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Isolation and antibiogram of pneumonic pasteurellosis causing microbes from nasopharynx of transport stressed Nigerian goats

Benjamin O. Emikpe[1,2], Lateef A. Tijani[3], Mariam O. Karim[4], Alao S. Omoniyi[4] and Ibikunle M. Akanbi[5]

[1]Department of Veterinary Pathology, University of Ibadan, Ibadan, Oyo State, Nigeria.
[2]School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[3]Department of Animal Production Technology, Lagos State Polytechnic, Ikorodu, Lagos State, Nigeria.
[4]Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria.
[5]Department of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

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This investigation reports the isolation and antibiogram of pneumonic pasteurellosis causing microbes, *Mannheimia haemolytica* (MH) and *Pasturella multocida* (PM) from the nasopharynx of transport stressed Nigerian goats. Nasal swabs were taken from 63 that were transport stressed (group A), 21 goats that were transport stressed followed by rest for two weeks (group B) and 21 resident goats which were not transported (group C). The characterisation, identification of the isolates was carried out using standard methods while the antibiotics sensitivity test was by disc diffusion technique. The rate of isolation and load of MH and PM from the nasal cavity was significantly higher in transport-stressed goats while there was no significance difference between groups B and C. The duration of journey did not significantly affect the isolation rate of the two organisms except the bacterial load of MH. Most isolates were susceptible to the quinolones (cefuroxime, ciprofloxacin and ofloxacin) and resistant to gentamycin, augmentin, nitrofurantoin, ceflazidime and ampicillin. Efforts should be geared towards improving state of Nigerian roads, animal transportation and the treatment plan that is commonly used for stabilising transport-stressed animals especially with the use of rest and appropriate antibiotics prophylaxis.

Key words: Goats, transport-stress, *Mannheimia hemolytica*, *Pasteurella multocida*, Nigeria.

INTRODUCTION

The management of goats in most parts of Africa is often extensive and it's primarily in the hands of women and children. They commonly serve as source of income and meat to the rural poor (Diallo, 2006). Goats thrive in most ecological zones but often than not, they predominantly domiciled in the arid and semi arid areas of Nigeria where there are vast expanse of land to support their production. The transportation of such animals from this area to other parts had become an age long, inevitable husbandry practice (Fazio and Ferlazzo, 2003; Minka and Ayo, 2007) especially in Nigeria where most of the food animals are often transported from the dry north to the humid south by road, either to be slaughtered or intensively reared. However there is no concrete information or policy on the transportation of animals in Nigeria, compared to the European Union.

*Corresponding author. E-mail: banabis2001@yahoo.com, benabis2001@gmail.com.

Transportation of animals as defined by the European directive, (European Union (EU) 2002) involves road transportation of live animals, in which the process of handling, loading and unloading had been regarded as stressful period than the journey itself (Minka and Ayo, 2007). During this period of transportation, the health and productivity of animals are significantly affected due to the deplorable state of major roads in Nigeria (Meludu, 2008) and the long distance in which the animals are subjected to.

The need to understand the behaviour, the physiological and pathological derangements associated with the transport of animal under these deplorable conditions when moved from one zone to the other cannot be overemphasised. This understanding will help in ensuring enforcement of code of practice/legislative control on animal welfare (Grandin, 1989) which will go a long way to ensure the safety of the transported animals and their handlers.

Numerous investigations abound on the welfare of livestock during road transportation and the effects on behavioural changes and physiological parameters (Kannan et al., 2000; Ayo et al., 2002; 2006, Odore et al., 2004; Minka and Ayo, 2007) but very little information are on the pathological derangements (Jasni et al., 1991; Emikpe et al., 2013) albeit the bacteriological changes.

In Nigeria, majority of the slaughtered and breeding goats transported down south by road (Minka and Ayo, 2007) are often predisposed to respiratory diseases which include peste des petits ruminants (PPR) and *Mannheimiosis* (*Pasteurellosis*, Shipping fever). The two diseases had been observed to co-exist in nature and the combined infection had been reported to be fatal (Emikpe et al., 2010).

