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Pneumonia as the cause of death in gilts experimentally infected with *Trypanosoma brucei*

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ABSTRACT

A study was conducted in 24 gilts to determine the effect of *Trypanosoma brucei* infection on their reproductive efficiency. The infected gilts developed clinical trypanosomosis following a prepatent period of 2-3 days with 1.8×10^6 trypanosomes per gilt. The clinical signs were observed intermittent fever, pale mucus membranes, short moist cough, moist rales, mucopurulent ocular discharges and hyperemia of the skin, reduced feed intake, and loss of body condition, recumbency, uncoordinated movements, posterior paresis and death of gilts. The cause of death in the pigs was pneumonia caused by *Escherichia coli*. Grossly, the lungs were severely congested and had undergone gray hepatization. Histopathologically, the lungs had thickened and congested alveolar walls, and were infiltrated by mononuclear cells which were noticed more in the lung parenchyma. The role of secondary bacterial infection in the pneumonia observed, orchestrated by immunosuppression, which is a classical attribute of trypanosome infection is discussed.

Key words: *Trypanosoma brucei*; Immunosuppression; *Escherichia coli*; Pneumonia; Gilts

INTRODUCTION

The majority of respiratory problems of grower to finisher swine are due to a combination of pathogens, hence the term porcine respiratory disease complex (PRDC). The more common viral causes are porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), pseudorabies virus (PRV), porcine circovirus type 2 (PCV II) and porcine respiratory corona virus (PRCV). The bacterial organisms involved in PRDC are *Mycoplasma hyopneumoniae, Pasteurella multocida, Streptococcus suis, Haemophilus parasuis, Actinobacillus pleuropneumoniae, Actinobacillus suis, and Salmonella* Choleraesuis (Battrel, 2000).

Escherichia coli is a normal flora of the intestine. It generally does not cause disease and may even contribute to normal function and nutrition. The organism only becomes pathogenic when it reaches tissues outside the intestinal tract particularly the urinary and biliary tracts, lungs, peritoneum and meninges, causing inflammation of these sites (Jawetz *et al*., 1987). The organism causes colibacillosis in all species of newborn farm animals and is a major cause of losses in this age group especially if they are deficient in immunoglobulins. Other diseases caused by this organism in farm animals are gut edema, enteric colibacillosis of feeder pigs, and mastitis. The gut edema, enteric colibacillosis occurs when there is proliferation of predominantly hemolytic serotypes of the organism within the small intestine (Blood and Radostits, 1989). This study presents a report of fatal bacterial pneumonia due to *E. coli* in gilts experimentally infected with *Trypanosoma brucei.*

MATERIALS AND METHODS

Experimental animals

Twenty four (24) cross breeds of piglets were bought from piggeries in Samaru village in Zaria, Kaduna State Nigeria and housed in clean fly proof pens in the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. Their baseline hematological data were obtained on arrival. The gilts were screened for endo- and ecto-parasites and ear notched for identification after which they were treated for nematodes and ecto-parasites with Ivermectin (Ivomec®) at a dose rate of 200 µg/kg body weight, administered subcutaneously. The piglets were fed compounded diet of 18% crude protein comprising maize (36.8%), soya bean (5%), ground nut (23.5%), rice bran (30%), beniseed (2%), bone meal (2%), premix (0.2%), table salt (0.5%) and water provided *ad libitum.* When the pigs were seven months old, they were randomly divided into two groups of 14 infected and 10 control animals.

Trypanosomes

The *Trypanosoma brucei* used in this study was obtained from the Nigerian Institute of Trypanosomiasis research Vom, Nigeria. It was originally isolated from a pure natural infection in cattle in Federe Kaduna state, Nigeria. The parasite was inoculated into mice and transported to the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. Prior to use, the infected blood of the mice was inoculated into 8 rats. The parasitemia in the rats was monitored and classified according to the method described by Woo (1969). When peak parasitemia was attained, the rats were anesthetized in a jar of chloroform and then bled via the heart. All the blood collected was pooled with ethylenediaminetetraacetic acid (EDTA) as anticoagulant in a beaker.

Inoculation of animals

About 2 ml of the infected rat blood containing approximately 1.8×10^6 *Trypanosoma brucei* organisms was inoculated into each animal in the infected group via the anterior vena cava while the ones in the control group were left intact. Following inoculation, all the animals were clinically examined and their blood samples collected daily using EDTA as anticoagulant and examined for levels of parasitemia and packed cell volume (PCV). Their rectal temperatures were also recorded daily. This continued until all the infected animals were positive for trypanosomes. Subsequently all the gilts were clinically examined, weighed and their blood collected twice weekly to determine PCV and levels of parasitemia throughout the experiment period of 167 days.

During the course of the study, five of the infected animals died between days 42 and 91 post infection (p.i.) respectively. Postmortem examination was carried out on all the dead animals. The lung samples of the dead animals were fixed in 10% formalin, trimmed, processed and stained with Ehrlich hematoxylin-eosin stain (H-E stain). The slides were then examined under the light microscope for histopathological lesions. The lung samples of the dead animals were streaked on blood agar and incubated at 37 ^{o}C and the colonies of the bacteria on the culture media were observed after 48 hours. These colonies were subsequently subcultured on eosin methylene blue agar (EMB). Gram staining of the bacteria was also done on clean slides and observed under the light microscope.

