Theileriosis in Pure and Crossbred Friesian Cattle in Khartoum State, Sudan: Clinico-Pathological Observations

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Summary
Atypical form of bovine tropical theileriosis, due to Theileria annulata, *was diagnosed in calves with Friesian blood raised in dairy farms in Khartoum State, Sudan. The most striking clinical signs were tumefaction of the eye ball, ataxia, stiffness of the neck, respiratory distress and mucohaemorrhagic diarrhoea. An average of 3-5 piroplasm forms in the RBC were observed in all cases with a range of 70-90% parasitaemia. The most prominent lesions were lymphoproliferative nodules in all organs and tissues including the omentum, mesenteric and retroperitoneal fat. On microscopy, the nodules showed the features of lymphoma with small, medium and large lymphocytes. Giemsa-stained preparations revealed the presence of* Theileria *schizonts in the majority of lymphocytes in the nodules.*

Introduction

Bovine tropical theileriosis, caused by *Theileria annulata*, represents a major threat to pure-bred and cross-bred cattle in the Sudan. It causes substantial losses both in animals and their products (Shommein,1976). In Sudan, bovine tropical theileriosis has assumend serious dimensions with the advent of large scale breeding programmes using exotic breeds for improvement of milk production. A mortality rate over 80% was observed among exotic cattle and their crosses; it is very high when compared with the more resistant indigenous local breeds (Anonymous, 1983). Pure exotic breeds and their cross-bred lines, therefore, live under the constant threat of tropical theileriosis. Nowadays, *T. annulata* is regarded as one of the most important parasites of cattle in the Sudan. This communication describes the

clinical signs and pathological findings induced by *T. annulata* in pure and crossbred Friesian calves.

Materials and methods

During 1991-1993, thirty-one calves (24 sick and 7 carcasses) were received in the pathology Department, Veterinary Research Laboratory, Khartoum for investigation. The calves, Friesian or cross-bred, were 5 to 90 days old and raised in dairy farms in Khartoum State, Sudan. The affected calves manifested lethargy, anorexia, pyrexia (40-42°C), stiffness of the neck, enlarged superficial lymph nodes, pale mucous membranes, dyspnoea, salivation, ocular and nasal discharge and mucohaemorrhagic diarrhoea. Ataxia and exophthalmia were occasionally noticed. In the terminal stage, most animals became recumbent and died within 5-9 days following the onset of clinical signs.

Blood smears and lymph node aspirates prepared from sick calves and their dams as well as impression smears made from tongue, oesophagus, abomasum, intestine, liver, gallbladder, kidney, heart, lung, brain, omentum, mesenteric and perirenal fat of necropsied calves were then stained with Giemsa and examined for presence of piroplasms and schizonts. Specimens from the same organs and tissues were fixed in 10% formalin and processed for paraffin wax sections, stained with haemotoxylin and eosin (H and E), Giemsa, and Masson's Trichrome (MT) stains. For immunofluorescence examination, piroplasm antigen slides were prepared from blood of 6 affected calves according to the method of Anonymous (1984). The direct fluorescent antibody (FA) technique was carried out according to Kimbes *et al.* (1973) using known antisera for *T. annulata* conjugated with fluorescein isothiocynate (Sigma Chemical Company St. Louis, MO, USA).

Results

At necropsy there was icterus, severe petechial and ecchymotic haemorrhages in the subcutaneous tissue and abdominal fat, hepatosplenomegaly and lymphadenopathy. Focal necrosis and haemorrhagic areas were seen in the mucosa of the abomasum, liver and kidneys. Pulmonary oedema, mucosal ulceration in the abomasum , heart flabbiness and distension of gallbladder with brownish bile were constant findings. Occasionally the gallbladder mucosa revealed necrotic foci. Greyish to brownish-red nodules of various size and shape were scattered throughout the tongue, heart and omental, mesenteric and retroperitoneal fat, especially around the kidneys. These nodules were firm in consistency and the cut surface was dry and lymphoma-like. Similar lesions were observed

on the serosal surface of the small and large intestines together with circular haemorrhagic nodules. In one calf lymphoma-like lesions were encountered in the oesophagus. Two calves showed severe petechial and ecchymotic haemorrhages throughout the gastrointestinal tract.

