



Outbreak of Peste Des Petits Ruminant in an Unvaccinated Sahel Goat Farm in Maiduguri, Nigeria

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ABSTRACT: An outbreak of peste des petits ruminant (PPR) among unvaccinated Sahel goats was investigated in a farm in Maiduguri, Nigeria. The morbidity rate of 63% and mortality rate of 17% was recorded on the farm, giving a case fatality rate of 27%. The infected animals manifested serous to mucopurulent oculonasal discharges, coughing, crust on the lips, erosive and necrotic lesions on the gums, dyspnoea, diarrhea, congestion and hepatization of the lungs, congestion in the large intestine and death. PPR virus antigen was detected in swabs and tissues of infected animals using agar gel immunodiffusion test (AGID) and virus was isolated from same samples. PPR virus neutralizing antibodies was also detected in 75% of goats and 60% of incontact sheep. This report represents the first confirmed PPR outbreak among Sahel goats in the North-Eastern region of Nigeria.

Keywords: *PPR outbreak, Sahel goat, Virus Neutralization test, agar gel immunodiffusion test, Nigeria*

INTRODUCTION

Peste des petits ruminant (PPR) is the most important singular cause of morbidity and mortality among small ruminants (Adeoye, 1984; Odo, 2003). It is a contagious transboundary disease that is widely distributed across the Sub-saharan Africa, Middle East, Arabian Peninsula and the Indian subcontinent (Odo, 2003; Diallo, 2006). The disease causes serious economic losses and remains a major deterrent to a successful development of small ruminant production in the countries where it occurs (Dhar *et al.*, 2002; Diallo, 2003; Yener *et al.*, 2004). It is one of the notifiable diseases of the OIE. Outbreaks of the disease has been reported in India (Shaila *et al.*, 1989), Southern Nigeria (Opasina and Putt, 1985), Arabian peninsula (Abu Elzeid *et al.*, 1990), Jordan (Leveré *et al.*, 2004), Isreal (Anon, 1993), Ethiopia (Reoder *et al.*, 1994), Turkey (Yener *et al.*, 2004), Pakistan (Ahmad *et al.*, 2005), Iran (Abdollahipour *et al.*, 2006), Congo

(OIE, 2006) Iraq (Dosky *et al.*, 2006), Tajikistan (Kwiatek *et al.*, 2007), etc. Out breaks of the disease are characterized by fever, erosive stomatitis, nasal and ocular discharges, pneumonia, diarrhea and death. These signs may not all be exhibited by infected animals during an outbreak, as symptomless infections have been reported (Diop *et al.*, 2005; Couacy-hymann *et al.*, 2007a & 2007b). Reports on disease outbreaks among Sahel goats are scarce. In this paper we report an outbreak of PPR among Sahel goats in Maiduguri, Nigeria.

CASE REPORT

A report of diarrhea, pneumonia and deaths among goats kept at the University of Maiduguri teaching and research farm, Maiduguri was made. The farm contains flocks of long-legged Sahel goats, sheep and herd of cattle. The clinical signs were only noticed among the goats. The animals were never vaccinated against PPR. The sick animals were treated with 20% oxytetracycline (Neimeth Nigeria Plc). Both the sick and apparently healthy goats in the flock were bled and ocular, nasal and rectal swabs were taken and autopsy was performed on the dead animals. The

Manuscript received: December 2007; Revision Accepted October 2008

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clinical signs observed among the infected goats were those of pyrexia (39.8-41.2 °C), anorexia, depression, ocular and nasal discharges, crust on the lips (Plate. 1), necrotic lesions on the gums, dyspnea, cough, diarrhea and death. All the animals showing the different clinical signs were aged between 6 to 18 months. The goats were never vaccinated against PPR.

Sample collection: Nasal, ocular and rectal swabs were collected from six each of the clinically sick and apparently healthy goats. The swabs were placed in sterile vacutainer tubes containing 0.25 ml of virus transport medium and transported to the Animal Virus research laboratory, University of Maiduguri on ice packs. Five milliliters (5 ml) of blood was collected from the entire flock (both clinically sick and apparently healthy animals) using sterile vacutainer needles and plain tubes. Blood was collected from the jugular vein of each animal. The blood samples were kept at room temperature to clot and the sera harvested and kept at -20 °C until used.

