Clinical and pathological responses of West African Dwarf goats (Fouta Djallon) infected with Nigerian strain of Wesselsbron virus

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BABA (S. S.), FAGBAMI (A. H.), OLALEYE (O. D.). Réponses cliniques et pathologiques des chèvres naines Djallonké infectées par une souche nigériane du virus de la maladie de Wesselsbron. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (4) : 329-335.

La pathogénicité du virus de la maladie de Wesselsbron a été recherchée chez des chèvres naines Djallonké infectées par voie sous-cutanée à l'aide de la souche nigériane du virus. Toutes les chèvres infectées se sont révélées sensibles. Deux des animaux ont developpé une maladie aiguë, fatale dans les 5 à 8 jours suivant l'inoculation. Les autres ont eu une maladie relativement longue durant 18 à 20 jours après la période d'incubation. La maladie était caractérisée par une diarrhée profuse, une déshydratation, une perte de poids et une mortalité de 100 p. 100. Les changements hématologiques associés comprenaient une polyglobulie en relation avec la déshydratation chez les malades. On a noté une leucopénie associée à une lymphocytopénie. Quant aux modifications pathologiques elles comprennent une nécrose hépatique étendue et des hémorragies jointes à une congestion étendue et une dilatation des vaisseaux sanguins méningés et cérébraux. *Mots clés* : Caprin - Chèvre naine d'Afrique de l'Ouest - Virus - Maladie de Wesselsbron - Infection expérimentale - Pathogénicité - Nigeria.

INTRODUCTION

Wesselsbron virus, a flavivirus has been responsible for severe disease outbreaks in sheep in South Africa (13). Clinical infection is characterized by high fever and severe leucopaenia in adults, abortion in pregnant ewes and high mortality in lambs and kids (11, 12, 13). In addition to these effects the virus may also be responsible for teratology in the developing foetus in sheep and pregnant cattle (3) and inapparent infections frequently occur in horses and pigs (1).

KEMP *et al.* (8) reported the first isolation of the virus from a camel during a routine virus surveillance in Northern Nigeria in 1968. Although the West African Dwarf sheep is susceptible to experimental infection with Wesselsbron virus (6), no clinical episode of Wesselsbron disease has been reported in Nigeria. The veterinary importance of the virus in Nigeria is therefore yet to be clearly defined.

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To further define the pathogenicity of Wesselsbron virus in domestic animals, attempt is made in this study to investigate the susceptibility of West African Dwarf goats to experimental infection with the Nigerian strain of Wesselsbron virus.

MATERIALS AND METHODS

Experimental animals

Five West African Dwarf goats (Fouta Djallon) aged 4-5 months and weighing 3.5-4.5 kg were used in the experiment. Four were females and one was male. The animals were kept in mosquito proof quarters and pen-fed *ad libitum* throughout the period of the experiment with freshly cut giant star grass (*Cyandon plectostacyns*) and commercially prepared concentrate pellets.

Prior to challenge, goats were put on antibiotic (Duphacycline™, Duphar B.V. Holland) therapy at a dosage of 1.5 ml per animal per day for 5 days. They were also treated for helminthiasis by oral administration of febendazole (Panacur™, Hoechst-Nig. Limited) at a dosage of 5 mg/kg.

The virus used

The Nigerian isolate of Wesselsbron virus Ib-AN 31956 isolated from the blood of camel was used in the experiment. It had undergone five intracerebral (i.c.) passages in mouse brain. Ten percent infected suckling mouse brain suspension prepared in Eagle's minimal essential medium (MEM) and centrifuge at 10,000 rev./min. served as stock virus. Titre of stock was $10^5 LD_{50}$/0.02 ml.

Experimental design

One of the animals was chosen as control by balloting and tagged (No. 5). The remaining animals were tagged Nos. 1-4 and were inoculated with Wesselsbron virus. The infected and control animals were kept in

different pens. All animals received similar feeding and management regimes.

