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Full Length Research Paper

Intranasal Inactivated Recombinant *Mannheimia hemolytica* Vaccine is not protective against naturally occurring Pneumonia in Nigerian Goats

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ABSTRACT

Pneumonia had been a major constraint to the development of small ruminant production and its control is expedient. This experiment was conducted to evaluate the protective effect of intranasal recombinant *Mannheimia hemolytica* vaccine against naturally occurring pneumonia in Nigerian goats. Twenty one goats were divided into five groups. Five goats each were in vaccinated groups while three goats were in the control groups. Group A was vaccinated once; group B was vaccinated twice at one week interval, and group D at two weeks interval, while group C and E were the positive and negative control groups. All goats including the vaccinated groups were challenged two weeks after the last vaccination by comingling with goats that had pneumonia for three weeks. The clinical, bacterial isolation, virus detection, lung consolidation, gross and histopathological score were employed using standard techniques. All data were analyzed statistically using the one-way analysis of variance (ANOVA). Group C and D had the highest clinical score following challenge while deaths were observed in all with the lowest in group B. The lung consolidation score and lesions in group C and D were significantly ($P<0.05$) severe than A and B. Similarly, the lesions in group B was lower than A ($P>0.05$). This study showed that the recombinant MH vaccine reduced the clinical manifestations especially when administered at a week interval more than once but was not sufficient to protect against naturally occurring caprine pneumonia commonly observed hence a need for modification to be effective in Nigeria.

Keywords: Intranasal route; pneumonia, Nigerian goats, recombinant *Mannheimia hemolytica* vaccine

INTRODUCTION

African small ruminant population is about 171 million and 34.5 million of which were found in Nigeria. Goats are the major source of meat, milk, hides, and skin (Williamson and Payne, 1984) and they play an important role in the welfare of smallholder arable farmer by providing income for women and children who are often entrusted with the care.

Pneumonia still remains a major constraint to small ruminant production in sub-Saharan Africa (Adamu *et*

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al., 2010, Elsheikh and Hassan, 2012, Tijjani, *et al*., 2012) and other part of the world (Yener *et al*., 2009, Momin *et al*., 2011, Azizi *et al*., 2013). Peste des Petit Ruminants and mannheimiosis are the most important viral and bacterial cause of caprine pneumonia in Africa. Although factors such as stress due to transportation and overcrowded housing do predisposes to caprine pneumonia but investigation into the role in this condition is still scanty (Elsheikh and Hassan, 2012, Emikpe *et al*., 2013). Early detection of caprine pneumonia remains a key to successful treatment but often the disease are detected at advanced stages where the response to antibiotics is poor and case mortality rates can be 100% (Weiser *et al*., 2009).

The prevention of mannheimiosis with the use of bacterine had been encouraging with the development of several commercial vaccines which include live attenuated leukotoxin, capsule, lipopolysaccaride, subunit vaccines, sodium salicilate extract and potassium thiocyanate (Mohamed *et al*., 2008, Weiser *et al*., 2009). Most of the available vaccines contain *M. haemolytica* A2 which had been reported to be poorly immunogenic, antigenic and cannot cross-protect against infections with other *M. haemolytica* serotypes (Singh *et al*., 2011).

In order to control this obvious problem, a vaccine which has potential to provide protection against *M. haemolytica* serotypes A2, A7 and A9 is essential. In search for this, a recombinant vaccine for mannheimiosis (RVM) was developed in our laboratory in year 2003 with the use of the outer membrane proteins (Omps) which were found to be immunogenic (Sabri *et al*., 2000). The vaccine induced satisfactory immune response (Sabri, 2006, Sabri *et al*., 2013) hence the need for an evaluation against naturally occurring pneumonia in different countries. To evaluate this, Nigeria was chosen because of the obvious challenges of pneumonia in small ruminants (Emikpe and Akpavie 2010a, Emikpe *et al*., 2013) and the use of bacterin is nonexistent. With concerted efforts being focused on the use of PPR vaccine alone in Nigeria with very little information on the possible use of MH bacterin, this experiment was conducted to evaluate the intranasal recombinant MH bacterin against naturally occurring pneumonia in Nigerian goats.

MATERIALS AND METHODS

Study location

The pens meant for small ruminants in the experimental animal unit of the Faculty of Veterinary Medicine, University of Ibadan was used for this study.

