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Extraocular Manifestations of Moraxellosis in a West African Dwarf Goat

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Abstract: A West African Dwarf (WAD) doe of about 4 years of age with primary complaint of abortion and blindness was presented to the University of Nigeria, Nsukka Veterinary Teaching Hospital (UNVTH). Clinical examination revealed ocular (bilateral keratoconjuctivitis, corneal opacity, mucopurulent ocular discharge, blindness) and oral (erosive stomatitis, lingual vesicles and dental attrition) lesions. Neither intracellular parasites nor inclusion bodies were found in the stained impression smears. Rapid Slide Agglutination Test (RSAT) was negative for brucellosis. Packed Cell Volume (PCV) (29%, reference value: 31±2.1) was normal. Serum biochemical analysis showed normal Alanine aminotransferase (ALT) (9 IU L^{-1}, reference value: 9.1±0.1), slightly increased Aspartate aminotransferase (AST) (27 IU L^{-1}, reference value: 24.3±1.4) and markedly increased Alkaline Phophatase (ALP) (38 IU L^{-1}, reference value: 11.9±0.1). Diagnosis of moraxellosis was based on isolation and identification of pure cultures of *Moraxella bovis* from cultured samples following standard protocols. Antibiotic sensitivity result showed that the isolate was resistant to chloramphenicol, ciprofloxacin, ampicillin and perfloxacin but susceptible to gentamicin, orfloxacin and tetracycline. Intramuscular injection of 5% oxytetracycline short-acting and multivitamin at dosages of 10 and 5 mg kg^{-1}, respectively for 5 days was very effective as the animal was completely healed by 4th day of treatment. This report indicates that extraocular manifestations could possibly occur in moraxellosis of WAD.

Key words: West african dwarf goat, moraxellosis, keratoconjuctivitis, abortion, extraocular manifestations

INTRODUCTION

Moraxellosis is an economically important contagious ocular disease of ruminants caused by Gram-negative bacilli *Moraxella bovis* (*M. bovis*) (Brown *et al*., 1998; Quinn and Markey, 2009). The spread of the disease within a ruminant herd is rapid (Brown *et al*., 1998; Ojo *et al*., 2009) and factors such as environment, season, concurrent pathogens, *M. bovis* strain and host immune system affect its occurrence and clinical severity (Brown *et al*., 1998). Moraxellosis occurs worldwide and has been reported in Africa, Asia, Australia, Europe and North America (Killinger *et al*., 1976; Punch and Slatter, 1984; Ojo *et al*., 2009). Clinically, it manifests majorly as keratoconjuctivitis which starts as unilateral or bilateral inflammation of the cornea and conjunctiva with moderate to severe hyperaemia, epiphora, excessive blinking, blepharospasm and photophobia but as the disease progresses if untreated there is bilateral panophthalmitis, mucopurulent ocular discharges with matting of the hair around the eyes, severe keratoconjuctivitis, corneal opacity, temporary or permanent blindness with descemetocele formation and possibly ocular rupture (Punch and Slatter, 1984; Brown *et al*., 1998; Biberstein and Hirsh, 1999; Akerstedt and Hofshagen, 2004; Ojo *et al*., 2009). However, concurrent extraocular manifestations such as polyarthritis, mammary gland and uterine infection in small ruminants have been suggested (Aiello and Moses, 2002).

Bacterial pathogens such as *Mycoplasma conjuctivae*, *Branhamella ovis*, *Chlamydia* sp., *Rickettsia* sp., *Listeria monocytogenes*, *Corynebacterium pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Arcanobacterium pyogenes*, *Proteus* sp., *Pasteurella haemolytica* and *Pseudomonas aeruginosa* have been reported to aggravate *M. bovis* infection in ruminants (Punch and Slatter, 1984; Takele and Zerihun, 2000; Akerstedt and Hofshagen, 2004; Jansen *et al*., 2006; Ojo *et al*., 2009). But reports have shown that these organisms most often in concurrent infections, enhance the establishment of the main etiologic agent, *M. bovis* (Brown *et al*., 1998), though they can also occur as primary pathogens in some cases of Infectious Keratoconjuctivitis (IKC) (Jansen *et al*., 2006).

Diagnosis of moraxellosis is based on clinical signs, isolation and identification of the aetiologic agent from the eyes of affected animal (Biberstein and Hirsh, 1999;

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Akerstedt and Hofshagen, 2004; Ojo *et al*., 2009; Quinn and Markey, 2009). However, virulent factors released by infectious agents usually destroy tissues and organs of affected animals and this could result to altered serum biochemical profile. Therefore, evaluation of some biochemical substances in infectious processes may provide information on the extent of systemic involvement and damage caused by the injurious agent hence could be of help in the diagnosis and adoption of treatment strategies.

