).

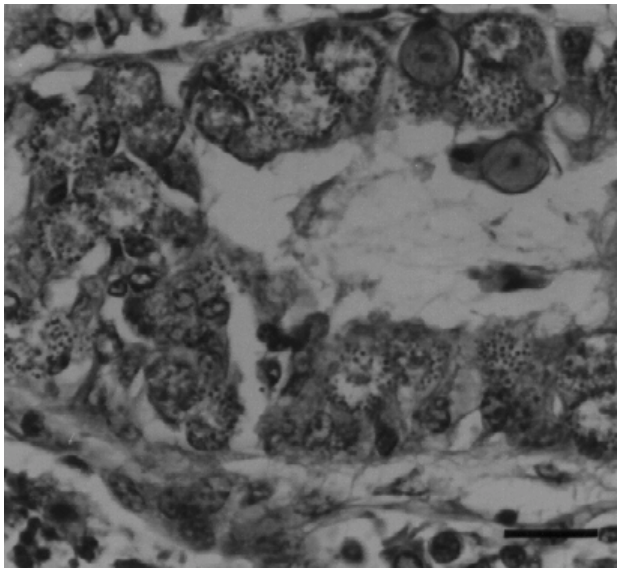

Figure 2. Section of colon, heavy infection of the glandular epithelium by macro and microgamonts with oocysts and debris cells in the lumen (bar = 25 μm).

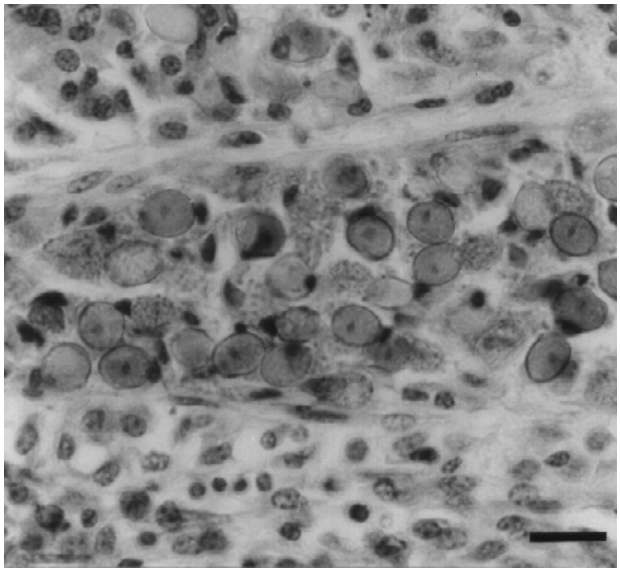

Figure 3. Complete destruction of the intestinal epithelium with many oocysts occluding the lumen (bar = 15 μm).

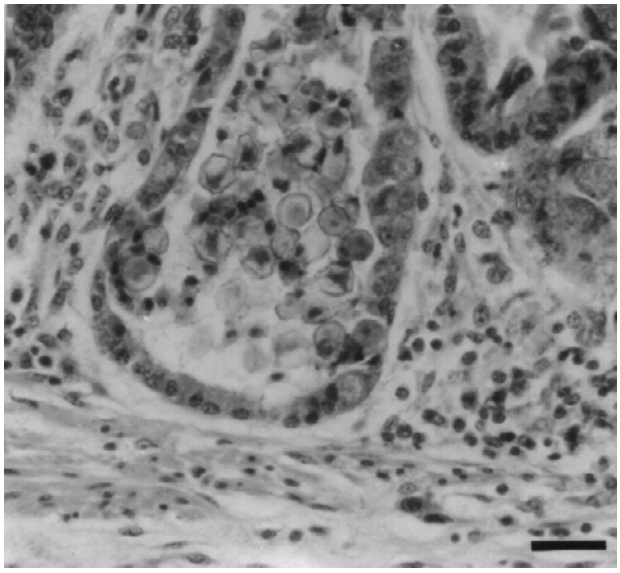

Figure 4. Numerous oocysts and debris cells in a dilated-cryptal lumen with flattening of the epithelium, mononuclear cells and eosinophils in the lamina propria (L), muscularis mucosae (M), (bar = 10 μm).

