関東地方の仔牛のクリプトスポリジウム感染およびマウスへの感染実験

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Cryptosporidial Infections in Calves in Kanto District, Japan, and Experimental Infections in Mice

Suguru MIYAJI, Yutaka SAKANASHI[1)], Hisashi ASAMI[2)], and Jun-ichi SHIKATA
First Department of Surgery, Teikyo University School of Medicine, Kaga, Itabashi-ku, Tokyo 173, [1)]Chiba Prefecture Toubu Livestock Hygiene Service Center, Kawaba, Tougane City, Chiba 283, and [2)]Asami Animal Hospital, Shimo-Miyaji, Chichibu City, Saitama 368, Japan
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Among calves, cryptosporidiosis has been reported to be common in America [1, 9], Europe [13], and Australia [7]. A causative agent of the disease, *Cryptosporidium*, is a member of the family Cryptosporidiidae, suborder Eimeriorina. It inhabits the microvillus border of the mucosal epithelium in the alimentary and respiratory tracts of numerous species of vertebrates [17]. Although subclinical infection is common in this disease, the infection is also frequently associated with diarrhea in a number of mammalian species, including man [2, 17]. It has recently been recognized as a zoonosis.

In Japan, *Cryptosporidium* has been reported in cats [3], chickens [6], brown rats [4], calves [5], swine [20], and humans [14]. This study reports the prevalence of *Cryptosporidium* among calves in the Kanto District, Japan, and the infectivity of the oocysts to mice.

When fecal samples of calves were examined directly by the sugar flotation technique, which is routinely used for detecting cryptosporidial oocysts, it was very difficult to find the oocysts. This was because much debris in the fecal material disturbed our microscopic observation. To resolve this difficulty, about 3 g of feces was pretreated by the ether sedimentation procedure reported by Oshima *et al.* [8], and this was followed by the sugar centrifugal-flotation method. Application of this two-step method made the detection of cryptosporidial oocysts easy, since the debris in the microscopic fields was reduced (Fig. 1). Thus, this method seems very suitable for detecting oocysts in feces of calves.

Sixty four fecal samples of Holstein calves (two to eight weeks old) were collected from 23 farms in Saitama and Chiba Prefectures, Japan, from December 1987 to October 1988. All samples were collected from distinct calves. Of the 23 farms, each of the two farms (8.7%) had one calf positive for cryptosporidial oocysts of a mean size of 4.7 × 4.2 (4.0–5.2 × 4.0–4.8) μm (Fig. 1). One of them was located in Saitama Prefecture, and the other in Chiba Prefecture. In the former farm one of three samples was positive, and in the latter one of two was positive. In order to get more information on the prevalence of the disease in the latter, 52 additional fecal samples were collected. Seven of the 52 samples were positive. Since one of the two samples mentioned above was positive, the total positive rate in this farm was 14.8% (8/54). Of the eight positive calves, two had mild diarrhea, one had severe diarrhea, and the others showed no clinical signs, such as diarrhea or debilitation. The calf that had severe diarrhea was shedding about 1×10^6 oocysts per gram of feces. This animal recovered after three weeks, and the discharge of oocysts also ceased.

Next we proceeded to the experimental infection in mice, to determine the infectivity of the oocysts discharged in the bovine feces. Four coccidia-free adult BALB/c mice (eight weeks old) and four other suckling litter-mate mice (one week old) were fed 1×10^4 oocysts each by stomach tube, and four suckling mice (one week

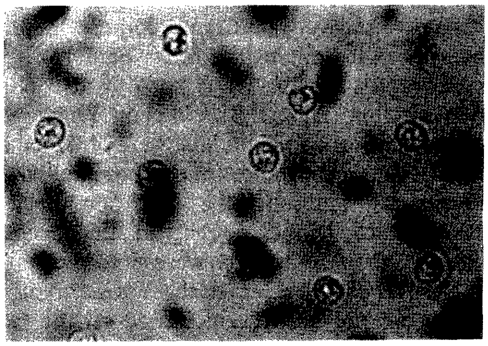

Fig. 1. Fresh *Cryptosporidium* oocysts isolated from a calf by the sugar centrifugal-flotation method. ×900.