Virus isolation: Virus isolation from the swabs was carried out on Vero cells (Vero R133 P₂₀) using a modification of the protocol by Couacy-hymann et al. (2007). Each tube containing the swabs was vortexed and the cotton bud of each swab was squeezed with sterile forceps and the medium filtered with Seitz filter of 0.22 µm pore size. One milliliter (1ml) of each pooled swab sample from similar organs were inoculated onto 24 hours old 75% confluent monolayer of Vero cells in a 25 cm³ flask and incubated at 37 °C for 1 hour with regular swirling every 10 minutes. Negative control flasks were inoculated with 1ml of plain G-MEM. All the flasks were then covered with 10 ml of G-MEM containing 10% fetal calf serum (FCS), 10% tryptose phosphate buffer, penicillin G at 100 IU/ml (Panpharma SD Fourgere France), streptomycin at 100mg/ml (Troge medical GMBH Hamburg Germany) and fungizone at 2.5µg/ml (Biochrom AG Berlin Germany). The samples were inoculated in duplicate. All the flasks were then incubated at 37 °C with 5% CO₂. Subsequently, the medium in the flasks were changed after 24 hours to maintenance medium containing 2% FCS. The flasks

were observed daily and their medium changed every 48 hours until CPE appeared. Flasks that failed to show or showed little CPE were frozen and thawed three times and the resultant harvest inoculated onto new flasks of Vero cells as described earlier. This was repeated twice for such samples. All positive samples were subjected to virus neutralization test against PPR positive antisera.

Serology

Agar gel immunodiffusion (AGID) test:- This test was used in detecting PPR virus antigen in swabs and tissues. It was carried out using a modification of the OIE (2004) protocol. Briefly a 1% agarose gel was prepared in Petri-dishes. The semi-solid agar prepared was punched using Ouchterlony template and the base of each well was sealed with molten agar to avoid the reagents sipping in between the agar and the plate surface. Test samples were dropped in the peripheral wells and the positive control sera was placed in the central well and the dish incubated at 37 °C in a humidified chamber and examined later for precipitin lines.

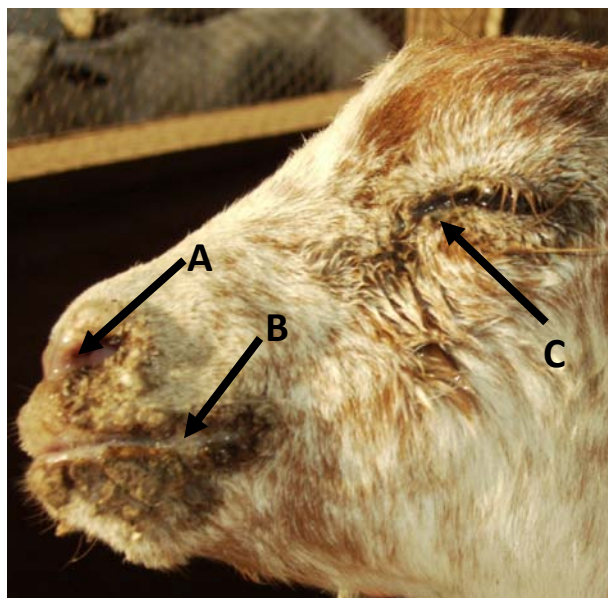


Plate 1: Suspected PPR virus infected goat showing: A: serous nasal discharge, B: oral crust and C: ocular discharge

Table 1: Results of the PPR antigen detection and virus isolation from PPR infected flock

Test	Nasal Swab		Ocular swab		Rectal Swab		Organs	
	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected
AGID	+ve ^a	-ve ^b	+ve	-ve	+ve	-ve	± ^c	NT ^d
Virus Isolation	+ve	-ve	+ve	-ve	NT	NT	NT	NT

^a = positive; ^b = negative; ^c = precipitin lines seen only after washing with 5% glacial acetic acid; ^d = Not tested

Table 2:

Sex distribution of PPR virus neutralizing antibody titres among PPR infected Sahel goats and in-contact sheep in Maiduguri, Nigeria

Reciprocal of end-point titre of PPR VN titre	Total number (%) positive					
	Goats (n = 113)			Sheep (n = 20)		
	Female	Male	Total	Female	Male	Total
5	3 (4.9)	3 (12.5)	6 (7.1)	2 (33.4)	1 (16.7)	3 (25)
10	10 (16.4)	3 (12.5)	13 (15.3)	0 (0)	1 (16.7)	1 (8.3)
20	3 (4.9)	2 (8.3)	5 (5.9)	0 (0)	0 (0)	0 (0)
40	1 (1.6)	0 (0)	1 (1.2)	0 (0)	1 (16.7)	1 (8.3)
80	9 (14.8)	1 (4.2)	10 (11.8)	2 (33.4)	0 (0)	2 (16.7)
160	12 (19.8)	2 (8.3)	14 (16.5)	1 (16.7)	1 (16.7)	2 (16.7)
320	8 (13.1)	11 (45.8)	19 (22.4)	0 (0)	0 (0)	0 (0)
640	6 (9.8)	1 (7.1)	7 (8.2)	0 (0)	1 (16.7)	1 (8.3)
1280	9 (14.8)	1 (4.2)	10 (11.8)	1 (16.7)	1 (16.7)	2 (16.7)
Total	61 (81.3)	24 (63.2)	85 (75.2)	6 (60)	6 (60)	12 (60)