The bacteria usually associated with caprine pneumonia especially pneumonic pasteurellosis are *Mannheimia haemolytica* (MH) and *Pasteurella. multocida* (PM) (Brogden et al., 1998, Emikpe et al., 2013). They are normal flora of the respiratory tract of goats (Emikpe et al., 2009) which can be pathogenic when the animal is subjected to stressful conditions. In Nigeria, with the deteriorated state of roads, transporting vehicles and the fact that animals are transported over more than 1 500 to 2 000 km which could span days, the possibility of being predisposed to respiratory infection is high.

With the investigations on the bacterial flora of the respiratory tract of Nigerian goats being on the pneumonic lungs (Tijjani et al., 2012a,b) and apparently normal nasal passage (Emikpe et al., 2009), it is expedient to understand the effect of transport stress on the nasal bacterial flora especially pneumonic pasteurellosis causing microbes, *Mannheimia haemolytica* (MH) and *Pasturella multocida* (PM). The aim of the present study was to investigate the effect of transport, as stress factor, on the isolation of pneumonic pasteurellosis causing microbes from nasopharynx of goats as well as the possible implications and subsequently method of control.

MATERIALS AND METHODS

Study area

Ibadan city is situated at latitude 07°20´ North and longitude 03°50´ East. Ibadan is one of the largest urban cities in Nigeria and West Africa with over one million residents and thousands of daily migrants. Bodija small ruminant market is a spacious market constructed by the Oyo State government. Sheep and goats from different parts of Nigeria are brought to the market and subsequently conveyed to different abattoirs, farms and households throughout the state.

Animals

A total of one hundred and five Nigerian goats were purposely selected based on the criteria used for this investigation which include transportation, duration of transport and age of the goats. Nasal swabs were taken from twenty one goats per location used (Niger, Kaduna, Sokoto and Miaduguri) which are state capital cities where goats are often transported to the study area. The goats were divided into three groups, group A: 63 goats with transport stress; group B: 21 goats with transport stress followed by rest for two weeks and; group C: 21 resident goats without any stress were used as control. The goats enrolled in this study were between two to three years old.

The distance of transportation ranged between from 350 to 1 800 km to the point of samples collection (Figure 1). The goats were fed with chopped dry grass and groundnut chaffs while water was supplied ad libitum before the commencement of the journey however; the goats were often not fed or given water in the course of the journey which could take two to four days.

Sample collection

Nasal swabs were collected from goats after arrival (group A), those that have rested for two weeks after arrival (group B) and from resident goats within the area, not stressed (group C, Control). Nasal samples were collected aseptically in all the groups by inserting sterile cotton tipped applicator sticks or swab into the nasal passage after proper cleaning and disinfection of the external nares. Each nasal swab on swab stick was carefully cut into a well labelled bottle containing 2 ml brain heart infusion broth. The swabs were transported in a deep fridge cold box to the laboratory for bacterial isolation.

Bacteriological examination

The characterization and identification of the bacterial isolates were carried out using standard methods as previously described by Quinn et al. (1994). Each nasal swab was removed from the bottle and streaked over the plates containing blood agar – base supplemented with 7% sheep blood and MacConkey agar. The streaking was further spread with inoculating loop to aid colony isolation. The plates were labelled and incubated aerobically at 37°C for 48 h (Carter, 1984). After taking note of cultural growth characteristics, positive cultures were subjected to Gram's staining properties and cellular morphology under 1000x objective of light microscope. Mixed colonies and Gram-negative bacteria were sub cultured on both blood and McConkey agars and incubated