Experimental Animals

Twenty one apparently healthy West Africa Dwarf goats (WAD) 6 months of age were used for the experiment. They were purchased from recognized private breeders in Ibadan and its environs. The animals were housed in a well partitioned pen on concrete floor, conditioned for 14 days before the commencement of study. Wheat bran, grass and water were provided *ad libitum* daily.

The animals were bled prior to vaccination to obtain a baseline data for some hematological values; they were later tagged and treated intramuscularly with a broad spectrum antibiotic (Oxytetracycline hydrochloride) at 1 ml/kg body weight/ per animal against bacterial infections. Subcutaneous injection of ivermectin (ivomec^{R}) was also given against parasitic infestations at dose rate of 1 ml/50kg body weight of the animal. The nasal swabs obtained from the animals were negative by cultural isolation for most respiratory pathogens which includes *Mannheimia haemolytica, Pasteurella multocida,* Mycoplasmma spp. Chlamydia spp. and Haemophilus spp. PPR antigen was also negative by Agar gel precipitation test prior to vaccine administration.

Vaccine

The vaccine contained cultures of the recombinant cell (Malaysian patent no. PI 2007 0305 on "*Mannheimia haemolytica* bacterial polypeptides and sequences, gene sequences and uses thereof" in the name of Universiti Putra Malaysia) prepared using pET-Blue-2 (Merck) were harvested and killed in 0.5% formalin-PBS overnight (Sabri, 2006). This was later rinsed in sterile PBS thrice to ensure the complete removal of the formalin. Finally, the recombinant cells were re-suspended in sterile PBS as stock vaccine seed. Adequate amount of sterile phosphate buffer saline (PBS) was added to the stock vaccine seed to give a final concentration of 1.0×10^5 CFU/ml. The sterility of the vaccine was tested by inoculating 0.1 ml of the vaccine onto blood agar followed by incubation at 37^0C for 24 h. The vaccine was considered sterile when no bacterial growth appeared on blood agar (Sabri, 2006).

Vaccination

The goats were divided into five groups with five goats each in vaccinated groups while three goats each serve as positive and negative control. Group A was vaccinated once; group B was vaccinated twice at one week interval, and group D at two weeks interval, while group C were the unvaccinated, challenged. The goats were vaccinated intranasally as described by Emikpe *et al*., (2013).

Challenge Infection

The vaccinated and control groups were challenged two weeks after the last vaccination by comingling with pneumonic goats to simulate the field experience.

The pneumonic goats used for the challenge were obtained from the market after being observed by clinicians to be pneumonic and the nasal swabs of the animals yielded high load of *Mannheimia haemolytica* by cultural isolation prior to the comingling. On introduction to the different groups, the goats were then monitored clinically.

The study was independently reviewed and approved by an ethical board of the Faculty of Veterinary Medicine, University of Ibadan and adequate measures were taken to minimize pain or discomfort.

Clinical Examination

Each of the animals was clinically examined daily and the clinical parameters evaluated included: clinical signs, the respiratory rate, and rectal temperature. The body weights were recorded weekly and the timing of the clinical signs of pyrexia, dyspnoea, weakness, nasal discharge, anorexia, other respiratory and non-specific signs were also noted daily. Post mortem examination was done on dead goats. Samples were obtained for bacteriology and pathology.

Microbiology

For bacteriology, swabs made from lung tissues from each tagged goat were placed in brain heart infusion broths which were incubated for 24 hours at 37°C before being brought out for sub-culturing into the agar. Each plate was labeled properly according to the tag numbers of the goats on the sample bottle. Each goat had two bacterial culture plates (Blood Agar and MacConkey Agar). The inoculated plates were arranged in candle jar and placed inside the incubator at 37°C for 18 – 24 hours. Pure cultures were later obtained after sub-culturing. Characterization and identification of the isolates were later carried out using standard methods (Odugbo *et al*., 2004a). PPR virus detection in tissue was attempted by employing agar gel precipitation test; this was based on the clinico-pathological presentation observed in the course of the experiment (Emikpe and Akpavie 2011).

Pathology

Post mortem examination was done on animals that died and those that were euthanized. Samples from the lungs were collected in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin for histological examination using x40 of the light microscope. For the lung pathology, the degree of consolidation or pneumonia was expressed as a percentage of the total lung volume (Odugbo *et al*., 2004b). Other lesions were recorded as they occur in the course of the experiment.