However, there is very little information in the available literature on moraxellosis in Nigeria and only a case of the disease has been reported in a WAD goat farm in the Western region of the country (Ojo *et al*., 2009). In this report, researchers describe unusual extraocular clinical manifestations in caprine moraxellosis in Nsukka, South-East Nigeria and the efforts researchers made to diagnose and treat the animal.

MATERIALS AND METHODS

A WAD goat about 4 years of age with primary complaint of blindness and abortion was brought to the University of Nigeria, Nsukka Veterinary Teaching Hospital in the month of June 2012. Anamnesis revealed that the animal developed the infection sometime in May 2012 and since then had being on treatment by the owner using visine® (potassium chloride and tetrahydrozoline hydrochloride) eye drop. Clinical parameters such as temperature, respiratory rate, heartbeat and pulse rate were checked twice daily until discharge. Mucous membranes, capillary refill time, skin rebound elasticity, skin lustreness, presence of ectoparasites, musculoskeletal system and palpable lymph nodes were examined daily until discharge. Blindness was assessed by Menace Blink Reflex and Obstacle Methods (Radostits *et al*., 2007; UCVOS, 2008). The animal's environment was also visited and examined.

Samples were collected asceptically in duplicates from the conjunctiva (conjuctival scraping), oral cavity and vagina using sterile swabs. They were used for direct impression smears (Giemsa and Gram staining) and for bacteriological culture. Blood was collected from the jugular vein in duplicate using anticoagulant-containing and non-anticoagulant-containing vacuutainers. Anticoagulant-containing blood was used for determination of Packed Vell Volume (PCV) by Microhaematocrit Method (Coles, 1986), thick film wet mount and Giemsa staining while the non-anticoagulant blood was allowed to coagulate and then centrifuged at 3,000 rpm to obtain serum used for colorimetrical assay of serum enzymes activity using QUIMICA APPLICADA (QCA, Spain) test kits. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activity was determined following a standard method (Reitman and Frankel, 1957) while Alkaline Phophatase (ALP) was assayed using a known method (Bassey *et al*., 1946). Rapid Slide Agglutination Test (RSAT) for detection of Brucella antibodies in the serum was performed using Brucella antigen (Cadmus *et al*., 2006).

Primary inoculation was done by streaking the samples unto 5% sheep Blood Agar (BA), Mac Conkey Agar (MCA) and supplemented Mycoplasma agar (Oxoid®). The BA and MCA plates were incubated at 37°C for 24 h in aerobic environment while the Mycoplasma agar was incubated for 72 h in microaerobic environment. After incubation, Mycoplasma agar was observed under stereomicroscope for the presence of microcolonies.

Preliminary identification of the isolate was done using morphological characteristics (colonial and microscopic) while the final identification was done by conducting biochemical tests such as oxidase, catalase, gelatine hydrolysis, nitrate reduction and fermentation of series of sugars (mannitol, inositol, sucrose, xylose, mannose, arabinose, rhamnose) following recommended protocols (Brown *et al*., 1998; Biberstein and Hirsh, 1999; Ojo *et al*., 2009; Quinn and Markey, 2009).

Antibiotic susceptibility test of the isolate was done following disc diffusion procedure (Bauer *et al*., 1966). The following antibiotic discs were used: ampicillin (10 μg), gentamicin (10 μg), tetracycline (30 μg), ciprofloxacin (10 μg), orfloxacin (10 μg), perfloxacin (30 μg) and chloramphenicol (10 μg) (Oxoid®). Homogenate of the isolate was made in 0.85% saline and adjusted to 0.5 McFarland's turbidity standard. Plates containing Mueller-Hinton agar were inoculated using sterile swab and incubated for 18 h at 37°C, after which the Inhibitory Zone Diameter (IZD) was measured and recorded. For each antibiotic disc, the test was performed in duplicate and the mean IZD calculated. Since, there is no Clinical and Laboratory Standard Institute (CLSI) interpretative standard for *M. bovis*, isolates with IZD ≤5 mm were considered resistant. The antibiotic used for treatment was selected based on the result of the sensitivity test.