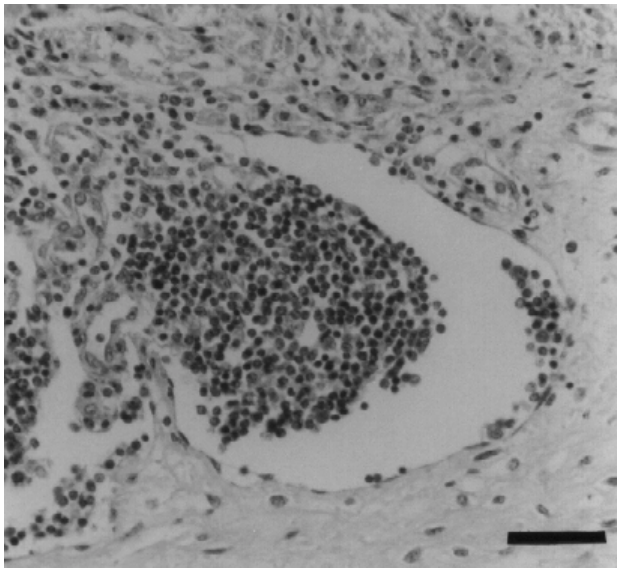

Figure 5. Lymphangitis and the accumulation of lymphocytes in the wall of a lymph vessel in the submucosa (bar = 10 μm).

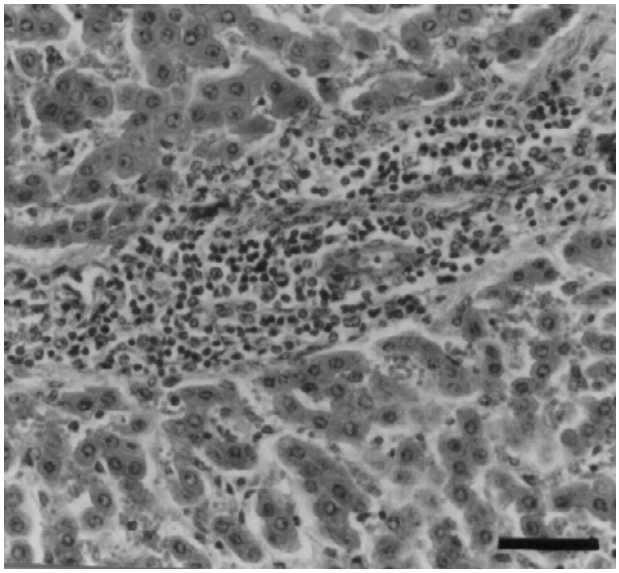

Figure 6. Infiltration of mononuclear cells (lymphocytes) in the portal area of the liver (bar = 10 μm).

circling movements. Necropsy was performed 4 hours after death. A fresh smear from mucosal scrapings of the large intestine was examined under a microscope. Brain, lung, heart, kidney, liver, spleen, mesenteric lymph nodes, ileum, colon and rectum were fixed in 10% neutral buffered formalin. The samples were conventionally processed and embedded in paraffin wax. Sections were made and stained with haematoxylin and eosin (H&E) for histopathological examination.

Case 2. Another 6-month-old, female calf from the nearby area also had bloody diarrhoea with anorexia. It was injected with 10 ml of trimethoprim-sulpha (Amphoprim, 200 mg/ml, Virbac, France) once daily for 2 consecutive days, and gradually improved. The faeces were collected and directly examined under the microscope.

Results and Discussion

Numerous oocysts were found in the smear of the intestinal scrape from case number 1 and from the faeces of case number 2. Those oocysts were round (Figure 1) and were morphologically similar to the oocysts of *Eimeria zuernii (E. zuernii)* (Pellerdy, 1974). The sizes of oocysts were 16.25 to 17.5 x 16.25 to 18.75 microns with a mean of 16.63 x 17.25 microns. The minimal sporulation time in 2.5% $K_2Cr_2O_7$ was less than 24 hrs. At necropsy, petechial to ecchymotic haemorrhage in the epicardium and thymus and mild, multifocal, ulcerative abomasitis were observed. The most prominent lesions were a severe, diffuse, petechial haemorrhage and thickening of the mucosa of all the caecum and colon, through to the terminal end of the rectum. Mild, diphtheritic typhlitis and colitis was also present but there were no gross lesions in the small intestine. The lesions were similar to those seen in experimental infections of *E. zuernii* in calves (Stockdale, 1977). In contrast to *E. bovis infection* (Friend and Stockdale, 1980) where severe lesions were also apparent in the ileum, the lesions in this case were confined to the large intestine, extending throughout the rectum. The findings of haemorrhagic typhlitis and colitis caused by coccidiosis must be differentiated from those seen in salmonellosis, malignant catarrhal fever, bovine viral diarrhoea and other diseases (cited by Blood and Radostits, 1989).