Microscopically, the lymph nodes manifested oedema, haemorrhage and diffuse proliferation of lymphoid cells. These cells, mainly actively dividing lymphoblasts, were encountered especially in the paracortical, medullary and subcapsular area which was greatly distended. There was intense infiltration of pleomorphic lymphoid tissue with lymphoblast cells often in mitosis in the heart, tongue, oesophagus, abomasum, small and large intestines, omental, mesenteric and retroperitoneal fat (Fig.1) In severely affected areas of these organs, there was diffuse infiltration of lymphomatous tissue (Figs. 2 and 3). Focal lymphoid infiltrations were seen in the trachea, lungs, liver, gallbladder and kidneys. Perivascular cuffing with aggregates of lymphoid cells were infrequently detected in the brain. Moreover, lung oedema and exfoliation of the oesophageal, abomasal and intestinal epithelial cells were evident. Irregular zones of necrosis were occasionally seen in some sections, whereas proliferation of fibrous tissues was demonstrated in the tongue and fat nodules using MT stain.

Giemsa-stained blood smears of both dams and calves showed intraerythrocytic piroplasms. In the dam, the parasitaemia was low (1-3/1000); whereas in severely affected calves, 3-7 piroplasms/ RBC were frequently seen together with schizonts-parasitised lymphoid cells. In calves, Giemsa-stained perparations from lymph nodes, tongue, spleen, liver, heart, omental, mesenteric and retroperitoneal fat, kidneys, lung, oesophagus, abomasum and intestine displayed mononuclear cell infiltration. Actively proliferating lymphoblasts often in mitosis, lymphocytes, macrophages, plasma cells and a few binucleated cells were observed. Inter- and intracellular macroschizonts were frequently seen (Fig. 4) whereas microschizonts were scarcely detected. Schizonts-parasitised lymphoid cells in mitosis and/or vacuolated cells were frequently encountered (Fig. 5). Lymphocytes parasitised by one or more schizont were not uncommon. Late in mitosis, the schizonts were located centrally in lymphoid cells (Fig. 6) or were shared by the two newly formed cells. The schizonts appeared to divide synchronously with the parasitised cells. Binucleated cells containing one or two schizonts were also detected (Fig. 7). Intranuclear schizonts encircled by a hallo were seen in cells with indented nuclei (Fig.8). Macrophages carrying either schizont or fragments of schizonts were sometimes encountered.

Piroplasms in all antigen preparations gave positive FA reaction with *T. annulata* antiserum.

Discussion

The clinical signs reported in the present investigation were similar to those described for acute form of *Theileria parva* infection (Irvin, 1983; Lossos, 1986) and peracute and acute forms of *T. annulata* and *T. lawrenci* infections (Lossos, 1986). A relatively unusual clinical manifestation recorded here was the bilateral tumefaction of the eyeball. This observation conformed well with that described by other authors (Khalifa and Kadhim, 1967; Baharsefat *et al.*, 1977).

The macroanatomical features of the disease described in this communication showed a certain similarity to those induced by *T. annulata* in crossbred calves (Khalifa and Kadhim, 1967; Shastri *et al.*, 1982) and by *T. parva* in adult cattle (Dumani and Ciftci, 1992; Irvin, 1983; Lossos, 1986). However, the lesions described in the present cases were more severe, nodular in nature and widely disseminated. This is possibly because the immune system of the newly born calves is not yet fully developed and cells of the neonates are highly susceptible to infection with *Theileria* parasites. A relatively uncommon macroscopic lesion recorded in the current study was a proliferative nodular lesion in the tongue and adipose tissue. Relevant literature on proliferative nodular lesions in adipose fat, apart from those reported by Ahourai *et al.* (1988), Baharsefat *et al.* (1977) and Rogers and Callow (1961), is meagre. The histological picture was uniform resembling lymphoma with intermixed cell types. Typical changes consisted of massive accumulation, in different organs and tissues, of actively dividing lymphoblastoid cells intermingled with lymphocytes, some macrophages, plasma cells and few binucleated cells. Identical picture was observed in Giemsa-stained preparations. This cellular infiltration is more intense and widely distributed than that previously described for *Theileria* infections in adult cattle (Irvin *et al.*, 1974; Jubb *et al.*,1985; Mugera, 1979; Mungua *et al.*, 1973).

Continuous multiplication of *Theileria* infected cells in tissue culture was previously described by Malmquist *et al.* (1970), Moulton and Malmquist (1971) and Pipano and Shkap (1990). Such a picture was observed in our current study. Though the cellular responses reflect the degree of the host defence mechanisms, however, the extensive proliferation of the cells and organisms observed in this study may be interpreted as lack of defence in neonates similar to that observed in tissue culture. On the other hand, infection in adult animals is possibly controlled by the defence mechanisms. This is in agreement with Irvin *et al.* (1974) and Irvin *et al.* (1975) who observed that *T. parva* - infected cells infiltrated extensively causing malignant neoplastic growth in irradiated mice. Infiltration of pleomorphic lymphoid cells, small, medium and large lymphocytes observed in this report resembled those described in malignant lymphoma in cattle (Bundza

et al., 1980; Moulton and Dungworth, 1978) in cats, dogs and cows (Valli *et al.*, 1981) and in other species (Moulton and Dungworth, 1978). This cellular change was described as a predominant feature in lymphoproliferative diseases of the fowl (Calnek and Witter, 1978). Histologically, these cellular changes were diagnostic for lymphosarcoma, the investigation of Pinder and Roelants (1983) showed that the kind of receptors in *Theileria* transformed lymphocyte differ from those of lymphoscarcoma.

