DEVELOPMENT OF MICROSCOPIC LESIONS AND THE ROLE OF CAUSATIVE AGENTS OF BOVINE PARASITIC OTITIS

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SUMMARY

The early and later stages of development of lesions of Bovine Parasitic Otitis (BPO) were studied by microscopic examination of tissue specimens taken from the wall of the external auditory meatus (EAM).The specimens were from nine, five to nine month old bull calves that had an experimentally induced BPO in an ongoing epidemiological study of the disease at Sokoine University of Agriculture (SUA). Early histopathological changes were also studied by inoculating *Rhabditis bovis(Rh. bovis)* into rabbit EAM. *Rhabditis bovis* is one of the suspected causes of BPO. The early microscopic lesions observed in the present study were spongiosis or hydropic degeneration followed by focal and later diffuse necrosis of upper epidermal layers of the EAM. Also observed were intraepidermal cleft formation and acantholysis. These are changes that would predispose to easy penetration of EAM epidermis by microorganism. Changes in the dermis/subepithelium were those of acute suppurative inflammation followed by healing and reparative changes in both the epithelial and subepithelial tissues. It is suggested that *Rh. bovis*, normally a free living soil nematode, predisposes the lining of EAM to bacteria infection through its feeding on some of the bacteria that form the normal flora of the EAM. This feeding behavior upsets the normal competitive inhibition in favour of building up of pathogenic/opportunistic bacteria to infective levels. At the same time the *Rh. bovis* excretory products and breakdown products of reproduction may play part, along with exotoxins of the pathogenic/opportunistic bacteria, in causing the early microscopic lesions in the epidermis. However, the major subsequent inflammatory changes in the lining epithelium and underlying tissues are largely bacterial in type. Further studies on the role of the causatives agents of BPO needs to be directed in these suggested mechanisms.

INTRODUCTION

The ear infection in cattle, commonly known as Bovine Parasitic Otitis (BPO), is characterized by presence of thick, dirty gray or brown, often foul smelling discharges from the ear. There are a number of publications on clinical manifestations, negative impact on productivity, probable etiology, epidemiology, pathogenesis and treatment of BPO (Kreis, 1964; Jibbo, 1966; Lwemo, et al, 1983; Msolla, et al 1985; 1986(a), 1986(b), 1987; Odongo and D'Souza, 1989; Shayo, et al, 1992; Msolla, et al, 1993; Shayo et al, 1994). However, records on the development of early and later microscopic lesions in BPO are few and lacking in detail. The work by Lweno, *et al* (1983) appears to be the only published report on histopathology of BPO. In their report, however, some details on microscopic changes and the possible histopathological development of lesions of BPO are not given. Also the cellular response in BPO as reported by Lweno, *et al,* (1983) is not typical of helminth infections in which eosinophil cells tend to be predominant (McEwen, 1992). There is therefore the need for further study of microscopic features during the early and later development of BPO. Such studies might also shed some light on the specific roles of the nematode *Rhabditis bovis (Rh. bovis)* and bacteria which seen to be involved in BPO (Shayo, 1997).

MATERIALS AND METHODS

The source of material for histopathological studies were nine, 5 to 9 month old cross bred bull calves (5, Friesian x Boran and 4, Ayrshire x Zebu). These calves had an experimentally induced BPO in an ongoing epidemiological study of the disease at Sokoine University of Agriculture (SUA). Specimens from these cattle were taken at different stages of the disease process. Tissues were taken at nine sites along the entire length of the ear canal and each specimen consisted of the entire thickness of the canal wall.

Studies on early pathological changes in BPO were also done by inoculating *Rh. bovis* worms into external ear canals of two rabbits after very mild trauma, by sterile iron wool, of the lining epithelium. Specimen from the two infected and one control rabbits were taken at 4 hours post infection. All specimens for microscopy were fixed in neutral buffered 10% formalin.

Before specimens were further processed for histopathology, those with bony tissues were decalcified in RDO (Du Page Kinetic Laboratory Inc., USA). All specimens were trimmed to appropriate size, dehydrated by passing in increasing concentrations of alcohol up to absolute alcohol, cleared in chloroform and embedded in paraffin wax. Sections were cut at 5 microns using a Reichert – Jung microtome. All tissue sections were stained with hematoxylin and eosin (HE).