The physiological parameters (temperature, respiration and pulse) were obtained before inoculation. Similarly haematological parameters including red blood cell (RBC) and white blood cell (WBC) counts (total and differential) were measured before inoculation.

Experimental procedure

Four animals (Nos. 1-4) were infected subcutaneously (s.c.) with 2.5×10^5 suckling mouse intracerebral (SMIC)LD_{50}. The control animal 5 received 0.05 ml of 10 p. 100 normal suckling mouse brain in MEM. The animals were examined daily for signs of clinical disease and daily rectal temperature were taken. Blood samples were obtained daily for 10 days for viraemia assay, red blood cell and leucocyte counts.

Clinical examination

The haematological parameters were determined as described by DUNCAN and PRASSE (5). The red blood cells and white blood cells were estimated using the improved Hawksley haemocytometer. For differential leucocyte count, blood smears were made from freshly collected samples and fixed in methanol. The fixed smears were later stained with Giemsa stain and examined with x 100 (Oil) objective. Leucocytes were identified until 100 cells were classified to type. The percentage of each leucocyte was multiplied by total white blood cell count to obtain the number of each leucocyte type per microlitre (µl) of blood.

The presence of virus in blood was determined by i.c. inoculation of suckling mice. LD_{50} end points were calculated by the method of REED and MUENCH (10).

Pathological examination

The carcasses of all the infected goats were systematically examined soon after death. Gross lesions were recorded and tissues for histopathology were fixed in 10 p. 100 buffered formalin. Section of paraffin embedded tissues were cut out at 5 microns and stained with haematoxylin and eosin (H & E).

RESULTS

Clinical observation

Goats infected with Wesselsbron virus developed clinical disease characterized by fever, serous oculonasal discharges, rough hair coat, weakness, dullness, diarrhoea, leucopaenia and 100 p. 100 mortality. Incubation period was 2 days in 2 of the infected animals and was followed by a rise in body temperature from a mean of 38.4 °C on inoculation day to 39.1 °C in one goat and 40.7 °C in the other. Fever lasted 1-3 days before death of the animals. After an incubation period of 4 days the other infected goats developed pyrexia with body temperature ranging from 39.3 °C to 40.4 °C. The febrile response lasted till day 10 P.I. The fever in one of them was quadriphasic which probably indicated the different periods of viraemia following remission in the liver and other organs. The control goat did not show any rise in body temperature throughout the period of the experiment (Fig. 1).

During the febrile phase of the disease, the animals became dull, weak and had rough hair coats. This stage was followed by serous oculonasal discharges and shallow and rapid respiration. At the peak of pyrexia, one of the infected goats (No. 1) became recumbent and died on day 5 P.I. The oculonasal discharges later became purulent and one of the infected animals (No. 3) became diarrhoeic. The diarrhoea became profuse after two days and probably resulted in death of the animal on the 3rd day of diarrhoea. The disease in the remaining two goats (2 and 4) were characterized terminally by rough hair coat, mucopurulent oculonasal discharges, coughing, nervousness, collapsed jugular vein, lateral recumbency and hypothermia (mean 37.3 °C). Apart from animal 1 which died on day 5 P.I. all infected goats had diarrhoea which lasted till death. Death occurred on days 5, 8, 22 and 24 P.I.

Viraemia

Virus was detected in the blood of all the infected goats 48 hours after infection and lasted for 24 hours.

Haematological changes

Table I shows the red blood cell counts of Wesselsbron virus infected and control goats at different periods of the experiment. The erythrocyte values of the infected goats were significantly ($P < 0.05$) higher than those of the control animal on days 4, 5, 6 and 7 P.I. Post inoculation level of erythrocytes remained unchanged in control goats.

The total and differential blood cell counts of all the infected animals are shown in table II. There was a mild increase in the total leucocyte values of the infected goats (2 and 4) from day 3 to 7 P.I. when compared with the values of the control goat during the same period. The difference was significant

TABLE I Mean values of red blood cells counts of infected and control goats at different periods.