Statistical Analysis

Statistical analysis was carried out with ANOVA and Duncan multiple range test of significance for means of the parameters recorded

RESULTS

Clinical Features

As shown in Figure 1, group A animals had a relatively stable respiratory rate except on the 4th and 7th week where there was a slight increase up to the 8th week, as compared to group B animals which also had a relatively stable respiratory rate that peaked at the third week, 7th and 8th weeks. Group C animals showed peaks at the 4th and 7th weeks. Group D animals showed similar peaks at the 5th, 7th and 8th week. As shown in Figure 2, all the groups had an increasing temperature until the 5th and 6th week where there was a decrease.

As shown in Table 1, there was a decrease in weight in group A, after the 4th week and in group D after the 5th week, while in groups C and D, it occurred at the 7th and 8th week respectively. The most pronounced clinical features observed across the groups were emaciation and dullness with group D and positive control group showing more severe clinical features.

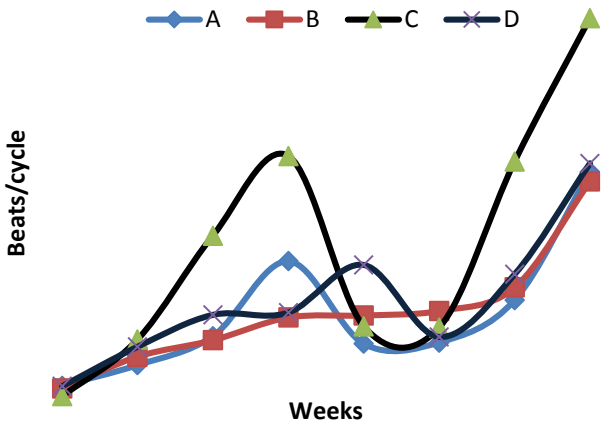

Figure 1:
Average respiratory rate changes of goats in the different treatments groups

Pathology

The gross pathology followed similar pattern with more pronounced lesion observed in group D and positive control group though the lesion in all the infected groups

were quite similar. The details of the grading is as presented in Table 3. At post mortem, the carcasses were mild to severely emaciated. The ocular and oral mucus membranes were pale. The trachea contained a copious amount of froth. The lungs were slightly to severely congested and oedematous. Different lobes of the lung showed focal to multiple randomly distributed areas of consolidation. The pleural surfaces showed deposits of fibrin and were attached to the rib cage. The spleen were moderately enlarged. There was hyperaemia of the entire length of the intestinal mucosa. The pneumonic goats used for challenge also showed very severe lesions when compared to the experimental groups.

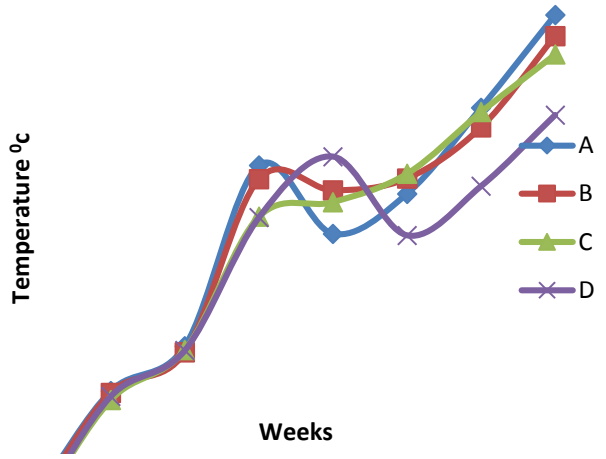

Figure 2:
Average weekly temperature changes of goats in the different treatments groups

The details of the grading of the histopathological changes were as shown in table 4 while the average pulmonary consolidation in the experimental groups was presented in Figure 3 with group B showing the least average consolidation score (0.41) and histopathological score.

Mortality Pattern

The mortality varied between 60 to 100% with three animals found dead in groups A and B, four in group D and positive control group C. There was no mortality in the negative control group.

Microbiology

Pure cultures of *M. hemolytica* were obtained from the lung samples of the goats in all the groups after subculturing. The homogenates of the lung samples in all the groups also produced a clear precipitin lines when agar gel precipitation test was employed.

