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Effect of intranasal recombinant *Mannheimia haemolytica* vaccination on some haematological indices of goats infected with peste des petits ruminants virus

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Abstract

There is dearth of information on the haematological changes associated with *Mannheimia haemolytica* vaccination in goats, hence this report which describes some haematological changes observed following vaccination with intranasal Recombinant *Mannheimia haemolytica* vaccine in goats naturally infected with peste des petits ruminants (PPR) virus. Twenty one (male, n=11; and female, n=10) goats were assigned to three vaccinated groups (A, B and D) with five goats per group (male: 3, female: 2), while the control group had 6 goats. Group A was vaccinated once intranasally, group B was vaccinated intranasally twice at one week interval and group D was vaccinated intranasally twice at two weeks interval . The control group (C) was not vaccinated. The vaccinated and control groups were challenged by comingling with pneumonic goats to simulate the field experience. PPR virus infection was later diagnosed in all the groups post vaccination. An average of four animals per treatment group in post-vaccination days were bled once weekly for six weeks (every week) to evaluate some haematological changes. The PCV values were within the normal range, while there was a decline in lymphocyte count at week 5, and a steady increase in neutrophil count in group A. In Group B, there was similar decline in lymphocyte count from the sixth week, while in groups C (Control) and D, the lymphocyte count declined at the 7th week, as the neutrophil counts increased. There were no significant changes in monocyte and eosinophil counts. The degree of changes in lymphocyte and neutrophil counts was mild in group B and marked in group D. This study revealed that intranasal vaccination of recombinant *Mannheimia haemolytica* vaccine in the presence of PPR virus outbreak results in mild hematological derangement when the goats were vaccinated with *Mannheimia haemolytica* bacterin at a week interval.

Keywords: Goats, Haematology, Intranasal Recombinant *Mannheimia haemolytica,* Peste des Petits Ruminants, Vaccination

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Introduction

African small ruminant population was estimated to be 171 million with about 34.5 million found in Nigeria. Goats are more commonly owned by farming units in Africa than other ruminants (FAO, 1991). They are a major source of meat, milk, and skin (Williamson & Payne, 1978). Goats continue to play an important role in the welfare of smallholder arable farmers as they improve the livelihoods of women and children often entrusted within their (Ikwuegbu *et al.,* 1995).

Diseases still remain the most important impediment to goat production in the tropics. Pneumonia is still one the diseases that poses a major constraint to small ruminant production in

Nigeria (Emikpe *et al*., 2013a, Emikpe *et al*., 2013b). common causes of bacterial pneumonia in goats in sub-Saharan Africa, parts of Asia and Arabian Penisular (Emikpe *et al*., 2010). Mannheimiosis is a well-known bacterial pneumonic disease of domestic and wild small ruminants and is characterized by pyrexia, mucopurulent nasal discharges, fibrinous pneumonia and ultimately, death (Emikpe & Akpavie, 2010). Factors such as stress and overcrowding had been identified as predisposing factors to caprine pneumonia, but investigation of their role in this condition is still not clear (Zamri-Saad *et al.,* 1989, Emikpe *et al*., 2014). Domestic animals especially goats are constantly subjected to some of the most stressful conditions in the humid zone where *Mannheimia haemolytica* complicated PPRV infection had been reported in goats (Emikpe *et al*., 2010, Emikpe *et al*., 2013a). This necessitates the need to produce an indigenous vaccine to curb the bacterial complication often associated with viral pneumonia in Nigerian goats. (Emikpe *et al*., 2013a).

Thus, the control and prevention of most bacterial respiratory diseases had focused on the use of intranasal vaccines which induce strong mucosal responses and protection in small ruminants (Zamri-Saad *et al*., 1989, Tenuche *et al*., 2013). The use and safety of vaccination against Mannheimiosis, even though common in some countries (Zamri-Saad *et al*., 1994, Purdy *et al*., 1997), had not been reported in Nigerian breeds of goats.

Previous reports showed that intranasal vaccination of recombinant *Mannheimia haemolytica* bacterine is not protective against naturally occurring pneumonia in Nigeria inspite of the ability to induce strong mucosal immunity in the respiratory tracts (Emikpe *et al*., 2013b, Tenuche *et al*., 2013), however there is dearth of information on the safety of *Mannheimia haemolytica* vaccination especially in countries where viral respiratory disease such as PPR is endemic. This report describes some haematological changes observed in intranasal recombinant *Mannheimia haemolytica* vaccinated goats that had Peste des Petits ruminants Virus infection in the course of evaluation of efficacy of the vaccine in goats.

