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Pathogenicity of *Mycoplasma mycoides* subsp. *capri* in calves previously infected with *Trypanosoma congolense*

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ABSTRACT

Pathogenicity of *Mycoplasma mycoides* subsp. *capri* (Mmc) for calves with and without *Trypanosoma congolense* (Tc) infection was undertaken. Four calves were inoculated with Tc and two of them were challenged with Mmc, while one calf was inoculated with Mmc only; another calf was inoculated with sterile broth as control. No overt disease was found in the Mmc-infected calf. The two calves with dual Tc/Mmc infections died 53.0 ± 1.4 days post-inoculation, showing marbling and consolidation of the lungs. Histopathologically there was widening of interstitial spaces by fibrin and inflammatory cells. The spleen and lymph nodes showed lymphoid depletion, haemosiderosis and erythrophagocytosis. However, the two Tc-infected calves recovered following trypanocidal treatment. The mean PCV value (mPCV), of each of the four Tc-infected calves (22.5 ± 2.9%, 23.0 ± 2.6%, 25.0 ± 2.1%, 25.4 ± 2.5%, respectively) was significantly ($P<0.05$) lower than that of the control (30.1 ± 1.0%) and Mmc-infected calf (30.2 ± 1.0%), respectively. Similarly, the mean rectal temperature (mRT) of each of the two calves (41.6 ± 0.8 °C, 40.5 ± 0.8 °C) with dual Tc/Mmc infections was significantly ($P<0.05$) higher than that of Tc-infected calves (38.1 ± 0.5 °C and 38.0 ± 0.3°C), control calf (37.8 ± 0.3 °C) and Mmc-infected calf (37.7 ± 0.3 °C), respectively. These findings confirm the pervasiveness of *Mycoplasma mycoides* cluster It is suggested that calves in trypanosome endemic areas should be given regular prophylactic trypanocides treatment to rid them of trypanosomes which can cause immunosuppression, thereby predisposing calves to caprine strains of Mycoplasma.

Key words: cattle, *Trypanosoma congolense*, *Mycoplasma mycoides* subsp. *capri*, pleuropneumonia

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Introduction

Mycoplasma mycoides subsp. *capri* is a member of *Mycoplasma mycoides* cluster, a group of six closely related mycoplasmas (COTTEW et al., 1987). *Mycoplasma mycoides* subsp. *capri* was reported to cause a pattern of diseases similar to those induced by *Mycoplasma mycoides* subsp. *mycoides* large colony (LC) specifically in goats, including contagious agalactia, arthritis and pulmonary diseases (VILLALBA et al., 1992; KUMAR et al., 1994; MADANAT et al., 2001). Furthermore, *Mycoplasma mycoides* subsp. *capri*, was recently incriminated in abortions recorded in Hungarian goatherds (SZEREDI et al., 2003).

Mycoplasma mycoides subsp. *mycoides* small colony (SC) is generally regarded as the causative agent of contagious bovine pleuropneumonia (CBPP) (VILEI et al., 2000). However, pleuropneumonia was produced in calves inoculated with *Mycoplasma capricolum* subsp. *capricolum* with and without *Trypanosoma congolense* immunosupression (AJUWAPE et al., 2004a; AJUWAPE et al., 2005). Similarly, NICHOLAS et al. (2005), reported an outbreak of systemic disease in Vaal rhebok (*Pelea capreolus*) caused by *Mycoplasma capricolum* subsp. *Capricolum*, a caprine pathogen, and they highlighted the pervasiveness of mycoplasmas in the *mycoides* cluster in small ruminants and the potential of interspecies transmission and disease when different animal taxa come in contact. Typical CBPP lesions in natural mycoplasmal pneumonia of cattle have also been associated with *M. dispar*, *M. ovipneumoniae*, *M. bovis*, and *Acholeplasma laidlawii* where no *Mycoplasma mycoides* subsp. *mycoides* SC was isolated in any of the samples (EGWU et al., 1996). In Nigeria, like many countries in Africa, small ruminants, including goats and sheep, are herded together with cattle by pastoralists and the close contact may enhance the transmission of microbial pathogens such as *Mycoplasma* (IKEDE and TAIWO, 1985; STONE, 2002).

Trypanosomiasis is a haemoprotozoan disease which is endemic in the tropical regions of Africa and have been associated with immunosuppression (RURANGIRWA et al., 1978; NANTULYA, 1990).