Period (days)	Number of infected animals (n)	Red blood cells × 10^6	
		Infected	Control
0	n = 4	8.42 ± 1.5	8.4
1 p.i.	n = 4	8.37 ± 0.42	7.6
2 p.i.	n = 4	8.97 ± 1.5	8.23
3 p.i.	n = 4	9.5 ± 1.2	7.72
4 p.i.	n = 4	9.97 ± 1.5	7.45
5 p.i.	n = 3	10.30 ± 1.3	7.2
6 p.i.	n = 3	10.0 ± 1.8	7.0
7 p.i.	n = 3	10.25 ± 1.5	8.2
8 p.i.	n = 2	7.9 ± 1.8	7.7
9 p.i.	n = 2	8.1 ± 2.0	7.7
10 p.i.	n = 2	7.1 ± 1.5	5.8
12 p.i.	n = 2	7.7 ± 2.5	6.5
14 p.i.	n = 2	6.7 ± 1.0	6.2

TABLE II Values of total white blood cell (WBC), neutrophils and lymphocytes of Wesselsbron virus infected and control goats at different periods.

Period days	Total WBC × 10^3					Neutrophils/µl					Lymphocytes/µl				
	Infected goat				Control goat	Infected goat				Control goat	Infected goat				Control goat
	01	02	03	04	05	01	02	03	04	05	01	02	03	04	05
0	11.0	10.6	11.4	11.2	11.5	432.0	428.0	486.0	412.3	488.2	559.0	565.0	500.0	586.0	494.7
1	11.2	10.8	11.0	11.0	11.2	430.0	416.0	462.0	412.0	465.4	609.6	615.1	550.0	636.0	572.2
2	11.2	10.4	10.8	11.0	11.1	451.0	442.5	492.0	437.4	459.8	578.0	604.0	538.6	625.0	622.0
3	10.98	11.4	11.2	12.6	11.8	453.0	462.4	488.0	476.3	472.6	596.6	623.4	557.0	643.4	596.0
4	10.6	12.2	11.2	12.4	11.3	496.0	484.0	509.5	500.0	480.0	605.5	632.4	566.3	652.5	627.0
5		12.6	11.8	12.6	11.3		658.5	698.0	686.0	479.6		654.4	589.4	674.0	631.5
6		10.9	11.8	11.98	9.6		696.4	800.0	776.0	429.6		689.0	623.5	707.0	599.0
7		10.8	11.2	11.86	11.2		764.0	786.0	756.0	462.8		624.8	558.5	641.0	448.0
8		11.0		11.4	9.9		350.0		386.0	412.5		558.4		575.0	568.0
9		10.0		10.4	10.1		344.0		356.0	483.4		566.5		575.0	543.0
10		9.8		10.6	10.1		353.0		355.0	478.5		586.5		598.4	545.0
12		10.0		10.0	9.5		346.0		359.0	482.4		513.4		525.0	564.0
14		9.6		10.8	9.5		352.0		362.6	476.7		486.4		512.0	578.1

($P < 0.05$) on day 5 P.I. After the initial leucocytosis, there was a gradual decline in leucocyte values of the infected goats (2 and 4) from about day 9 P.I. There was a sharp increase in neutrophil values of the infected goats 2 and 4 from day 5 P.I. to day 7 P.I. A significant ($P < 0.05$) difference occurred between pre-infection values of the goats when compared with control goat at the same period. There was fluctuation in the lymphocyte values, but there was a general gradual decline in lymphocyte values.

