BOVINE CRYPTOSPORIDIOSIS

By

J. Pohlenz

Institute of Veterinary Pathology

Veterinary School, University of Zurich

Cryptosporidiae are protozoa of the suborder Eimeriorinae representing the genus of Cryptosporidium. The different stages of life-cycle may occur at one site in the small intestine, where trophozoites, schizonts, macrogametes and cysts develop on shortened villi in the microvillus area on top of enterocytes. They are in close contact to the epithelial cells and reduce the absorptive capacity by pushing away the enzyme containing brush border. These protozoan in a size of one to four microns are described to occur in at least 12 animal species including man. They are thought to be species specific, but there is controversy to this species specifity in literature.

Life cycle and way of transmission are not yet totally known and their pathogenicity is in discussion. In general they are known to occur as intestinal parasites, but recently they have been described as pathogens in the respiratory tract in turkeys.

Though these protozoa were already described in 1907 by TYZZER in mice, the first description in the bovine species was given in 1971 by PANCIERA. Since that description, there have been several reports in single cases from USA and Canada, and there are several unpublished data that Cryptosporidia occur in neonate calves in Europe too.

Today Cryptosporidia are thought to be one of the etiologic factors causing or contributing to neonatal diarrheoa in calves.

Assiut Vet. Med. J. Vol. 7 Suppl. 1, 1980.

Just recently MORIN from Canada could demonstrate that these parasites occurred in more than 30% of diarrhoeic calves out of 51 calves investigated systematically. They are found either in association with rota-, corona and EVD-virusses or alone in calves in the first two weeks of life. These results correspond to those which we could find in herds from the State of Iowa in 1977.

Out of our experimental work there will be pointed out 3 aspects in this report as follows:

1) Possible life cycle of Cryptosporidium,
2) Transmission of Cryptosporidium from calf to calf,
3) Diagnosis of Cryptosporidiosis in calves.

1- From our investigations we concluded that oocysts occur either on the intestinal epithelial cell or they are discharged with feces in the surroundings. This concept of first stage of development was just recently demonstrated by ISEKI from Japan for Cryptosporidium felis from domestic cats. Mature oocyst containing 4 sporozoites are released either inside the host to reenter the epithelium, or they enter a new host from outside. 4 sporozoites containing oocysts are demonstrable from feces in calves by pelleting them with agar or by scanning or transmission EM. Having contacted the epithelium the parasites develop as trophozoites. They contain a well developed nucleus and in later stages of maturation a golgi apparatus. The asexual development results in schizonts which contain 8 individual parasites. These however settle down again on epithelial cells or form gametes. The macrogametes are detectable from a different number of electrondense bodies. Microgametes are difficult to find and we failed to demonstrate them in our materia from calves. The sexual cycle results in oocyst formation.

2- Since we found that Cryptosporidia are detectable in the lower small intestine and colon we took material from ileum

of two donar calves which were known to be affected from fecal examination. These calves in the age of 11 and 17 days were taken from a herd where diarrhoea has been a problem since several years and where Cryptosporidia were persistent at least for 10 months. Ileal mucosa was scraped and diluted with saline containing bovine serum. The inoculum was mixed from both calves and diluted so that every calf of four recipients got 100 ml by stomach tube orally. The four recipients were born in a plastic bag, colostrum deprived and free from cryptosporidia on day two when they were inoculated. All 4 calves which were given the cryptosporidia infected scraping developed profuse watery diarrhoea and had rectal temperatures from 39° to 40,2° degreos Celsius by 48 hours after inoculation. Feces were tinged with flecks of blood or fibrin 48 to 72 hours after inoculation. Later fecal samples contained only fibrin and increased mucus. Diarrhoea persisted in all 4 recipients until they were killed on day 3, 6, 9, and 12. Two animals became exsiccotic on day 5 and 7 and needed to be treated in the usual manner by fluid supply intravenously.

By histologic examination Cryptosporidia were detectable in the small intestine in all animals in a different intensity. In the caecum and in colon they were adherent in three respectively in two calves. From these experiments we could demonstrate, that Cryptosporidia originating from the bovine species are transmissible to newborn colostrum-deprived calves. We are aware of the fact, that our way of transmitting these parasites is unphysiologically but at that time when these experiments were carried out, we did not know of the existence of oocysts. We could detect those during these experiments. In addition to that we know, that in our experiments virusses may have been transmitted. But Cryptosporidia are known to occur along with virus infections.

3- The most imortant question for the daily work in laboratory is how to detect these parasites:

When it is possible to obtain intestinal tissue at death of the victim, there will be no problem in demonstrating these parasites in the brush border in histologic sections. They stain with Hamatoxilin-Eosen faintly pink, and are 1 to 4 micron in diameter. They are detectable in the lower small intestine and normally adhered to enterocytes in shortened villi. We never found them in long and unaffected villi . In one micron sections different stages of life cycle are detectable by higher power magnification. In the daily work of the pathologist, where calves are necropsied beeing dead since more than a few hours, scrapings from intestinal mucosa stained with Giemsa's stain after methanol fixation are more effective. The parasites take a light-blue colur with some intensely red stained nuclei.

In our experiments we compared the effectivity of scrapings with histologic examination. One piece of ileum was taken at necropsy and fixed in formalin for histologic examination, respectively a scraping carried out. The material was stored at room temperature and the procedure repeated after 6 and 12 hours. Already 6 hours after death Cryptosporidia are only demonstrable in two out of six calves - and not demonstrable at 12 and 24 hours. In scrapings however they are detectable in nearly all examples.

In addition to prepare scrapings we stained fecal samples by Giemsa's stain in the same way. Stirring fecaes from diarrhoeic calves by cotton sticks and producing a smear make et easy to detect the parasites.

In conclusion it is necessary to point out, that cryptosporidia may contribute to diarrhea or even cause diarrhoea. They are extracellular parasites which decrease intestinal

enzyme activity by pushing away the brush border. They are transmitted from calve to calve and do not seem to be dependent on an intermediate host. More research is needed to evaluate their pathogenicity. Their occurence in about 20 to 30% of calves with diarrhoea makes it necessary to observe these parasites more exactly. We think they have been overlooked for a longer time.