As part of an on-going research on pathogenicity of caprine strains of "*Mycoplasma mycoides* subspecies *mycoides*" for cattle, this investigation was carried out to determine the pathogenicity of *Mycoplasma mycoides* subsp. *capri* for indigenous cattle breed (Sokoto Gudali) infected with and without *Trypanosoma congolense*.

Materials and methods

Mycoplasma mycoides subsp. *capri* Ib7 (local isolate, OJO, 1976) caprine strain obtained from Professor Ojo was used. The organism was grown in medium N (FREUNDT, 1983).

Trypanosoma congolense was obtained from the National Institute of Veterinary Research, Vom, Plateau State, Nigeria. The *T. congolense* was inoculated into Webster

mice and when the parasitaemia was 3.78×10^3 trypanosomes/mL. blood, the blood was collected for inoculation.

Animals. Six 9-month-old Sokoto Gudali calves purchased in Sokoto and transported to Ibadan by road were used. On arrival at Ibadan they were housed at the Large Animal Facilities of the Veterinary Teaching Hospital, University of Ibadan. The animals were kept in 3.96m × 3.96m concrete pens and treated with diminazene aceturate (Berenil®) intramuscularly, at a dose rate of 3.5 mg/kg body mass (BM), against haemoprotozoan parasites (*Trypanosoma* spp.). Additionally, on arrival at the University of Ibadan Veterinary Teaching Hospital they were dosed orally with tetramizole (Nilverm® I. C. I., Pharmaceuticals, U. K.) antihelmintic at a dose rate of 66 mg/kg BW. They were allowed to rest for 6 weeks, after which the animals were confirmed negative for the presence of haemoprotozoan parasites, especially *Babesia*, *Trypanosoma* and *Anaplasma* species and intestinal helminths by standard methods (SOULSBY, 1982). Clinical samples were obtained with the aid of sterile swabs from the eyes, ears, nostrils and rectum of each calf and examined microbiologically for *Mycoplasma* species (ERNØ and STIPKOVITS 1973a, b) and other bacteria (BARROW and FELTHAM, 1993).

Pathogenicity test. Four Sokoto Gudali bull calves (SG01 SG02 SG03 SG04) were respectively given 1.0 millilitre of 3.78×10^3 *T. congolense*/mL blood, intravenously through the jugular vein. Blood was collected from each bull every three days through the jugular vein to determine level of parasitaemia and packed cell volume (PCV) of the blood of each bull calf by standard methods (JAIN, 1986).

When the PCV of the blood of each bull calf was about 20%, two of the calves (SG03 and SG04) were treated with 10 mL of 2.3 g of diminazene aceturate intramuscularly. The remaining 2 calves (SG01 SG02) were inoculated intratracheally respectively with 10 mL of 1×10^9 CFU/mL. of *M. mycoides* subsp. *capri*. One Sokoto Gudali (SG05) calf was inoculated intratracheally with 10 mL of 1×10^9 CFU/mL of *Mycoplasma mycoides* subsp. *capri* only, while the calf (SG06) given 10 mL of sterile medium N intratracheally served as control.

The rectal temperature of each bull calf was recorded daily. Blood samples were collected from each animal every three days until termination of the experiment to monitor the PCV of the animals and to check for parasitaemia in each calf.

Necropsy. Post-mortem examination was carried out on the dead animals. Tissues from the organs, including the lungs, liver, spleen, kidneys, and lymph nodes showing macroscopical lesions, were collected for histopathological and bacteriological examination. Histopathology tissues were fixed in 10% phosphate-buffered formalin. Thin sections (5 μm) of the tissues were made and stained with haematoxylin and eosin for histological examination. Tissue specimens for bacteriology were put in sterile universal bottles and kept at -20 °C until cultured.

Statistical analysis. Packed cell volume (PCV) and daily rectal temperature values of the infected groups of calves and the control groups were compared by the Duncan's Multiple Range Test using Statistical Analysis System (SAS, 1987) computer programme. Tests were carried out at 95% ($P<0.05$), 99% ($P<0.01$) and 99.9% ($P<0.001$) levels of confidence.