Necropsy findings

Gross pathology

Three of the carcasses (Nos. 2, 3 and 4) examined had lesions including dehydration with sunken eyes and prominent ribs. In addition, there was mucopurulent oculonasal discharges and the hindquarters were soiled with faecal material. There was also diffuse congestion of the lungs and the trachea and bronchi

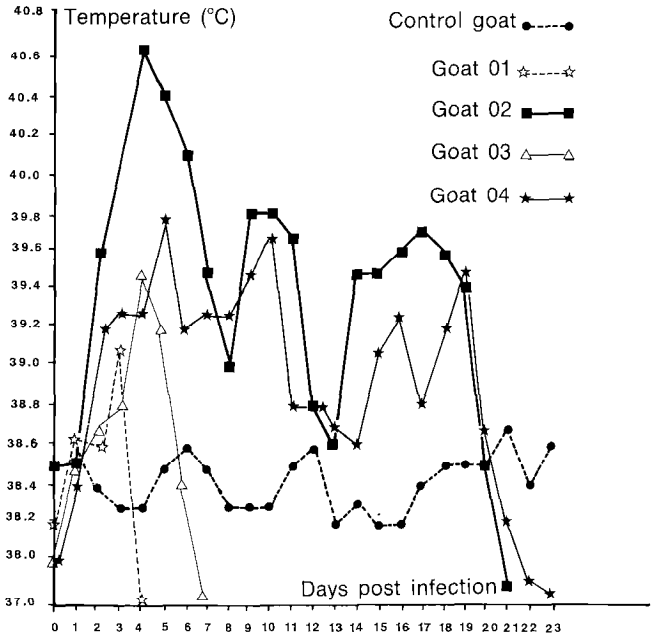

Fig. 1 : Daily temperature of WSLV infected and control goats.

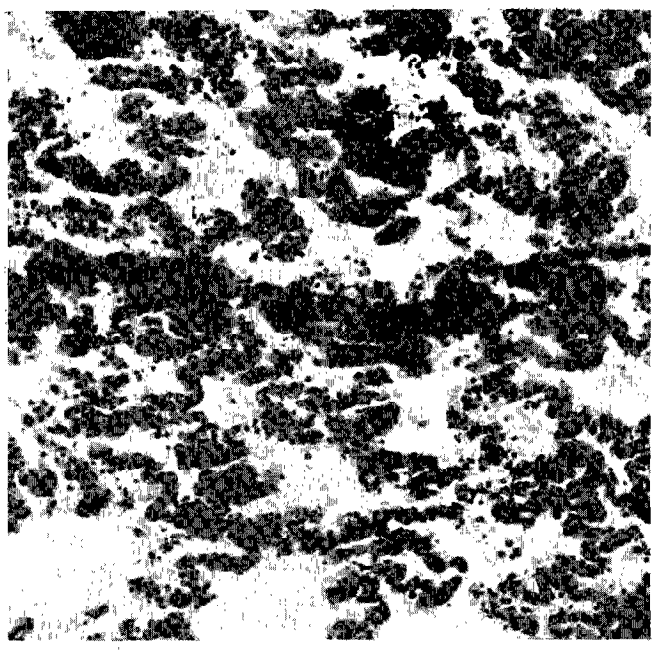

Photo 1 : Showing pulmonary congestion and oedema. x 125 H & E.

were filled with frothy exudate in two carcasses (2 and 4). There was serous atrophy of the omental and coronary fat in carcasses 2 and 4 and the liver was congested and friable with whitish necrotic sports in two carcasses (Nos. 2 and 4). The subcutaneous and intestinal blood vessels were prominent in the two carcasses.

Microscopic lesions

There was dilation and engorgement of the pulmonary blood vessels, and moderate accumulation of oedema fluid in the alveoli in two infected animals (2 and 4) (Photo 1). There was also widespread lymphocytic cuffing of the pulmonary vessels in animals 2 and 4 (Photo 2). Few giant cells were also seen in the alveoli in the two carcasses. The liver of the two animals (2 and 4) showed severe and widespread hepatic necrosis and haemorrhage. There was depletion of lymphoid follicle of the spleen in three of the carcasses (Nos. 2, 3 and 4). The lymphoid depletion was mild in one of them (3). The brain of two carcasses (Nos. 2 and 4) showed dilation of the meningeal and cerebral blood vessels. The blood vessels were also hyperaemic (Photo 3). There was mild gliosis and presence of eosinophilic intracytoplasmic inclusion bodies in the neuronal cells of the deep cerebrum (Photo 4). The adrenal gland in one of the carcasses showed dilation