RESULTS

The earliest microscopic changes observed were in the upper layers of the epithelium of the outer two thirds of the external auditory meatus (EAM). These were focal areas of either cellular hydropic change or actual separation of epithelial cells (spongiosis) (Fig. 1). Later these changes became wide spread. In other foci there was necrosis of upper epithelial cells (Fig. 2 and 3). Following these changes there was observed cleft formation in the upper layers of the epithelium (Fig. 3) which started as thin irregular lines of separations of cells. Later the separated cell layers got lifted off from the lower epithelial layers. At this stage of cleft formation there was also poymorphonuclear cells (PMN) infiltration among the epithelial cells. In some cases the PMN were sequestered in some of the epithelial cavities formed from the clefts.

The above microscopic changes were also the early changes in the inner one third of EAM where the lining epithelium is of the noncornified stratified type. However, these changes, in this area, occurred later than in the outer two thirds of the EAM. The inner one third of the EAM became affected later by spread of infection from the outer regions of the EAM. Also the above pathological processes were more pronounced in the inner one third of the EAM.

In describing pathological changes in tissues underlying the epithelial lining of the EAM, the term dermis applies to the outer two thirds of the EAM whose histology resembles that of the skin of the inner aspect of the pinna. The term subepithelium applies to the inner one third of the EAM which does not resemble the skin (Semuguruka, *et al*, 1999).

While the above changes were taking place in the lining epithelium, the following were observed in the dermis/subepithelium. A mild PMN and very light mononuclear cells infiltration in the upper dermis/subepithelium, immediately below the lining epithelium, was observed along with the hydropic change or spongiosis. In the lower dermis/subepithelium and among the glandular tissues (dermis) no tissue reaction was evident. With cleft and cavity formations in the epithelium, PMN and mononuclear cells infiltration, mostly macrophages and lymphocytes, became more evident in the dermis. Although the cellular infiltration was diffuse, there was also aggregation of these cells about blood vessels. The PMN predominated in upper dermis whereas the mononuclear cells seemed slightly more in the lower dermis. Similar changes were seen in the subepithelium but PMN cell infiltration was more intense here particularly in the upper subepithelial connective tissue lying between the downward projecting epithelial ridges (Fig. 4a). Also in the connective tissue of the inner one third of the EAM, the small blood vessels, often located close to the surface of the epithelium, were much dilated (Fig 4b).

In the lower dermis where both sebaceous and ceruminous glands are located, there was also PMN and mononuclear cell infiltration. Some glands of both types showed early changes of necrosis. Some of the ceruminous glands appeared more dilated.

After epithelial cleft formations and or cavitation there was epithelial desquamation. The desquamated epithelium together with amorphous acidophilic material of degenerate PMN cells, sections of worms and other detritus, overlaid the underlying remaining epithelium

(Fig 5). With desquamation, the EAM epithelium became thinner. In the tissues underlying the epithelium, in all parts of the EAM, there was increasing prominence of the mononuclear cell infiltration (macrophages, lymphocytes as well as some plasma cells) in a diffuse manner (Fig 6) but also in small aggregations.

Later changes to the above were of a recovery or chronic nature. In the epithelium overlying the dermis there was evident parakeratosis and hyperplasia with much ridge formation (Fig 7). A few PMN scattered among the epithelial cells were still evident at this stage. In the immediate underlying dermis and connective tissues of the dermal papillae (between epithelial ridges) there was a prominent diffuse infiltration by mostly lymphocytes, plasma cells and macrophages (Fig 7). Very few PMN cells could be seen among these cells. An aggregation of similar mononuclear cells could be seen about blood vessels in these areas. In the lower parts of the dermis, including areas between the glands, was a diffuse infiltration of mononuclear cells and very few PMN.