Results

The mean PCV value (mPCV), of each of the four *Trypanosoma congolense*-infected SG calves (22.5 ± 2.9%, 23.0 ± 2.6%, 25.0 ± 2.1%, 25.4 ± 2.5%) was significantly ($P<0.05$) lower than that of the control (30.1 ± 1.0%) and (30.2 ± 1.0%) recorded for calf inoculated with only Mccp (Table 1). The mean rectal temperature (mRT), of each of the two calves (41.6 ± 0.8 °C, 40.5 ± 0.8 °C) infected with *Trypanosoma congolense* and *Mycoplasma mycoides* subsp. *capri* was significantly ($P<0.05$) higher than that of 38.1 ± 0.5 °C and 38.0 ± 0.3 °C) with *Trypanosoma congolense* infections the control (37.8 ± 0.3 °C) and (37.7 ± 0.3 °C) recorded in calf inoculated with only *Mycoplasma mycoides* subsp. *capri* and sterile broth, respectively.

Table 1. Comparative PCV (%) and rectal temperature (°C) of Sokoto Gudali calves inoculated with *Mycoplasma mycoides* subsp. *capri* with and without infection by *Trypanosoma congolense*

GRP	SG01^{T}*	SG02^{T}*	SG03^{T}	SG04^{T}	SG05*	SG06**
PCV	22.5 ± 2.9^{a}	23.0 ± 2.6^{a}	25.0 ± 2.1^{a}	25.4 ± 2.5^{b}	30.1 ± 1.0^{c}	30.2 ± 1.0^{c}
n	20	18	23	23	23	23
Temp	41.6 ± 0.8^{a}	40.5 ± 0.8^{a}	38.1 ± 0.5^{b}	38.0 ± 0.3^{b}	37.8 ± 0.3^{bc}	37.7 ± 0.3^{c}
n	52	54	60	60	60	60

Data expressed as mean ± standard deviation; n = Number of Replicates; Means on the same column with the same superscripts are not significantly different ($P<0.05$); * Calves inoculated with *Mycoplasma mycoides* subsp. *capri*; ** Calf inoculated with medium N (Control). Calves SG01 and SG02 died on 53.0 ± 1.4 days p.i. Calves SG03 and SG04 were treated with Berenil® 33 and 35 days p.i., respectively.

The four bull calves (SG01, SG02, SG03 and SG04) infected with *T. congolense* became progressively weaker, moderately emaciated and developed relative anaemia, whereas the PCV of the calves (SG05 and SG06) that were inoculated with *M. mycoides* subsp. *capri* only and sterile N broth respectively did not fall until termination of the experiment. The 2 bull calves (SG01 and SG02) infected with *T. congolense* and later inoculated with *M. mycoides* subsp. *capri* became severely weak, emaciated, and exhibited dry rales and respiratory distress. Both developed bilateral swollen and painful hock joints and later

became recumbent. From the 48th day post-infection onwards they could not stand, until they died 4 and 6 days later, respectively. The PCV values of two (SG03 and SG04) bull calves inoculated with *T. congolense* and subsequently treated with Berenil®, showed steady recovery after treatment.

Table 2. Pathological findings in calves experimentally infected *T. congolense* and subsequently challenged with *Mycoplasma mycoides* subsp. *capri*

Lesion	SG01	SG02
Fibrin strands in the thoracic cavity	+	+++
Pulmonary marbling / consolidation.	++	+++
Congestion of the pulmonary blood vessels	+	++
Enlarged lymph nodes / Spleen	++	++
Histopathological widening of interstitial spaces by fibrin and inflammatory cells	+	+++
Histopathological necrosis loss of splenic architecture and diffuse haemosiderosis	++	+++

+ weak; ++ moderate; +++ marked

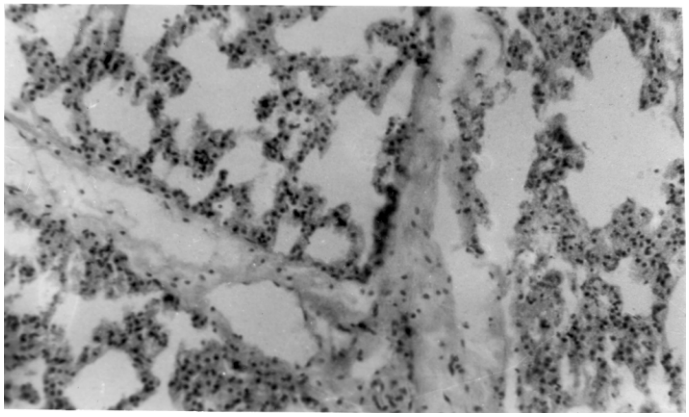

Fig. 1. Histological section of pneumonic lung showing oedema, widening of interlobular and interalveolar septae and inflammatory cells trapped in the fibrin; × 250