Table 1
Average Weekly weight of goats in the different treatments groups

	Treatments (Mean±SD)			
WEEKS	**A**	**B**	**C**	**D**
1	5.60 ±0.55^{a}	5.40 ±1.14^{a}	6.33 ±1.53^{a}	5.00 ±0.70^{b}
2	6.20 ±0.45^{ab}	6.00 ±1.00^{ab}	7.00 ±1.00^{a}	5.00 ±0.71^{b}
3	7.40 ±0.55^{a}	6.80 ±0.84^{a}	7.00 ±2.00^{a}	6.40 ±0.55^{a}
4	7.00 ±0.71^{a}	7.80 ±0.45^{a}	6.00 ±3.00^{a}	6.60 ±0.55^{a}
5	5.60 ±0.89^{b}	7.40 ±0.55^{a}	7.50 ±0.71^{a}	6.80 ±0.45^{a}
6	6.00 ±0.00^{ab}	7.00 ±0.00^{a}	6.00 ±1.41^{ab}	5.50 ±0.58^{b}
7	5.00 ±0.00^{a}	5.20 ±0.84^{a}	5.00 ±1.41^{a}	5.00 ±0.00^{a}
8	4.00 ±0.00^{a}	5.00 ±0.82^{a}	4.50 ±0.71^{a}	4.50 ±0.71^{a}
Total	5.97 ±1.16^{a}	6.29 ±1.23^{a}	6.09 ±1.69^{a}	5.77 ±0.99^{a}

The negative control group did not show any significant weight loss

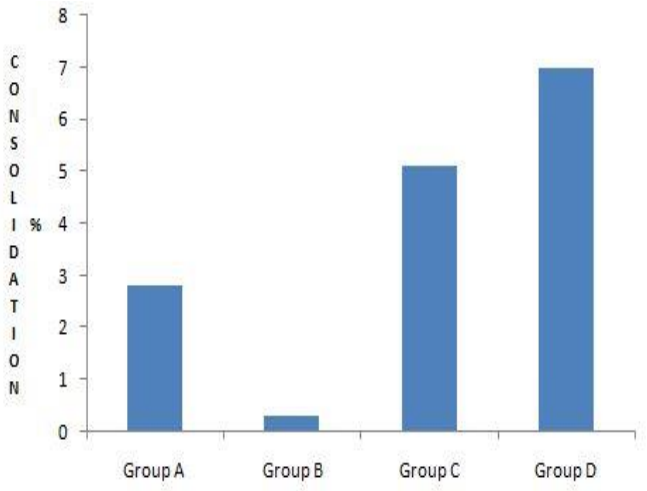

Fig. 3
Average Lung consolidation of goats in the different treatments groups

DISCUSSION

This study describes the protective evaluation of the recombinant *M. hemolytica* vaccine against the naturally occurring pneumonia in Nigeria. The evaluation was by using the clinico-pathological features and lung consolidation as a basis of protection.

Table 2
The occurrence of clinical features of goats in the different treatments groups post challenge (in days)

Clinical features	Positive Control C	GRP A	GRP B	GRP D	PPRV+MH (Emikpe and Akpavie 2013)	MH (Emikpe and Akpavie 2011)
Rough hair coat	5-14	12-14	-	10-14	2-7	3-7
Nasal Discharge (serous)	5-10	--	7-8	8-10	2-8	6-10
Nasal Discharge (mucoid)	10-13	----	-	10-13	6-44	8-15
Cough	5-14	7-10	-	8-14	5-14	8
Pyrexia	4-10	6-10	9-12	4-14	5-6	5-6
Staggering	9-14	8-10		9-14	-	-
Emaciation	4-10	7-10	11-14	10-14	-	-
Ocular discharge	--	--	-	8-10	2-8	22-25
Death	7 (80%)	10 (60%)	3,4(60%)	7 & 11, 13 (80%)	1, 12 and 15	8 and 12

The negative control group did not show any significant clinical features

Table 3
The frequency of gross lesions in goats in the different treatments groups (A, B, C, and D)

NO.	Gross Lesions	Group A (vac once)	Group B (vac 2x/1wk)	Group D (vac 2x/2wks)	Group C (positive control)
1	Rough hair coat	1/5	2/5	3/5	3/3
2	Emaciation	2/5	2/5	5/5	2/3
3	Dehydration	-	1/5	3/5	2/3
4	Ulcerative stomatitis	-	-	3/5	2/3
5	Diarrhea	5/5	2/5	5/5	3/3
6	Ocular discharges	1/5	1/5	-	-
7	Pulmonary congestion and oedema	2/5	3/5	4/5	3/3
8	Pulmonary haemorrhages	2/5	1/5	-	1/3
10	Acute Broncho- pneumonia	3/5	4/5	3/5	2/3
11	Splenomegally	1/5	1/5	-	1/3