RESULTS AND DISCUSSION

Initial clinical examination (on the day of presentation) revealed that the animal was pyrexic (temperature, 43.2°C) had elevated heart, pulse and respiratory rates of 115 beats/minute (bpm), 110 bpm and 25 cycles/min (cpm), respectively. The elevated parameters returned to normal physiological values of 39.5, 110 bpm, 80 bpm and 24 cpm, respectively 3 days

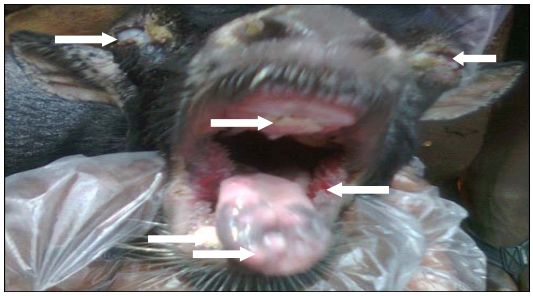

Fig. 1: Ocular (bilateral keratoconjutivitis, corneal opacity, mucopurulent ocular discharge) and oral (erosive stomatitis, lingual vesicles and dental attrition) (arrows) in a WAD goat

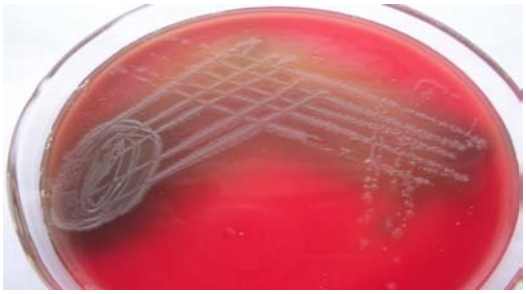

Fig. 2: Pure culture of β-haemolytic *Moraxella bovis* isolated from conjuctiva, oral cavity and vagina of WAD goat on blood agar

into treatment. Regional physical examination showed that the animal had severe bilateral keratoconjuctivitis, corneal opacity with mucopurulent ocular discharge, lingual vesicles on the tongue apex, erosive stomatitis and dental attrition (Fig. 1). The hair coat lacked lustre, no evidence of dehydration or nervous involvement and the eye mucous membrane was congested. The animal neither blinked nor removed its face when an obstacle was brought close to its eyes and it hit itself to an obstacle placed along its way indicating blindness. The palpable lymph nodes (prescapular and popliteal) were normal-sized and there was no evidence of enteritis. In the animal's environment, large numbers of houseflies and Stomoxys were found.

The PCV was 29% and reference value: 31.3±2.1. Biochemical assay result compared to reference values (Daramola *et al*., 2005) showed normal Alanine aminotransferase (ALT) activity of 9 IU L^{-1}, reference value: 9.1±0.1; slightly increased Aspartate aminotransferase (AST) 27 IU L^{-1}, reference value: 24.3±1.4 and markedly increased Alkaline Phophatase (ALP) 38 IU L^{-1}, reference value: 11.9±0.1. The RSAT was negative (no agglutination) for brucellosis. No intracellular or blood parasite was observed from the wet mount and stained impression smears. No growth (microcolonies) was observed in the Mycoplasma agar under stereomicroscope and MCA. The growth on blood agar from all the cultured samples yielded pure culture of bacterium that was rough, greyish, flat, dry, firm, umbonate and β-haemolytic (Fig. 2) and microscopically was Gram-negative plum bacilli arranged in pairs. Biochemical tests showed that the isolate produced catalase and oxidase, reduced nitrate, hydrolysed gelatine and was non-fermentative. Researchers identified the isolate as *Moraxella bovis* based on these characteristics.

The isolate was resistant to ampicillin, chloramphenicol, ciprofloxacin and perfloxacin but

Fig. 3: Complete disappearance of ocular and oral lesions at 4th day of treatment (arrows)

sensitive to tetracycline, gentamicin and orfloxacin. Short-acting (5%) oxytetracycline and multivitamin preparation at the dosage of 10 mg kg^{-1} were injected once daily for 5 days intramuscularly. By the 2nd day of treatment, the ocular lesions began to clear and by the 4th day, the animal recovered completely (Fig. 3).

Researchers are not aware of any previous report of caprine moraxellosis in which extraocular lesions (lingual vesicles, erosive stomatitis and dental attrition) and abortion occurred. Ojo *et al*. (2009) observed only ocular lesions in a WAD goat herd in Abeokuta, Western Nigeria which is similar to that of other reporters in other countries. However, Norman and Elissaldle (1979) reported abortion in laboratory animals (rats, mice, guinea pigs and rabbits) experimentally-infected with *M. bovis* and related it to the number of viable organism injected, species of animal and stage of pregnancy.