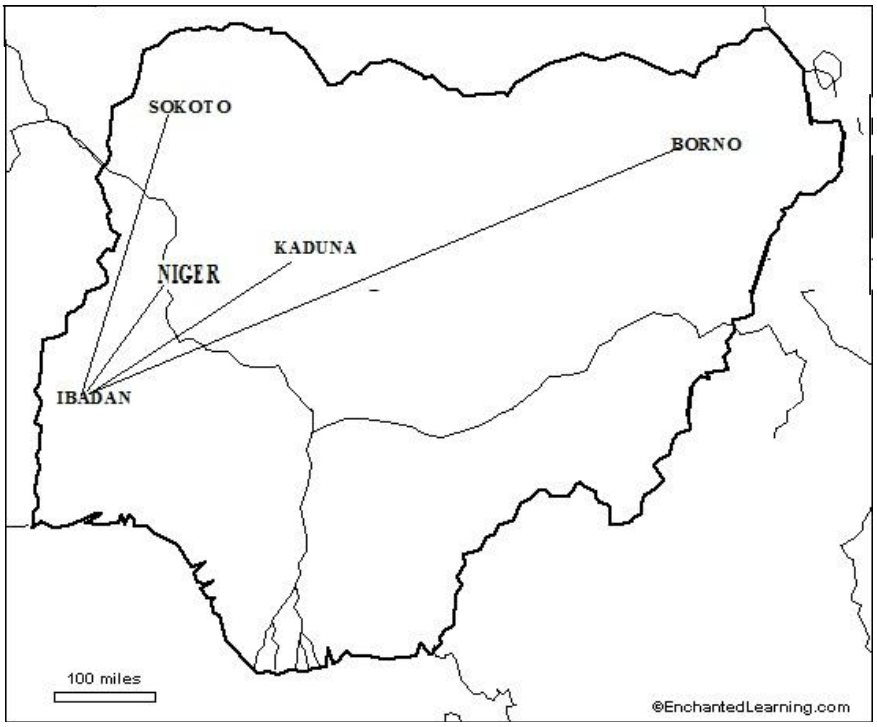

Figure 1. The areas where goats are often transported to the study area.

Table 1. Effect of transport stress on the isolation rate and nasal *Mannheimia haemolytica* and *Pasteurella multocida* load.

Grouping	*Mannheimia haemolytica* isolation rate (%)	Load × 10^8	*Pasteurella multocida* Isolation rate (%)	Load × 10^8
Transported	70	70.5 ± 5.5	95	47.2 ± 4.5
Two weeks after	70	11.3* ± 2.3	100	21.5* ± 3.4
Control (not transported)	80	6.6* ± 1.5	90	15.3* ± 2.5

*Not significantly different (p>0.05).

aerobically for further 24 h. Pure culture of single colony type, from both blood and McConkey agars were transferred onto nutrient agar- slants for a series of biochemical tests including catalase, oxidase and fermentative/oxidative tests for final identification following standard procedures (Quinn et al., 1994). All the isolates of *P. multocida* were catalase and indole positive and did not grow on MacConkey agar while those of *Mannheimia hemolytica* grew on MacConkey and did not produce indole (Tefera and Smola, 2002). Further identification using API test kit was not attempted.

For antibiotic sensitivity test, newer and some common anti-biotics were used. The antibiotics include cephalosporins (cefuroxime, ciprofloxacin and ofloxacin), gentamycin, augmentin, nitrofurantoin and ampicillin. The disc diffusion technique was employed and inhibition observed as clear zones around the antibiotics. Inhibition zones were measured using meter rule and measurement greater than 0.5 cm was regarded as susceptibility (Emikpe et al., 2009).

Statistical analysis

Descriptive statistics was used to summarise the data generated from the study. The effect of transport and duration were evaluated using one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006, version 15.0). Duncan's multiple range tests were used to compare differences among individual means.

RESULTS

The frequency and the load of MH and PM isolation were varied within the groups as shown in Table 1. The isolation and load of MH and PM from the nasal cavity were significantly *(P<0.05)* higher in transport stressed goats than goats rested for two weeks or not subjected to transportation stress.

The effect of duration of journey on the isolation rate and nasal bacterial load were shown in Table 2. The duration of journey did not significantly *(P < 0.05)* affect the isolation rates of MH and PM, while the MH load was significantly affected by the distance of journey.

Table 2. Effect of the distance of journey on the isolation rate of nasal *Mannheimia haemolytica* and *Pasteurella multocida* load.