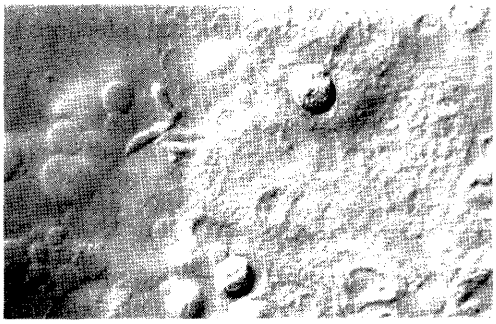

Fig. 2. Nomarski interference-contrast photomicrograph of developmental stages of *Cryptosporidium* in mucosal scraping of small intestine of an experimentally infected mouse (5 days post infection). ×1,200.

Fig. 3. Endogenous stages of *Cryptosporidium* on microvillar brush border of ileum of an experimentally infected mouse (5 days post infection). Giemsa's staining. ×1,200.

old) were used as a non-infected control group. In all of the suckling mice fed oocysts, cryptosporidial infection was established, and the mice discharged oocysts in their feces from day 5 through day 17 after inoculation. Various endogenous stages of *Cryptosporidium* were observed in the microvillar brush border of the ileum when the mouse was necropsied 5 days after infection (Figs. 2, 3). No clinical changes, such as diarrhea or weight loss, were observed in the infected suckling mice. In the four adult mice fed oocysts, and also the four non-infected suckling mice, neither oocysts nor endogenous stages of *Cryptosporidium* were detected.

Tzipori *et al.* [18] reported that bovine cryptosporidia are not host-specific based on the fact that oocysts from calves were infectious to one-day-old lambs, pigs, mice, rats, chickens, and guinea pigs. In the present study, although suckling mice (one week old) were susceptible to *Cryptosporidium* isolated from a calf, adult mice (eight weeks old) were resistant. An age-related susceptibility of *Cryptosporidium* was reported by Sherwood *et al.* [12], but Reese *et al.* [10] said that adult mice as well as the newborns were infected with *Cryptosporidium* of human and calf origin.

Upton and Current [19] reported large, 7.4×5.6 μm, and small, 5.0×4.5 μm, forms of cryptosporidial oocysts from calves, and they considered the former to be *C. muris* and the latter *C. parvum*. The large and the small forms of oocysts in brown rats (*Rattus norvegicus*) were reported to be 8.4×6.3 μm and 5.3×4.8 μm in size, respectively [4]. Iseki [4] and Tyzzer [15, 16] reported that *C. muris* inhabited only the gastric glands, and *C. parvum* only the small intestine. In the cryptosporidial parasites detected in the present study, the size of the oocysts was about 4.7×4.2 μm and the endogenous stages were observed only in the ileum of experimentally infected mice. So the present cryptosporidial species detected in calves was regarded as *C. parvum*.

In America [1, 9], Canada [11], Europe [13], and Australia [7], cryptosporidiosis has been reported to be widespread in calves. Anderson and Hall [1] reported that 56% of 73 herds of calves in Idaho had one or more calves with cryptosporidial infection. In the present study, 2 (8.7%) of 23 farms were positive for cryptosporidial infection. Compared with the result of Anderson and Hall [1], the prevalence rate in Japan is lower. However, in the present study, the number of farms examined was less. To clarify the prevalence and intensity of cryptosporidial infection in Japan, more large-scale surveys are needed.

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要　約

関東地方の仔牛のクリプトスポリジウム感染およびマウスへの感染実験(短報)：宮地　俊・坂梨　裕[1]・浅見寿[2]・四方淳一(帝京大学医学部第一外科学教室，[1]千葉県東部家畜保健衛生所，[2]浅見獣医科医院)——関東地方の23牧場中2牧場で仔牛(2-8週齢)のクリプトスポリジウム感染が認められ，陽性牧場における仔牛の陽性率は14.8%と高率であった．ウシ由来の *Cryptosporidium* は1週令のBALB/cマウスに感染が成立した．