The goat in this case was 3 months pregnant during which high nutritional requirement is needed for foetal development but because it was blind and had oral lesions, feeding would have been difficult if not impossible. This may have resulted to stress-induced abortion and emaciation. The animal could also have hit itself to obstacles in its environment due to blindness and

this may have resulted to threatened abortion. Since, the infection was chronic, it may be that hyperthermia resulting from bacteraemia (Akerstedt and Hofshagen, 2004) caused the abortion. This report may however suggest that the isolated *M. bovis* strain have the capacity to adhere to epithelial cells of mucous membranes and by systemic circulation invaded the animal causing uterine infection. It has been suggested that in goats, mammary gland and uterine infection may also occur simultaneously with keratoconjunctivitis (Aiello and Moses, 2002). *M. bovis* strains with rough colonies (virulent strains) have pili which are important structural attributes that enable them adhere to cell surfaces (Schurig *et al*., 1984; Brown *et al*., 1998; Biberstein and Hirsh, 1999).

The presence of large number of houseflies and Stomoxys in the animal's environment suggests that these arthropods may have transmitted the disease to the goat (Killinger *et al*., 1976; Gerhardt *et al*., 1982; Brown *et al*., 1998). These flies are usually found in abundance during rainy season (March to September) in Nigeria. The humid tropical climate of Nigeria favours their development.

Apart from the fact that we isolated *M. bovis* as the sole aetiopathogenic agent from the cultured samples, the results of the tests enabled us to rule out other pathogens that were earlier reported in IKC of goats. Result of RSAT made us to rule out brucellosis. Since, there was no nervous sign, listeriosis was not considered and this organism is rarely incriminated in IKC (Akerstedt and Hofshagen, 2004) and there was no nervous symptom. Trypanosomosis was ruled out because the palpable lymph nodes were not enlarged, wet mount and stained blood films were negative for haemoprotozoans and treatment with antibioitics was very effective. Moreover, WAD goats rarely do suffer from trypanosomosis since they are genetically trypanotolerant (Chiejina *et al*., 2009). Peste des Petit Ruminants (PPR) endemic in Nigeria causes erosive stomatitis in goats but was ruled out because there was no evidence of dehydration and enteritis (diarrhoea). The fact that the disease progressed despite the use of visine® which is a vasoconstrictor (Skilling *et al*., 2005) by the owner indicated that the drug was not effective against the aetiology since it was bacterial. In fact, the visine® seems to have exacerbated the infection by constricting the blood vessels supplying the eyes, thereby preventing the phagocytic cells from reaching the site of infection. This probably may have enabled the organism multiply more invading the oral and vaginal cavities. Healing without treatment (self-limiting infection) after few weeks (Akerstedt and Hofshagen, 2004) could have occurred if the owner did not apply visine®.

The slightly increased AST may have resulted from red blood cells haemolysis than from liver damage (Meyer *et al*., 1992) since the *M. bovis* strain, researchers isolated a haemolytic strain and ALT level was normal. Liver damage in ruminants often result in high serum AST levels and only slightly raised ALT (Alemu *et al*., 1977; Igado *et al*., 2011). Destruction of erythocytes may also have contributed to the hyperthermia. The high level of ALP may not be unconnected to the dental attrition because its activity increases in cases of dental and bone diseases in animals (Meyer *et al*., 1992). WAD goats have been suggested to have the tendency for Compensatory Accelerated Production (CAP) of PCV in case of infection (Daramola *et al*., 2005) and CAP has been shown to return PCV to normal following an infection (Dargie and Allonby, 1975).

Isolation of *M. bovis* from oral and vaginal swab samples in this present case, may suggest that this bacteria can adhere and survive in the oral and vaginal cavity of goats. As observed also in this study, ocular including nasal carriers of *M. bovis* have earlier been reported in ruminants (Brown *et al*., 1998).

The multidrug resistance exhibited by the isolate is similar to the report of Ojo *et al*. (2009) and calls for concern because *M. bovis* may have the capacity of transferring resistance genes to other bacterial species. The resistance may not be unconnected to the fact that most antibiotics have been tremendously abused. They are usually used in sub-therapeutic doses as growth-promoters in animal feeds which are often used in supplemental feeding of these ruminants. Also, they are used in treatment of animals and humans without conducting sensitivity tests by quacks in Nigeria. Therefore, the organism may have been exposed earlier to these drugs. This study indicates that in chronic moraxellosis of WAD goats, extraocular lesions could occur.

CONCLUSION

Although, it has been well documented that moraxellosis manifests clinically as keratoconjuctivits in ruminants and concurrent extraocular manifestations such as polyarthritis, mammary gland and uterine infection have been suggested in goats, no report has documented oral lesions and abortion as possible clinical manifestations in WAD goats. Brucellosis is usually considered the major abortion-causing infectious disease of ruminants in Nigeria but other diseases such as trypanosomosis, listeriosis and a myriad of other infectious and non-infectious diseases endemic in Nigeria

have also been reported to manifest clinically as abortion and keratoconjuctivitis in ruminants (Kummeneje and Mikkelsen, 1975; Saror, 1980).

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