Duration of journey	MH isolation rate (%)	Load × 10^8	PM isolation rates (%)	Load × 10^8
2 days	80	$52.0^{(a)} \pm 4.3$	100	$47.2^{(a)} \pm 4.5$
3 days	50	$26.7^{(a)} \pm 3.5$	83.3	$21.5^{(b)} \pm 3.3$
4 days	70	108.0 ± 7.4	80.0	$56.0^{(a)} \pm 4.2$
Control (not transported)	0	6.6 ± 2.1	90.0	$15.3^{(b)} \pm 2.4$

*Same alphabet in the same group are not significantly different $p>0.05$.

Table 3. Anti microbial sensitivity pattern of the isolation rate of nasal *Mannheimia haemolytica* and *Pasteurella multocida*.

Bacteria	AMP	CAZ	CRX	GEM	CPR	OFL	AUG	NIT
Mannhemia haemolytica	R	R	S	S	S	S	R	R
Pasteurella multocida	R	R	PS	S	S	S	R	S

R: resistant, S: sensitive, PS: partially sensitive, AMP: ampicillin 25 µg, CAZ, ceflazidime 30 µg, CRX cefuroxime 30 µg, GEN gentamicin 30 µg, CPR ciprofloxacin 10 µg, OFL, ofloxacin 5 µg, AUGaugmentin 30 µg, NIT nitrofurantoin 300 µg.

The antibiotics resistance pattern showed that the isolates were resistant to ampicillin, gentamycin and augmentin, but susceptible to cefuroxime, ciprofloxacin and ofloxacin (Table 3).

DISCUSSION

The significant higher percentage of isolation and load value of MH and PM in the nasal cavity of transport stressed goats in this study suggest their potential risk in the establishment of pneumopathy in stressed animals (Brogden et al., 1998; Emikpe et al., 2013). *Mannheimia haemolytica* and *Pasteurella multocida* were isolated immediately after the arrival of the transported goats which suggested that transportation could enhance the isolation rate of the organisms from the nasal cavity of transported goats. Duration of journey might increased the chance of isolation as opined by some workers (Zamri-Saad et al.,1989) as time is required for active proliferation and persistence of the bacteria in the nasal cavity. The mechanism of the persistence and proliferation is that stress factors with or without viral infection suppress the mucociliary clearance (Brogden et al., 1998) and an abrupt shift from commensals to pathogenic (Gonzalez and Maheswaran, 1993) form which may account for their involvement in most pneumonia associated with stress.

Resting allowance of two weeks after arrival could alleviate the transport-stress and reduce the proliferation of *Mannheimia haemolytica* and *Pasteurella multocida* in the nasopharnyx with time (Pass and Thompson, 1971). This observed marked reduction in bacterial load and isolation rate possibly due to acclimatization may account for the usual two weeks stabilization of animals after stressful farm operations and in quarantine procedure (Zamri-Saad et al., 1989).

In this study, we observed no significant *(P<0.05)* correlation between PM load or isolation rate and the distance of journey while in MH, there was a significant *(P<0.05)* correlation between bacterial load and the distance of journey, this observation further lend credence to the fact that MH is more associated with transport stress than PM in Nigeria unlike the reverse in Malaysia (Emikpe et al., 2013). The possible effect of distance and the period of resting phases in the course of the journey on the bacterial load may need to be further investigated.

In this study, the drug resistance pattern was similar for the two bacteria with isolates being susceptible to the cephalosporins (cefuroxime, ciprofloxacin and ofloxacin) while most were resistant to gentamycin, augmentin, nitrofurantoin, ceflazidime and ampicillin. The antibiotic sensitivity pattern observed further revealed that the bacteria are resistant to expensive and newer antibiotics and were resistant to common antibiotics which are easily bought off the shelf without appropriate prescription from practicing veterinarians and are often prone to adulteration.

Conclusion

Efforts should be geared towards improving the treatment plan that is commonly used for stabilising transport-stressed animals especially with appropriate antibiotics prophylaxis. The prophylaxis may possibly involve vaccination strategies which may help in the control of bacte-

rial pneumo-pathy associated with road transport in small ruminants. Effort should also be geared toward improving the state of the roads in Nigeria to reduce the stress associated with road transportation.

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