At post-mortem, lungs were oedematous, swollen and showed diffuse marbling. The visceral pleura was thickened and cloudy, while there was moderate to severe congestion of the dorsal part of the diaphragmatic and cardiac lobes. The spleens were enlarged and the bronchial, prescapular and prefemoral lymph nodes were soft and enlarged (Table 2). However, the synovial fluid of the hock joints was clear. Histopathological changes included pulmonary marbling and oedema, severe thickening of the visceral pleura and widening

of interstitial spaces by fibrin and inflammatory cells (Fig. 1). The spleen and lymph node showed necrosis loss of the architecture and diffuse haemosiderosis (Fig. 2).

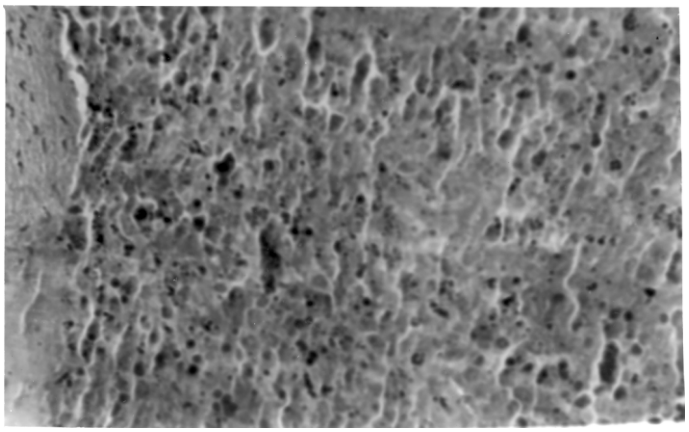

Fig. 2. Histological section of spleen from calf infected with *Trypanosoma congolense* and subsequently challenged with *Mycoplasma mycoides* subsp. *capri* showing haemosiderin pigments; × 450

Bacteriology. Mycoplasma mycoides subsp. *capri* was isolated in pure culture from the synovial fluid, liver, lungs, spleen and bronchial lymph node of the two animals (ERNØ and STIPKOVITS, 1973a, b). No *Mycoplasma* was isolated from the clinical samples collected from the eyes, ears, nostrils and rectum.

Discussion

In Nigeria, different investigators have reported normal PCV values in indigenous Zebu cattle. For instance, SAROR and COLES (1975) documented a normal mean PCV value of 30.0 ± 3.0%, while IKEDE and TAIWO (1985) recorded normal a mean PCV value of 31.2 ± 4.7% for normal parasite-free White Fulani, Red Bororo and Sokoto Gudali. In trypanosomiasis PCV value remains an index of anaemia since no evidence of haemodilution has been found in trypanosome-infected cattle (MURRAY and DEXTER, 1988). The PCV values recorded for the negative control calf and the calf inoculated with only *Mycoplasma mycoides* subsp. *capri* alone in this study fell within the values reported above. The mean PCV of calves given *M. mycoides* subsp. *capri* and ordinary medium respectively, showed no significant difference ($P>0.05$). Thus, it appears that *M. mycoides* subsp. *capri* may not adversely affect the values of erythrocytic series. This may not be unconnected with the fact that this Mycoplasma species is not associated with haemagglutinating properties,

unlike the avian mycoplasmas: *M. meleagrides*, *M. gallisepticum* and *M. synoviae*, or human pathogen *M. pneumoniae* (ALAFIATAYO et al., 1990). The study of haematological changes in mycoplasma infections could provide additional diagnostic criteria and assist in further understanding of the pathogenic and body defense mechanisms in livestock mycoplasmosis.

