FIRST DETECTION OF CRYPTOSPORIDIUM SP. OOCYSTS
IN CALF FAECES BY FLOATATION METHOD

I. PAVLÁŠEK

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague

Dedicated to Prof. V. Dykt DSc on the occasion of his 70th birthday

Abstract. Oocysts of coccidia of the genus Cryptosporidium were detected for the first time in the faeces of naturally infected calves using centrifugation flotation method with saturated solution of ZnSO₄ and Bremer's flotation solution. Since the detection of oocysts in the faeces is very difficult, a new method has been prepared. The oocysts recovered from the faeces were sporulated, spherical or slightly elliptical, with a smooth, colourless and thin wall. In the fresh preparation after flotation the oocysts measured 5.4 x 4.6 µm on the average, whereas in the smears stained after Giemsa, their average size was 4.8 x 4.3 µm. A typical feature of the oocysts is a residual body appearing as a dark point at one pole of the oocyst when observed by optical microscope. The oocyst contains four sporozoites forming a C-shaped rim around the residual body. No sporozoites were recorded. The residual body stains blue and the nucleus dark red by Giemsa's method.

Coccidia of the genus Cryptosporidium have been still more reported from various species of both domestic and free-living animals. They were recorded even in man.

The genus Cryptosporidium was established by Tyzzer (1910) on the basis of the species Cryptosporidium muris found in stomach glands of laboratory mouse (Tyzzer 1907). A characteristic feature of this genus is the extracellular life cycle during which develops an oocyst with four sporozoites, without formation of the sporocytes. The genus includes five species.

The first case of Cryptosporidium infection in cattle (8-month-old heifer) was described by Panisiera et al. (1971) in the USA. Barker and Carbonell (1974) found coccidia of this genus in 2- and 3-week-old calves. Meuten et al. (1974) in a 14-day-old calf and Schmitz and Smith (1978) in 5-day-old calf. In Great Britain, coryptosporidiosis was first reported by Pearson and Logan (1978) in a 7-day-old bull. Pohlenz et al. (1978a, 1978b) demonstrated Cryptosporidium sp. in newborn calves in the USA and Canada. Nagy et al. (1979) recorded the first case of cryptosporidiosis in Hungary in two calves at the age of 13—14 days. Pavlášek (1981) found Cryptosporidium sp. in forcibly slaughtered calves in Czechoslovakia. Some authors, as Morin et al. (1978) and Snodgrass et al. (1980), studied the effect of cryptosporidial infections together with rotaviruses and coronaviruses in acute diarrhoea of newborn calves.

The oocysts of Cryptosporidium sp. have not yet been isolated from the faeces of infected calves and, except the record of oocysts of Cryptosporidium felis Ieki, 1979, in faeces of naturally and experimentally infected cats, not even in any other host.

This paper deals with the first finding of oocysts of coccidia of the genus Cryptosporidium in calf faeces by means of two flotation solutions.

MATERIAL AND METHODS

The faeces for parasitological examination were taken from the rectum of calves destined for a forced slaughter. After slaughter, samples were scraped from different parts of jejunum and ileum. The tissues around the scrapes were fixed in AAF (100 ml of 40% formol, 50 ml of glacial acetic acid, 850 ml of absolute alcohol).
Saturated solution of zinc sulphate of specific gravity 1.18 (sp. gr. 1.18) and Brez's floatation solution (Brez 1957) consisting of 3 portions of saturated solution of MgSO₄, 3 portions of saturated solution of Na₂SO₄, and 1 portion of tap water of specific gravity 1.3 were used as floatation solutions. The faeces were thin, watery and of yellow-brown colour.

**Faeces examination process**

1. About 2–3 ml of liquid faeces were transferred to a centrifuge tube by means of Pasteur pipette. The tube was filled with the floatation solution up to 5 mm below the margin, the mixture was pipetted. Around with a glass rod and centrifuged at 2 000 r.p.m. for 5 min. After centrifugation, a) 3 loops of float supernatant were taken from the surface membrane, put on a slide and covered with a cover glass, b) the floatation solution was added up to the margin of the tube, the cover glass was carefully placed on it for 15 min and then transferred to the slide. The same process was carried out with both floatation solutions.

2. The sediment was examined by the method of Iseki (1979).

3. 2–3 ml of faeces were fixed in methanol in the centrifuge tube for 10 min. Then the mixture was centrifuged at 2 000 r.p.m. for 3 min and the supernatant was carefully removed by the pipette without damaging the sediment. After addition of 4 ml of Giemsa stain in 1 : 9 dilution, the sediment was covered by a glass rod and stained for 20–45 min. The following process was the same as after fixation. Then 10 ml of the respective floatation solution were added to the sediment, stirred by the glass rod and again centrifuged at 2 000 r.p.m. for 5 min. The oocytes were removed from the surface membrane in the same manner as in point 1.

4. The material was smeared on a slide, fixed with methanol and stained after Giemsa for 10 min. Both fresh and permanent preparations were examined at the magnification of 800–1 000 x. The Cryptosporidium sp. was detected in our previous studies also by means of scanning electron microscopy. The tissus was critical point dried, coated with gold and examined in TESLA BS 300 microscope produced in Czechoslovakia.

**RESULTS**

Various developmental stages of Cryptosporidium sp. were found on the surface of calf ileum by both light microscope at histological examination (Plate I, Fig. 1) and scanning electron microscope. Three craters (Plate I, Fig. 2) seem to be the sites which remained after the schizonts or oocytes released into the intestinal lumen.