Histopathologically, the villous epithelium of the

ileum was completely absent and a mild haemorrhage was evident in the remainder of the lamina propria. Numerous inflammatory cells were present in the lamina propria without any evidence of coccidial infection. These changes might be the consequence of an initial infection by coccidia. Various stages of *E. zuernii* were observed but only in the mucosa of the colon and rectum, where gamonts and oocysts were seen in the deep mucosa. The lesions found in both parts of the large intestine were similar. In many places, villous epithelial cells were almost absent and the villi had become flattened. A thin diphtheritic membrane covering the top of the villi was occasionally seen. The membrane was composed of inflammatory, debris cells, fibrin and oocysts. These histopathological lesions were similar to those in experimental infections with *E. zuernii* (Stockdale, 1977), thus assisting our diagnosis. Developing gametogony stages of *E. zuernii*, including macrogamonts, microgamonts and oocysts, were prominent in the intestinal mucosa. The infiltrates of inflammatory cells in the lamina propria were dominated by lymphocytes and plasma cells, whereas neutrophils, eosinophils and macrophages were only occasionally observed. Multifocal haemorrhages were also commonly observed in the lamina propria.

In the middle layer of the mucosa, the epithelial cells, in some areas, were entirely infected with gametocytes (Figure 2). As the epithelial cells were disrupted, many oocysts were occluding the lumen of the gland (Figure 3). In experimental infections, schizogony appeared to cause little reaction in the small intestine but the gametogony stage, taking place about 18 to 20 days post infection, can cause severe pathological changes in the large intestine (Stockdale, 1977). Gametocytes were the main forms seen in the mucosa of this case, indicating that the damage to the mucosa was caused by the sexual stages of the coccidia. Such stages are highly pathogenic, leading to destruction of the epithelium and haemorrhage into the intestinal lumen. We also found a severe infection in the crypt epithelium, adjacent to the muscularis mucosae (Figure 4). Oocysts, with debris cells, were seen in some cryptal lumens. These findings have been previously called crypt abscesses (Stockdale, 1977). The intestinal submucosa was oedematous and infiltrated by lymphocytes, plasma cells, eosinophils and macrophages. Lymphangitis was occasionally seen in some areas of the submucosa (Figure 5). Inflammation of the submucosa has been described earlier in experimentally infected calves (Stockdale, 1977) and also occurs in infections by other species of Eimeria (Friend and Stockdale, 1980).

The hepatitis was characterised by infiltrates of lymphocytes, plasma cells and a few neutrophils, mainly in the portal area, as well as in the liver parenchyma (Figure 6). Infiltration of inflammatory cells into the liver may be the consequence of bacterial complications or an immunological response to antigens of coccidia. Sections of the brain showed severe congestion, with multiple small foci of haemorrhages, in the cerebrum and the cerebellum. Such lesions may be associated with the clinical signs of seizures in this calf. Nervous disorders, associated with coccidiosis in young cattle have been reported in Australia, Europe and America but their pathogenesis is not clearly understood (Isler et al., 1987[a] and Jubb, 1988). However, Isler et al. (1987[b]) proposed that neurotoxin may be involved in the pathogenesis of a nervous form of coccidiosis.

Generally, the management of native cattle in this region of Thailand, conforms to the backyard system and all cattle are confined in the same pen, as in our cases. It is known that adult cattle are carriers of coccidia and serve as a potential source of infection (cited by Blood and Radostits, 1989). Newly born calves may receive sporulated oocysts, from contaminated faeces or bedding, when temperature and moisture are optimum. From the history data, we found that diarrhoea of calves has occurred sporadically in this area and it is possible that coccidiosis caused by *E. zuernii* could account for such cases in this region. Further investigations are required to verify the true incidence of bovine coccidiosis in the area.

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