The calves with dual (*Trypanosoma congolense* and *Mycoplasma mycoides* subsp. *capri*) infection showed pleuropneumonia. Likewise, the mean rectal temperature of each calf with *T. congolense* and *M. mycoides* subsp. *capri* infection was significantly higher than SG03, SG04, SG05 and SG06, respectively. Furthermore, on investigation no errant disease or clinical signs were produced in the bull inoculated with *M. mycoides* subsp. *capri*. These findings agree with earlier reports that *T. congolense* and *T. vivax* produced immunosuppressive effect on the secondary immune response of cattle to *M. mycoides* subsp. *mycoides* (RURANGIRWA et al., 1978; ILEMOBADE et al., 1982). Immunosuppressive effect of trypanosomiasis was incriminated in outbreaks of contagious bovine pleuropneumonia in vaccinated herds in Nigeria reported by OSIYEMI et al. (1985). A similar finding was documented by AJUWAPE et al. (2004b) in calves inoculated with *Mycoplasma capricolum* subsp. *capripneumoniae*, a caprine strain of Mycoplasma, whereas *Mycoplasma capricolum* subsp. *capricolum* produced typical pleuropneumonia without *T. congolense* immunosuppression (AJUWAPE et al., 2005).

Pure culture of *M. mycoides* subsp. *capri* was isolated from the liver, spleen, lungs, bronchial lymph node and synovial fluid collected at necropsy from the calves that died as a result of dual *T. congolense* and *M. mycoides* subsp. *capri* infections. This agrees with the documented pervasiveness of mycoplasmas in the *mycoides* cluster in small ruminants and the potential of interspecies transmission and disease when different animal taxa come into contact NICHOLAS et al. (2005).

The majority of natural Trypanosome infections are chronic and often aparasitaemic, a large number of which may go undetected by parasitological methods (NANTULYA, 1990). It is suggested that calves in trypanosome endemic areas should be given regular prophylactic treatment with trypanocides to prevent calves' immunosuppression which predisposes to caprine strains of Mycoplasma such as *Mycoplasma mycoides* subsp. *capri*.

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SAŽETAK

Istražena je patogenost vrste *Mycoplasma mycoides* subsp. *capri* u neinvadirane teladi i teladi invadirane praživotinjom *Trypanosoma congolense*. Četiri teleta bila su invadirana vrstom *Trypanosoma congolense*. Od toga su dva teleta naknadno bila zaražena vrstom *Mycoplasma mycoides* subsp. *capri*. Jedno je tele bilo zaraženo samo vrstom *Mycoplasma mycoides* subsp. *capri*, a jedno kontrolno tele dobilo je sterilnu hranjivu podlogu. Nije ustanovljeno očitovanje bolesti u teleta inficiranog mikoplazmom. Dva teleta inficirana objema spomenutim vrstama uginula su 53. odnosno 54. dana nakon inokulacije pokazujući mramoriranost i konsolidaciju pluća. Patohistološki je ustanovljeno proširenje i fibrinizacija intersticijskih prostora i upalne stanice. U slezeni i limfnim čvorovima ustanovljena je limfoidna deplecija, hemosideroza i eritrofagocitoza. Dva teleta invadirana praživotinjom *Trypanosoma congolense* oporavila su se nakon terapije tripanocidnim lijekovima. Srednja vrijednost hematokrita svakoga od četiriju teladi invadirane tripanosomom (22,5 ± 2,9%, 23,0 ± 2,6%, 25,0 ± 2,1%, 25,4 ± 2,5%) bila je značajno manja ($P<0,05$) nego u kontrolnog teleta (30,1 ± 1,0%) i mikoplazmom inficirana teleta (30,2 ± 1,0%). Slično je i srednja vrijednost rektalne temperature svakog teleta s dvojnom infekcijom (41,6 ± 0,8 ^{0}C, 40,5 ± 0,8 ^{0}C) bila značajno ($P<0,05$) veća nego u teladi invadirane tripanosomom (38,1± 0,5 ^{0}C i 38,0 ± 0,3 ^{0}C), kontrolnog teleta (37,8 ± 0,3 ^{0}C) i teleta inficiranog mikoplazmom (37,7 ± 0,3 ^{0}C). Rezultati upozoravaju na širenje skupine *Mycoplasma mycoides*. Preporučuje se da se teladi u enzootskim područjima redovito daju tripanocidna sredstva da bi se očistila od tripanosoma, koje mogu uzrokovati imunosupresiju i time olakšati infekciju teladi kozjim sojevima mikoplazme.

Ključne riječi: govedo, *Trypanosoma congolense*, *Mycoplasma mycoides* subsp. *capri*, pleuropneumonija