The negative control group did not show any significant lesion

Table 4
The frequency of histological lesions in goats in the different treatments groups (A, B, C, and D)

Nos	Lesions	A	B	C	D
1	Pulmonary oedema	3/5	4/5	1/3	4/5
	Oedema with fibrin	2/5	1/5	2/3	
2	Pulmonary congestion	5/5	5/5	3/3	3/5
3	Sloughing of bronchiolar epithelium and necrosis	2/5	1/5	-	3/5
4	Perivascular cuffing (lymphocytic)	-	2/5	-	-
	Peribronchiolar cuffing (lymphocytic)	1/5	1/5	-	-
5	Thickening of alveolar septae	5/5	5/5	3/3	4/5
	Proliferation of type 2 pneumocytes	-	2/5	-	2/5
6	Infilteration of inflammatory cells				
	Predominantly lymphocytes	2/5	4/5	2/3	2/5
	Predominantly neutrophils	3/5	1/5	1/3	2/5
7	Giant cells	5/5	5/5	-	-
8	Haemorrhages	3/5	1/5	2/3	1/3
9	Thickening of the pleura	2/5	1/5	2/3	1/4
10	Thickening of interlobular septae	3/5	2/5	3/3	1/4

The negative control group did not show any significant lesion

The natural occurring pneumonia in goats has observed in this study is that due to bacterial complicated PPR virus (PPRV) infection which had been earlier reported to be of a combined PPRV and *M. hemolytica* infection (Emikpe and Akpavie 2010b). This was also observed in the course of PPRV experimental infection in West African dwarf goats (Emikpe and Akpavie 2011).

Natural and experimental combined PPRV and *M. hemolytica* infection is often characterized by sudden deaths which do occur within a day in experimental animal model while in uncomplicated MH infection, it could be as early as 12 hours after the first sign of illness (Odugbo *et al*., 2004b, Emikpe and Akpavie 2010b). Animals in groups A, B and D after vaccination showed clinical signs though at later stages but are comparable to that observed in experimental combined PPRV and *M. hemolytica* infection (Emikpe and Akpavie 2012). The control group also showed similar signs as expected with death occurring in all the groups between day 7 - 13 post challenge.

Group B showed the least clinical signs with a mild increase in temperature and relatively stable respiratory rate; this suggested that the treatment in group B may have an ability to reduce the occurrence and duration of the clinical features except for death which was recorded in all the groups though at a later period in group B. The variation may also be due to individual variation in antibody titres as speculated by some workers (Akan *et al*., 2006). The observed mortality showed the inability of the recombinant MH vaccine irrespective of the frequency of administration to protect against the naturally occurring pneumonia in Nigeria. Although in group B where the vaccine was administered twice at a week interval, the lung consolidation was drastically reduced but the treatment was not capable of preventing the mortality observed.

The persistent gross lesion observed in the entire group was that of acute pneumonia with a characteristic fulminating acute fibrinous bronchopneumonia being observed in a goat in group B as compared two goats in positive control group C and three goats in groups A and D.

The degree of lung consolidation in all the groups showed group B having the least percentage consolidation possibly suggesting adequate mucosal immunity while the treatment in group D was least protective. These findings further support the report of Akan *et al*, (2006) that a vaccine (One Shot ultra 8) did not significantly reduce the lung lesion in sheep than when given twice at two weeks interval.

The consistent histopathological change was that of an acute broncho-interstitial pneumonia with giant cells, which was observed in animals before and after inoculation especially in all the groups. This finding further reaffirms that most pneumonia in Nigerian goats are complicated viral pneumonia as earlier reported by some workers (Obi, 1984). This was also substantiated by the average lung consolidations observed in the control animals which were similar to that observed in experimental PPRV and MH infection (Emikpe and Akpavie 2012).

Conclusions

The recombinant *M. hemolytica* vaccine alone is not sufficient to combat the fulminating naturally occurring pneumonia commonly encountered in Nigerian small ruminant population. Efforts should therefore be geared towards a combined PPR and MH vaccine which could possibly have prospects of reducing the mortality associated with this condition in Nigeria.

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