Coccidia of the genus Cryptosporidium were found by all mentioned methods in the faeces of naturally infected and forcibly slaughtered calves. The oocytes isolated from the faeces by means of floatation solutions are shown in Plate II, Fig. 3a. They are widely ellipsoidal to spherical, with a smooth, colourless and relatively thin wall, measuring 4.5–6.3 x 3.5–5.4 μm (average 5.4 x 4.6 μm). The values were obtained by measuring 100 oocytes at the oprological examination of faeces. The average index of the oocytes was 0.85. The size of oocytes in the stained solution was 3.5–6.0 x 3.5–5.0 μm (average 4.8 x 4x3 μm). The average index of these oocytes was 0.89.

The oocytes were without sporozoites and their characteristic feature was the residual body. They found it were 4 sporozoites arranged in form of C, inside which was a nucleus visible in fresh preparations. In some cases there was a great number of granules inside the sporozoites. Plate II, Fig. 3b shows the oocytes of Cryptosporidium sp. in phase contrast which facilitates the detection of these coccidia in the faeces.

The result of the detection of cryptosporidiosis in faeces by the methods of fixation, staining and floatation used by us is shown in Plate II, Fig. 4. The sporozoites are clearly visible in the undamaged oocytes.

The oocytes obtained after centrifugation from the surface membrane of the floatation solution, washed in water and fixed and stained after Giemsa are shown in Plate II, Fig. 5. The cytoplasm and residual body stained blue, the nucleus stained red.

**DISCUSSION**

The cryptosporidial infections have been lately reported in various species of hosts at histological examinations of jejum, ileum and colon tissues. Various forms of these coccidia, which are considered by some authors to be the sporozoites, are described on the basis of stained scrapes from intestinal mucosa. The presence of cryptosporidium was detected by both transmission and scanning electron microscopy. For example, Vetterling et al. (1971) described Cryptosporidium caviae in laboratory guinea pigs Cavia porcellus and stated that no oocytes were detected by any of the floatation and sedimentation methods. According to Meuten et al. (1974), there was no method for the diagnosis of cryptosporidiosis and further studies were necessary for the detection of developmental stages which could be found in the faeces of infected calves. Oocytes and other developmental stages of cryptosporidiosis were found by Baker and Carbonell (1974) at the examination of intestinal tissue in calves by electron microscopy. The authors wrote that it could not be ascertained whether the cysts getting into the contents of intestines are complete or whether they release sporozoites into the lumen and thus cause a superinfection. Pohleven et al. (1978a) found organisms measuring 2–4 μm in their faeces, flesch and calves suffering from cryptosporidiosis and regarded all of them as oocytes. Also Snodgrass et al. (1979) recorded similar organisms in calf faeces on the basis of our examinations we assume that the organisms described by the above authors are not oocytes, but most probably developmental stages of Cryptosporidium, which, in mass infections, can be released from the surface of intestine into the lumen and excreted with the host faeces.

Iseki (1979) has succeeded in demonstrating oocytes of Cryptosporidium felis in cat faeces using a saturated solution of ZnSO₄. The oocytes measured 5 x 4.5 μm.

As it is mentioned above, we managed to detect oocytes of Cryptosporidium in diarrheal faeces of examined calves by means of floatation solutions commonly used for the diagnosis in medical and veterinary parasitology. At coprological examination, the average size of the oocytes was 5.4 x 4.6 μm. They were somewhat smaller (4.8 x 4.3 μm) from the material stained with Giemsa's solution. Most probably the oocyst wall is contracted during the fixation with methanol.

The diagnosis of these coccidia at coprological examination is difficult due to the small size of the oocytes and to their transparency. Also the presence of various impurities floating together with the oocytes makes the focus very unclear during the microscopic observations, particularly in severe diarrhoea. The detection of oocysts was more easier when using the method described in Material and Methods (point 3) and phase contrast. In any case, however, the preparations should be examined at the magnification of 800–1 000 x.

The intensity of oocysts was rather high (10–15 oocysts in a viewing field at magnification ×100). Iseki (1979) reported that cats naturally infected with Cryptosporidium felis expelled with the faeces even several millions of oocysts.

Our findings demonstrated that the oocytes are excreted with the faeces of infected animals and that they can be isolated by means of commonly used coprological methods of floatation centrifugation. This is very important for the detection of this parasite in infected animals and for the studies of its distribution under various conditions. The isolation of oocysts from the faeces enables to elucidate the life cycle of cattle cryptosporidiosis on the basis of experimental infections and their participation in the dying of calves.
РЕЗУЛЬТАТ
Описа строения "Cryptosporidium" и его обнаружение в исследуемом материале.


Fig. 1. Cryptosporidium sp. (arrow) in ileum of calf. Giemsa stain (×600). Fig. 2. SEM of ileum infected with Cryptosporidium. a, b, c, — developmental stages of Cryptosporidium, d — craters after schizonts or released oocysts. (×3,200).

Fig. 3a, b. Oocysts of Cryptosporidium sp. obtained by flotation. a — bright field microscope, b — phase contrast microscope. (×1,800). Fig. 4. Oocysts of Cryptosporidium sp. obtained by flotation from previously fixed (by methanol) and stained (after Giemsa) faeces. (×2,000). Fig. 5. Oocysts of Cryptosporidium sp. (arrows) isolated from faeces by flotation, additionally fixed (by methanol) and stained (after Giemsa). (×1,800).