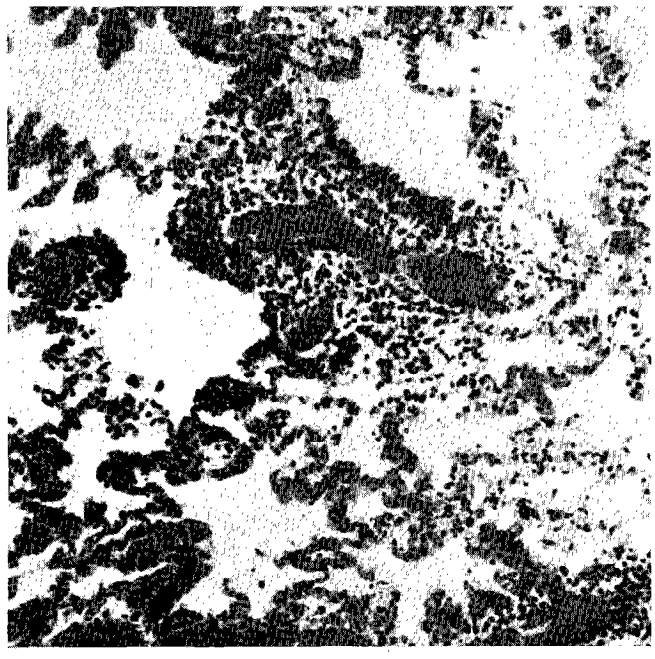

Photo 2 : Showing perivascular lymphocytic cuffing of pulmonary vessels. x 125 H & E.

and engorgement of blood vessels, this could be attributed to the generalised vascular reaction to infection with Wesselsbron virus.

Photo 3 : Brain showing dilated and engorged meningeal blood vessels. x 250 H & E.

Photo 4 : Showing mild neuronal degeneration and a neurone in the deep cerebrum with intracytoplasmic inclusion body. x 400 H & E.

DISCUSSION

The short incubation period characteristic of natural and experimental Wesselsbron virus infections (2, 6, 11, 13) was observed in this study. The clinical changes observed in Wesselsbron virus infected goats complement and confirm those reported by WEISS (13), COETZER *et al.* (4), COETZER and THEODORIDIS (2). However, diarrhoea which is common to most of the infected goats in this study is not a common finding. It was observed that infected goats that survived the initial phase of the disease developed diarrhoea which was progressive till the death of the animals. High mortality observed in this age group of infected goat was consistent with findings of COETZER *et al.* (4) and COETZER and THEODORIDIS (2).

The presence of circulating virus in blood of goats infected with Wesselsbron virus agrees with earlier findings. Earlier report on the haematological changes of Wesselsbron disease by FAGBAMI (6) was based mainly on total leucocyte counts in experimental infections. The present study showed that changes occurred in the erythrocyte values of Wesselsbron virus infected animals. Also the study of FAGBAMI (6) showed a slight fall in total white blood cells characterized by leucopaenia. Although leucopaenia occurred in all infected animals in this study, there was an initial leucocytosis before the animal became leucopaenic.