In the inner one third of the EAM the changes were similar to those in the outer two thirds of EAM. As at other stages of the pathological process, the changes in this area were out of phase with those in the outer two thirds of the EAM owing to later spread of infection here. However, in this inner one third of EAM the PMN infiltration was still obvious among the epithelial cells and in connective tissues between the downward projecting epithelial ridges at which mononuclear cells were less prominent. In the immediate and lower subepithelial tissues the situation was different. Mononuclear cells infiltration was diffusely prominent but in some places the cells formed nodular structures with mostly lymphocytes plus few plasma cells and macrophages.

In both the cartilaginous and osseous supported parts of the EAM, even though recovery or chronic type of changes were taking place, the lumen of the meatus still had an exudate composed of cellular debris, worm sections and other detritus. Worm sections were not in direct contact with the underlying epithelium and were not seen among the epithelial tissues (Fig 5) in either early or later stages of the development of the lesions. As the lesion advanced further the stratified character of the epithelium was restored though in some cases it was obviously hyperplastic and no PMN cells could be detected among the epithelial cells even in the inner one third of the EAM. However, in the tissues underlying the epithelium there was still prominent presence of mononuclear cells, either diffusely, linearly or nodularly disposed. Linear and nodular formations of mononuclear cells was particularly prominent in the inner one third of the EAM. Also evident now was increase in connective tissue in the dermis/subepithelium while mononuclear cell infiltration decreased markedly as the pathological (healing) process progressed. This increased tissue formation here caused thickening of the wall and reduction of the lumen of the canal.

DISCUSSION

The early microscopic lesions observed in the cornified stratified squamous epithelium of the external auditory meatus (EAM) are similar to the spongiosis, hydropic degeneration, intraepidermal cleft formation and acantholysis seen in acute or subacute dermatosis as described by Yager, *et al* (1992). These changes were followed by focal and later diffuse necrosis of the superficial layers of the epithelium. Along with these early microscopic changes in the epithelium, particularily at the stage of intraepidermal cleft formations, polymorphonuclear (PMN) cells, mostly neutrophils, appeared among the epithelial cells, later becoming sequestered in cavities formed from the clefts. This appears to be similar to the exocytosis and acantholysis seen in inflammatory dermatosis (Yager *et al, 1992)*. While these changes were occurring in the epithelial cover, in the dermis/subepithelium there was mild PMN cells and very light mononuclear cells infiltration particularly at the stage of cleft/cavity formations in the epithelium. This seems to indicate that the causative agents or their products were already reaching the deeper parts of the epithelium as well as the dermis/subepithelium.

The early microscopic lesions observed in the EAM which have histopathological features similar to those of acute or subacute dermatosis may be caused by microbial infections, chemical agents or even ectoparasites. In BPO, the suspected etiologic agents are the nematode *Rhabditis bovis* and certain groups of bacteria which belong to the normal microflora of cattle EAM (Lweno, *et al, 1983;* Msolla, *et al*, 1989; Shayo, 1997). In the study by Shayo (1997), *Rh. bovis* in combination with *Actinomyces pyogenes (A. pyogenes)* or *Pseudomonas aerogenosa (P. aerogenosa)*, produced moderate to severe BPO. However, the bacteria alone failed to produce BPO although *Rh. bovis* alone caused a mild form of the disease. In experimental infection of rabbits, in the present study, *Rh. bovis*, given alone, produced the early microscopic lesions in the epithelium within four hours post infection. It appears therefore that *Rh. bovis* has some primary role in the causation of BPO. However, the later lesions that were observed, namely prominent PMN (mostly neutrophils) cells infiltration in the epithelium and dermis/subepithelium and the foul smelling dirty gray or brown exudate seen grossly in BPO, are suppurative changes often associated with bacterial involvement in an inflammatory process (Slauson and Cooper, 1982).