Statistical test used

Data obtained from the study was computed and analyzed using Student's *t*-test.

RESULTS

Clinical observations

After a prepatent period of 2 to 3 days, all the infected gilts eventually developed clinical trypanosomosis. There was a steady increase in the levels of parasitemia of the infected pigs which was followed by fluctuations. Peak parasitemia was recorded between days 7 and 35 p.i. The rectal temperatures of all the infected animals increased during the course of the infection. This was also followed by fluctuations. The highest mean temperature attained by the infected animals was 40.3 ^{o}C. During this period, the temperatures of the control animals were within the normal range. The difference in the ranges of temperature between the infected and the control animals was significant ($P<0.05$). There was a gradual decrease in the PCV of the infected pigs. This was first noticed on day 14 p.i. in one of the pigs. The lowest PCV value recorded was 17%. The mean PCV values of the infected animals were significantly different from those of the control ($P<0.05$). Other clinical signs observed in the infected gilts were, intermittent fever, pale mucus membranes, short and moist cough, moist rales, mucopurulent ocular discharges and hyperemia of the skin, reduced feed intake, loss of body condition, recumbency, uncoordinated movements, posterior paresis and death of 5 pigs.

Gross pathological findings in the lungs

The lungs of three of the gilts were severely congested especially the cardiac and apical lobes. In the remaining two gilts, their lungs had areas of gray hepatization which were indicative of pneumonia (Fig. 1).

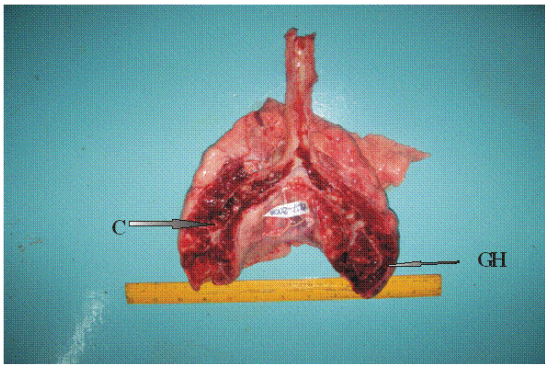

Fig. 1. Lung of a *T. brucei* infected gilt that died of *E. coli* pneumonia showing congestion (C) and gray hepatization (GH).

Histopathological findings in the lungs

Severe inflammatory lesions were observed in the lungs of the infected gilts. This was characterized by mononuclear cellular infiltration, which was noticed more in the parenchyma. The lungs also had thickened and congested alveolar walls (Fig. 2).

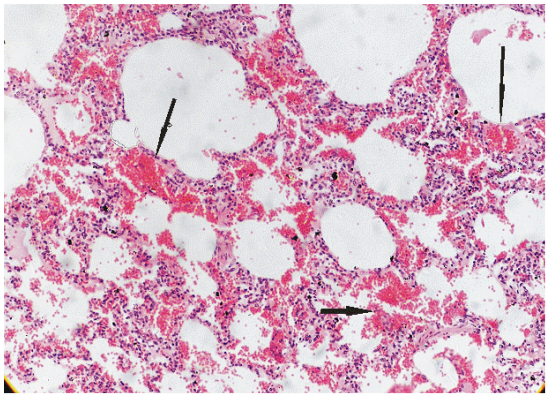

Fig. 2. Photomicrograph of the lung of a *T. brucei* infected gilt. Note thickened and congested alveolar walls (arrows) H-E stain, x147

Microbiological results

The colonies on blood agar were creamy, raised, and smooth showing beta hemolysis. The subculture on EMB agar had a greenish metallic sheen. Microscopically, gram negative rods were identified.

DISCUSSION

Trypanosomosis is known to suppress the immune system of infected animals (Onah and Wakelin, 1999; Onah and Wakelin, 2000; Morrison, *et al*., 1985; Sileghem *et al*., 1994). During the course of the infection, the immune response mounted by the host is often overpowered by the ability of the parasite to undergo antigenic variation and in the process; it evades the host's immune response (Spriggs, 1985). This is followed by immunosuppression where the host's ability to resist other infections is lowered. The *T. brucei* used in this experiment caused clinical trypanosomosis in all the infected gilts killing 5 of them as a result of pneumonia due to *E. coli.* The *E. coli* caused fatal pneumonia in the infected pigs because their immunity was depressed.

During this study, the animals that were used were inoculated only once and they were fed with high quality diet and they had no concurrent infection. Also they were crosses which are usually raised under the traditional husbandry systems and are known to possess a broad genetic variability of indigenous livestock breeds in tropical African countries. This enables them to have valuable traits which include disease resistance, ability to survive under stressful environmental conditions such as poor nutrition and high temperature (FAO 1988a and 1988b).

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