Our histological record coincided with that of Ahourai *et al.* (1988) who demonstrated, in some instances, that *T. annulata* infection in calves resulted in lymphoproliferative nodules confusable with juvenile bovine leucosis. The authors attributed these changes to the phenomenon of immunological tolerance. The binucleated cells or Reed-Sternberge-like cells noticed in some slides were possibly formed either by fusion of two cells or incomplete ceilular division; binucleated cells were seen in malignant lymphoma (Erdman *et al.*, 1992). Intranuclear schizont is possibly encircled by nucleus during synchronous division of schizonts and cells rather than representing a stage in the development of the parasite.

Two of the calves under study died when they were 5 and 7 days old respectively. The route of infection in these two calves was not known, but was possibly due to prenatal infection. The dams of the calves were from enzootic areas and were carriers of the parasite. The age of the calves compared to the severity and nature of the lesions supported intrauterine transmission. This corroborates with the findings of Purothit *et al.* (1983) and Waltschowishi and Pawiov, (1970) who demonstrated *Theileria* organisms in different organs of aborted foetuses and erythrocytes of neonate calves. Hence it is necessary to screen calves immediately after birth for *Theileria* infection and early treatment of sick calves be undertaken especially in areas where bovine tropical theileriosis is enzootic. Early treatment of sick neonate calves will reduce the mortality rate and cutdown the losses inflicted by *Theileria* infections.

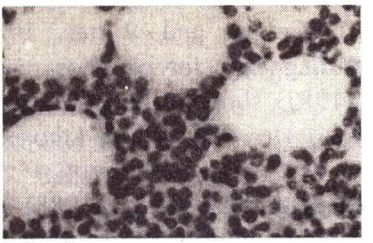

Fig. 1

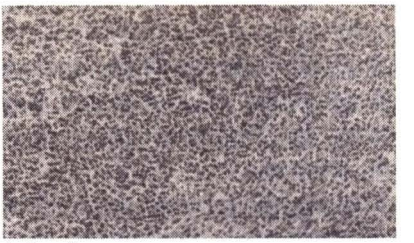

Fig. 2

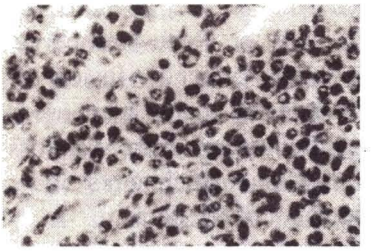

Fig. 3

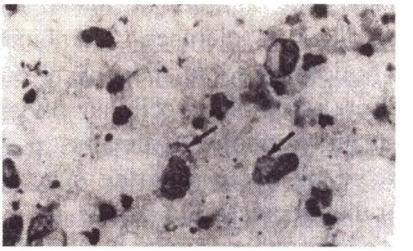

Fig. 4

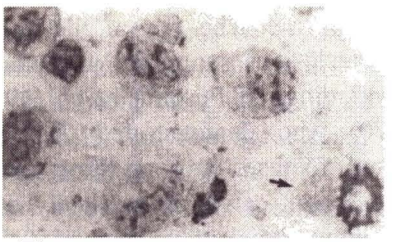

Fig. 5

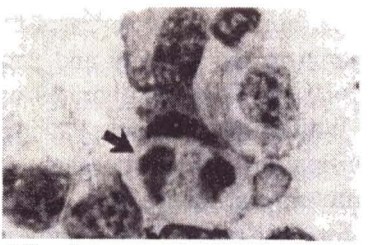

Fig. 6

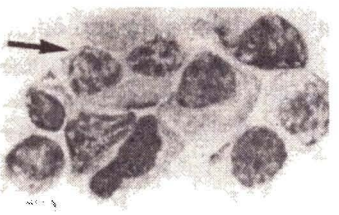

Fig. 7

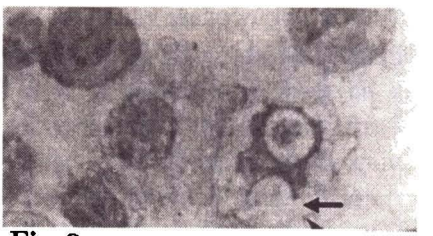

Fig. 8

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