The cornified stratified squamous epithelium of the EAM, like that of the skin, is known for its effectiveness as a barrier to microbial, ectoparasitic, chemical and thermal agents of disease, as long as it remains healthy and intact (Stenn, 1988). In the case of the skin, lack of moisture, constant desquamation of the surface corneocytes and ecological pressures exerted by normal flora limit pathogenic microbial colonisation (Yager, et al, 1992). The healthy and intact epidermis of the EAM is probably protected in a similar manner. Shayo (1997) isolated different types of bacteria from EAM of normal cattle and those with BPO. Bacteria isolated included *A. pyogenes, P. aerogenosa* and many other types that form the normal flora of the EAM either as permanent or transient populations. *Rh. bovis*, however, belongs to the genus *Rhabditis* which are free living nematodes often found in soils of decaying organic matter and manure in cattle sheds (Thorn, 1961; Gooday, 1963; Msolla, *et al*, 1986a, 1989). *Rhabditis* nematodes, like many other free living soil nematodes, survive by feeding on bacteria and other materials in decaying matters in the soil. In early studies on the *in vitro* cultivation of *Rh. bovis*, dependency on bacteria as feed for these worms was shown by the fact they survived longer if bacteria were added to the media (Lweno, *et al*, 1983). These worms may be introduced into cattle ears by scratching the ears with soil contaminated hoofs, from dip wash or by flies (Lweno, *et al*, 1983; Msolla, *et al*, 1986, 1989). Once these worms are introduced into the cattle ears they survive by feeding on the normal bacterial flora in the ears and probably also on the epithelial cells that desquamate as part of normal cell turn over. By feeding on some of the bacteria in the ears, the competitive inhibition that usually keeps the population of some pathogenic or opportunistic bacteria very low is upset. Bacteria like *A. pyogenes* and *P. aerogenosa* are thus able to build up their populations to levels that overcome the normal defenses of the epithelium of the EAM, leading to the development of BPO. Thus by feeding on bacteria *Rh. bovis* predesposes the EAM to infection by resident or introduced opportunistic/pathogenic bacteria. Another probable predesposing factor may lie in the presence of excretory materials from the worms. In the soil, when the free living nematodes consume bacteria, ammonium (NH_4^+) compounds are released. Apparently bacteria contain much more nitrogen than the nematodes require (Ingram, 1999). This probably happens also in cattle EAM with *Rh. bovis*. The build up of NH_4^+ compounds and other metabolites like carbon dioxide(CO_2) may contribute to the break down of the integrity of the epidermis and thereby predespose to easy invasion by bacteria. *Rh. bovis* has a short life cycle often completed within 48 hours during which time the viviparous *Rh.bovis* disintegrates to release its progeny (Lweno, *et al*, 1983). There is thus a rapid build up of breakdown products of reproduction whose accumulation may lead to the maceration/necrosis of the epidermal cover and its subsequent invasion by bacteria. Therefore, with the help of *Rh. bovis* to increase their populations and the damage to the epithelium, the bacteria may easily set up the inflammatory processes in the tissues of the EAM as was seen in the present study.

Bacteria like *A. pyogenes* and *P. aerogenosa* produce exotoxins, for example, haemolysins and proteases, that can induce necrosis (Carter and Changappa, 1991) and inflammation. *P. aerogenosa* are opportunistic bacteria that easily invade defective epidermis and can cause degenerative changes in epithelia (Gyles, 1983). Thus the early microscopic lesions in the epidermis of the EAM in BPO may also be the result of the action of exotoxins and other toxic metabolites of these bacteria. Therefore it is possible that both *Rh. bovis* and these bacteria contribute to the initial breakdown of the integrity of the epidermis. However, the build up of these invasive bacteria needs the presence of *Rh. bovis* in the EAM. Even then the subsequent tissue reaction characterized mainly by suppurative inflammation, is a bacterial induced process. In both early and later stages of development of lesions in BPO there was no obvious eosinophil cell reaction that often characterizes worm invasion of tissues (McEwen, 1992). Actual presence of worm parts in the wall of the EAM were not encountered at anytime during the microscopic study of lesions of BPO. *Rh. bovis* - worms seem to have no tissues invasive behaviour.

Hyperplastic changes in the epithelium and marked presence of mononuclear cells, mostly lymphocytes and some plasma cells in the dermis/subepithelium, distributed diffusely or nodularly are an indication of containment of the irritants by the body immunologic defenses. Such defenses can be triggered by bacterial, viral or even helminth invasion. This marked presence of mononuclear cells as well as increased connective tissue due to repair led to encroachment and reduction the lumen of the EAM in some cases that were observed in the present study. This would likely cause dysfunction in the EAM. This may partly be the explanation for the holding of the head low and sideways by some cattle with BPO.