Examination of the carcasses showed that gross and histopathological lesions were limited to respiratory and alimentary organs as well as lymphoid tissues and the brain. The lesions were similar to those reported by WEISS *et al.* (13) and LE ROUX (9) in lambs and by COETZER and THEODORIDIS (2) in association with Wesselsbron virus infection in lambs and kids. It was observed that animals that died at the early phase of the disease had mild necrosis of the hepatocytes while those that died later showed severe and widespread hepatic necrosis and haemorrhage. There were also differences in the splenic and nodular changes between the animals that died during the early phase of the disease and those that survived the infection for longer period. While there was severe depletion of lymphoid follicles in the spleen of those that suffered late mortalities, such changes were mild in the two goats that died on 5th and 8th day P.I. This suggests a destructive effect of Wesselsbron virus on lymphocytes especially with lymphopaenia that characterized the later phase of the infection. The pulmonary lesions were different from those reported by LE ROUX (9). There was dilation and engorgement of the pulmonary blood vessels, and moderate accumulation of oedema fluid in the alveoli. The cerebral lesions were also different from those reported by WEISS *et al.* (13), it was found that animals that died in the later phase of

the infection had dilation and hyperaemia of the meningeal and parenchymatous blood vessels. There was also mild gliosis and presence of eosinophilic intracytoplasmic inclusion bodies in the cells of the cerebrum.

CONCLUSION

The high mortality rate in West African Dwarf goats observed in this study calls for serious concern in this environment, because goats are numerically the most abundant among the ruminants animals in Nigeria (7). The goat population is made up of 3 breeds : the Maradi (Red Sokoto), the Sahel, both found in the North and the Fouta Djallon (West African Dwarf goat), found in the South. It is possible that Wesselsbron virus is of great economic and veterinary importance in this country. It is suggested therefore that particular attention should be paid to studies involving this virus in order to ascertain its veterinary significance in Nigeria and also serve as a differential diagnosis in epizootics involving sheep and goats in Nigeria.

BABA (S. S.), FAGBAMI (A. H.), OLALEYE (O. D.). Clinical and pathological responses of West African Dwarf goats (Fouta Djallon) infected with Nigerian strain of Wesselsbron virus. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (4) : 329-335.

The pathogenicity of Wesselsbron virus was investigated in West African dwarf goats infected subcutaneously with the Nigerian strain of the virus. All infected goats were susceptible to infection. Two of the infected animals developed an acute disease with death occurring within 5-8 days post inoculation (P.I.). The others had a relatively protracted disease lasting between 18-20 days following the incubation period. The disease was characterized by profuse diarrhoea, dehydration, weight loss and 100 p. 100 mortality. The associated haematological changes include relative polycythaemia which was associated with dehydration in infected animals. There was leucopaenia characterized by lymphocytopoenia. The pathological changes include, widespread hepatic necrosis and haemorrhage coupled with widespread congestion and dilation of meningeal and cerebral blood vessels. *Key words* : Goat - West African Dwarf goat - Wesselsbron disease - Virus - Experimental infection - Pathogenicity - Nigeria.

BABA (S. S.), FAGBAMI (A. H.), OLALEYE (O. D.). Respuestas clínicas y patologicas de cabras nanas Djalonke infectadas por una cepa de Nigeria del virus de Wesselsbron. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (4) : 329-335.

Se investigó la patogenicidad del virus de Wesselsbron en cabras nanas Djalonke infectadas por vía subcutánea mediante una cepa de Nigeria del virus. Fueron receptivas todas las cabras infectadas. Dos de ellas desarrollaron una enfermedad aguda, fatal durante los 5 a 8 días después de la inoculación. Demás tuvieron una enfermedad relativamente larga durante 18 a 20 días después del periodo de incubación. Se caracterizaba la enfermedad por una diarrea abundante, una deshidratación, una pérdida de peso y una mortalidad de 100 p. 100. Las modificaciones asociadas incluían una policitemia en relación con la deshidratación en los enfermos. Se observó una leucopenia con una linfocitopenia. Las modificaciones patologicas eran : una necrosis hepática extendida y hemorragias añadidas a una congestión importante y una dilatación de los vasos sanguíneos meningeos y cerebrales. *Palabras claves* : Ganado cabrío - Cabra nana Djalonke - Enfermedad de Wesselsbron - Virus - Infección experimental - Patogenicidad - Nigeria.

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