Materials and Methods

Animal

The experimental protocol had been previously described (Emikpe *et al*., 2013b). Briefly, twenty one (male, n=11; and female, n=10) West African Dwarf (WAD) goats obtained from a recognized breeding farm, six months of age, of average weight of 7 kg were used for the experiment. They were conditioned for 14 days before the intervention and vital signs (rectal temperature, pulse and respiratory rates) were monitored daily to ascertain that they remained afebrile and free of any clinical signs of disease. The animals were then randomly assigned to four well partitioned, fly proof pens of the Veterinary Pathology Department, in the experimental animal unit of the Faculty of Veterinary Medicine, University of Ibadan. The animals were fed daily with cut grass; supplemented feed and clean drinking water was available *ad libitum*. Each intended vaccinated group (A, B and D) had five goats (male: 3, female: 2) while the control group (C) had 6 goats.

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

Vaccine

The vaccine used was obtained from Malasia. It 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).

Experimental procedure

Group A was vaccinated once intranasally. This was carried out by introducing a single spray of the vaccine directly into each nostril. The same was done for groups B (vaccinated twice at one week interval) and D (vaccinated twice at two weeks interval). Group C served as the control group and was not vaccinated. The experimental goats were not experimentally infected with PPR virus, however the vaccinated and control groups were challenged by comingling with pneumonic goats to simulate the field experience. Peste des Petits ruminants (PPR) virus infection was later diagnosed all the groups post MH *(Mannheimia haemolytica)* vaccination by clinical features of pneumonia and histopathological changes consistent with PPR as observed in lungs and intestinal tissues of goats from all the groups (Emikpe & Akpavie, 2011).

Haematology

All or surviving animals in post-vaccination days were bled by jugular venepuncture once every week over a period of 8 weeks. 2mls of blood were collected on each occasion into containers with ethylenediamine tetra acetate (EDTA) for hematological studies. The packed cell volume (PCV) was determined by the microhaematocrit method while the differential leucocyte counts were determined from Giemsa stained blood smears (Jain, 1986)

Statistical analysis

Statistical analysis was carried out with ANOVA and Duncan multiple range test of significance (< 0.05) for means of the parameters recorded (SPSS-20) (Petrie & Watson, 1999).

Results

All results of the packed cell volume (PCV) are shown in Table 1. At the 4th week, there was an increase in PCV in all the groups except group B (23.60%), it was highest in group D (34.60%). At the 8th week, group C had the highest PCV (35.00%) of all the groups**.** There was a continuous decline in PCV in groups A and D after the 5th and 6th weeks, respectively.

There was a decline in lymphocyte count (Table 2) in group A after the 5th week which continues to drop. The neutrophil count (Table 3) showed an increase within the same period.

Group B) showed a similar decline in lymphocyte count from the sixth week and by the 8th week there was an obvious improvement in the lymphocyte count and a decline in the neutrophil count. Groups C and D however maintained a normal lymphocyte count value which only started to decline at the 7th week as their respective neutrophils increased. There were no significant findings with respect to the changes in monocyte and eosinophil counts.

Table 1: The packed cell volume changes in the different vaccinated groups of goats

	Groups (Mean PCV ±SD)			
WEEKS	A	B	C	D
1	25.80±2.95^{a}	26.00±3.16^{a}	25.50±4.95^{a}	28.77*±2.07*^{a}
2	26.80*±7.06*^{a}	24.60*±2.70*^{a}	30.33*±0.58*^{a}	29.60*±6.80*^{a}
3	24.60*±6.77*^{a}	28.80*±3.63*^{a}	31.00*±1.00*^{a}	31.00±3.60^{a}
4	31.75*±6.02*^{ab}	23.60*±5.37*^{b}	31.50*±2.12*^{ab}	34.60*±3.13*^{a}
5	34.00*±0.00*^{a}	31.40*±5.68*^{a}	32.50*±2.12*^{a}	24.50*±4.44*^{a}
6	32.00*±0.00*^{a}	21.80*±6.06*^{b}	27.00*±0.00*^{ab}	26.33*±3.06*^{ab}
7	29.00*±0.00*^{a}	24.67*±6.03*^{a}	26.50*±4.95*^{a}	22.50*±2.12*^{a}
8	27.00*±0.00*^{ab}	26.00*±9.90*^{ab}	35.00*±0.00*^{a}	20.50*±0.71*^{b}
Total	28.04*±5.48*^{ab}	25.91*±5.43*^{b}	29.80*±3.86*^{a}	27.81*±6.19*^{ab}