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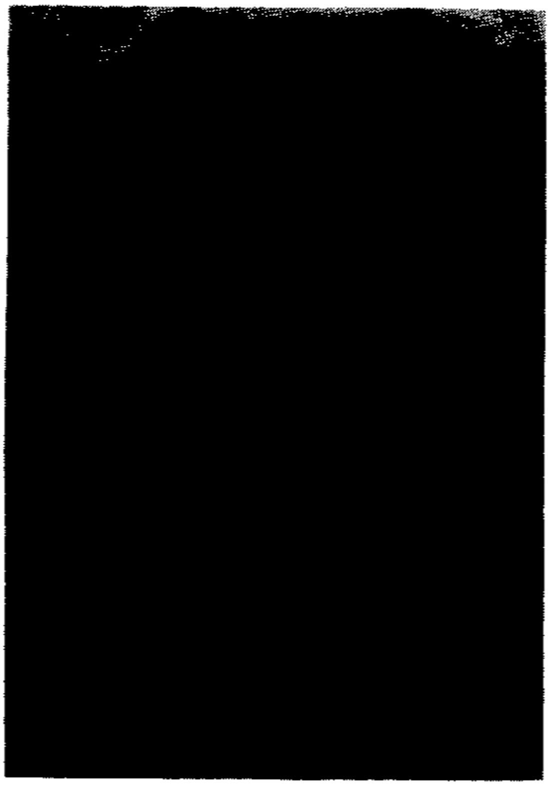

Fig. 1. External Auditory Meatus. Focal areas of spongiosis (asterix) in the upper part of the cornified stratified squamous epithelium. X200.

Fig. 2. External Auditory Meatus. Necrosis in the upper part of the cornified stratified squamous epithelium. X400.

Fig. 3. External Auditory Meatus. Cleft formation in the cornified stratified squamous epithelium (arrows). Foci of necrosis (asterix) are also present in the upper parts of the epithelium. X200.

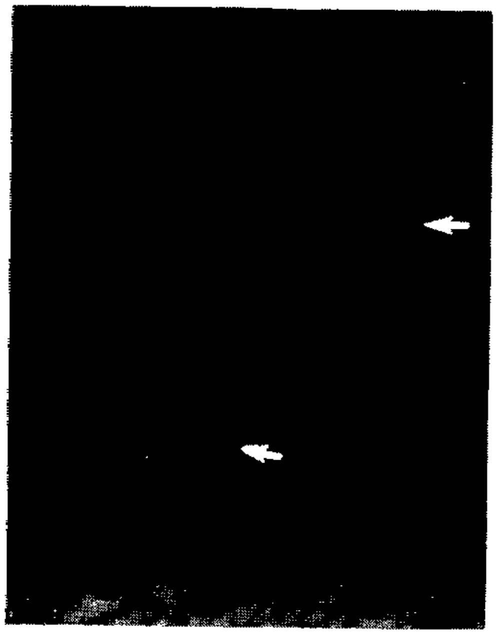

Fig. 4a. External Auditory Meatus. Inner one third with noncornified stratified squamous epithelium. Prominent polymorphonuclear cells infiltration between downward projecting epithelial ridges (arrow) and just below the ridges. X200.