Note: Mean±SD across a row with different superscripts are significantly different with a>b (< 0.05)

Table 2: The average lymphocyte changes in the different vaccinated groups of goats

	Treatments (Mean±SD)			
WEEKS	A	B	C	D
1	43.20*±16.16*^{a}	61.80*±18.19*^{a}	50.00*±22.63*^{a}	57.6*±3.36*^{a}
2	47.00*±14.32*^{a}	62.20*±9.39*^{a}	60.67*±11.02*^{a}	65.20*±13.63*^{a}
3	47.20*±18.19*^{a}	53.20*±14.04*^{a}	60.00*±5.00*^{a}	61.40*±16.90*^{a}
4	43.25*±7.93*^{a}	31.00*±15.26*^{a}	56.00*±11.31*^{a}	55.80*±23.89*^{a}
5	49.00*±0.00*^{b}	53.20*±17.70*^{b}	86.50*±3.54*^{a}	72.25*±8.88*^{ab}
6	36.00*±0.00*^{a}	41.40*±32.61*^{a}	69.00*±18.39*^{a}	47.67*±29.74*^{a}
7	35.00*±0.00*^{a}	41.00*±18.08*^{a}	39.50*±2.12*^{a}	45.00*±28.28*^{a}
8	35.00*±0.00*^{a}	48.00*±19.80*^{a}	38.00*±0.00*^{a}	46.00*±35.36*^{a}
Total	43.33*±12.35*^{b}	49.51*±20.23*^{ab}	57.33*±18.40*^{a}	58.05*±21.36*^{a}

Note: Mean±SD across a row with different superscripts are significantly different with a>b. (< 0.05)
Mean separation done with Duncan Multiple Range test

Table 3: The average neutrophil changes in the different vaccinated groups of goats

	NEUTROPHIL			
	Treatments (Mean±SD)			
WEEKS	A	B	C	D
1	51.80±15.64[a]	32.20±15.94[a]	46.50±23.33[a]	32.20±13.72[a]
2	49.80±12.76[a]	35.60±9.71[a]	32.00±7.00[a]	32.20±13.72[a]
3	50.80±18.05[a]	44.40±12.95[a]	33.33±6.65[a]	37.00±11.02[a]
4	54.50*±7.77*[a]	66.20*±15.06*[a]	42.00*±12.73*[a]	43.00*±24.81*[a]
5	49.00*±0.00*[a]	43.20*±17.51*[a]	14.50*±6.36*[b]	23.00*±9.45*[ab]
6	62.00*±0.00*[a]	56.80*±33.03*[a]	30.00*±21.21*[a]	51.00*±31.18*[a]
7	62.00±0.00[a]	54.00±23.58[a]	44.00±9.90[a]	45.50±37.48[a]
8	63.00±0.00[a]	50.00±21.21[a]	56.00±0.00[a]	50.50±34.65[a]
Total	53.78*±12.00*[a]	47.26*±20.63*[ab]	37.47*±16.09*[b]	38.71*±22.52*[b]

Note: Mean±SD across a row with different superscripts are significantly different with a>b. (< 0.05)
Mean separation done with Duncan Multiple Range test

Discussion

This report appears to be the first report describing some haematological changes observed in intranasal Recombinant *Mannheimia haemolytica* vaccinated goats that had Peste des Petits ruminants Virus infection. In this study, the differential cell count was used to ascertain the presence or absence of inflammatory response in the leukogram of all the groups. The reduced lymphocytes below normal values in group A at two weeks and the increasing neutrophil counts suggests a possible acute bacterial infection. In group B, there was an increase in lymphocyte count and a decline in the neutrophil while groups C and D maintained a similar lymphocyte count value till the sixth week with increased neutrophil count at 7th week. This observation is similar to that of Ganheim *et al.* (2003) where *Mannheimia haemolytica* infection was reported to induce an increase in leukocyte count especially neutrophils with low lymphocyte count. The degree of changes in lymphocyte and neutrophil values in all the groups showed that group B had the mildest inflammatory response possibly suggesting adequate immunity while that of group D showed a severe acute inflammatory response.

In conclusion, this study has revealed that intranasal vaccination of recombinant *Mannheimia haemolytica* bacterine does not cause severe hematological derangement when the goats were vaccinated with *Mannheimia haemolytica* bacterine at a week interval even in the presence of PPR outbreak, while severe derangement was observed in animals vaccinated at two weeks interval.

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