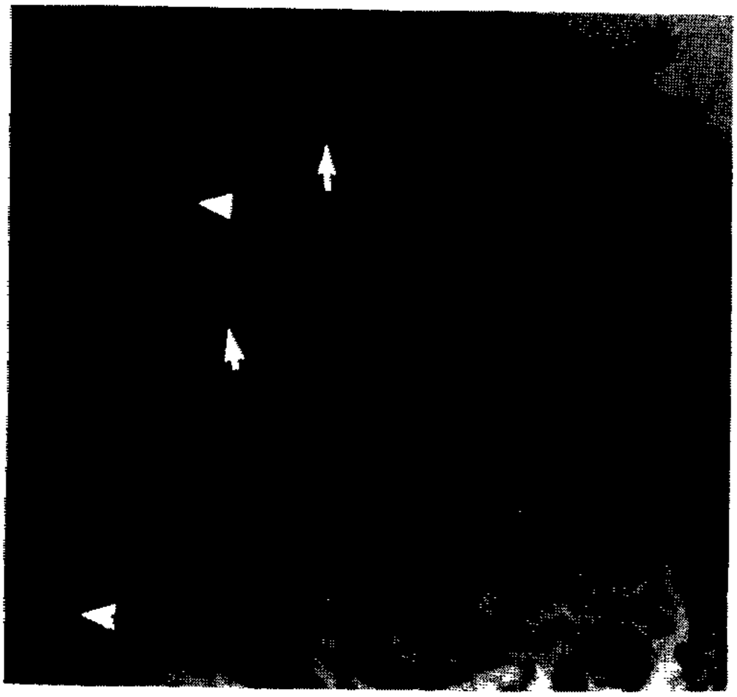

Fig. 4b. External Auditory Meatus. Inner one third. Subepithelial connective tissue (star) between epithelial ridges (arrow head). Oblique section. Blood capillaries are (close to epithelial surface) are dilated (arrow) and polymorphonuclear cells are present in the connective tissues. X400.

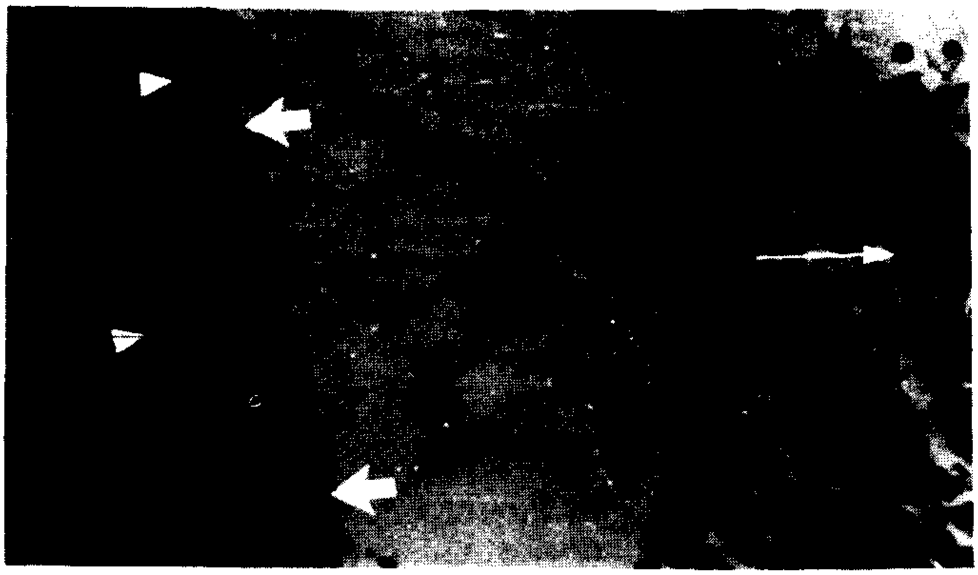

Fig. 5. External Auditory Meatus. Outer two thirds. Desquamated epithelium, amorphous acidophilic materials, degenerate polymorphonuclear cells, worm sections (long thin arrow), and other detritus, overlie much thinned epithelium (thick arrow). The empty space (star) is an artifact. There is prominence of mononuclear cells (arrow head). X200.

Fig. 6. External Auditory Meatus. There is prominent diffuse mononuclear cell infiltration (arrow head) in the dermis. These are mostly lymphocytes, macrophages and some plasma cells. Note worm sections (arrows) in the overlying desquamated epithelium. X400.

Fig. 7. External Auditory Meatus. Parakeratosis, hyperplasia and prominent epithelial ridge formation (short arrow) characterized the later microscopic changes. There is prominent mononuclear infiltration in connective tissue between epithelial ridges and in the immediate underlying dermis (arrow head). Some blood vessels are surrounded by mononuclear cells (long arrow) in the lower dermis. X200.