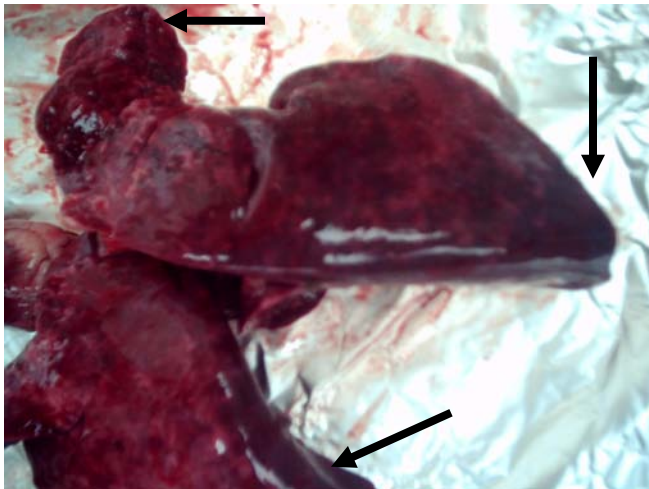


Plate 2:

Lungs of suspected PPR infected Sahel goat showing areas of congestion on all the lobes (arrows)

Virus neutralization (VN) test: - This test was used to detect for PPR antibodies in the sera collected from the flock. The test was carried out according to the protocol described in OIE (2004) manual. Briefly, the test sera were heat inactivated at 56 °C for 60 minutes in a water bath. A two fold serial dilution of the test sera in complete medium (G-MEM containing 10% FCS) was carried out in sterile flat bottom Nunc

microtitre plates by adding 160 µl of complete medium in the first rows of microtitre plate and 100 µl in the subsequent wells. Forty microlitres (40 µl) of each test serum was added in duplicate to the wells containing the 160 µl medium and mixed to give a 1:5 dilution. The 1:5 dilution sera was then looped across the plate by transferring 100 µl from the first well to the next well up to the last well. After diluting the sera, 100 µl of 10⁵ TCID₅₀ of PPR virus (Capripesovax®) was added to each well and the plate incubated at 37 °C with 5% CO₂ for 1 hour. Fifty microlitres (50 µl) of Vero cells (4x10⁵ cells/ml) was then added to each well and the plate incubated at 37 °C and 5% CO₂. The plates were observed daily until CPE appeared.

Findings

A morbidity rate of 63%, mortality rate of 17% and case fatality rate of 27% was recorded among Sahel goats on a farm. At postmortem the lesions observed were those of oral crusts, nasal and ocular discharges (Plate. 1), congested lungs (Plate 2), necrotic ulcerations of gums, pinpoint hemorrhages on the liver and congestion in the intestine. The nasal and ocular swabs collected from the sick animals produced CPE on Vero cells 6 days after inoculation. The CPE was inhibited when the harvest was titrated against PPR

positive antisera, thus confirming the isolate as PPR virus. The swabs and 1:3 suspension of the organs were positive against PPR positive antisera using AGID (Table 1). Seventy-five percent (75%) of goats and 60% of sheep on the farm were carrying PPRV neutralizing antibodies of varying titres (Table 2). Gender distribution of PPR neutralizing antibodies among both species showed no statistical difference (Table 2). The age distribution of the PPR VN antibodies among the goats showed an ascending pattern with increase in age, the < 1 year old (58%), followed by the > 2 years old (72%) and the 1 – 2 years old (83%); but the sheep showed higher prevalence among the < 1 year old (80%), followed by the >2 years old (60%) and least in the 1 – 2 years old (40%) (Table 3).

DISCUSSION

Although PPR outbreaks have been reported in some parts of Nigeria and serological evidence reported all over the country including North-Eastern arid zone, this is the first confirmed outbreak of the disease among Sahel goats in the region. We have observed in this report the clinical signs of pyrexia, depression, anorexia, coughing, nasal and ocular discharges, crust on the lips, caseous necrotic lesions on the gums, diarrhea and death, consistent with PPR infection. These observations are similar to those reported by Reoder *et al.* (1994) among Ethiopian goats. The occurrence of a fatal fast spreading disease characterized by stomatitis, diarrhea and pneumonia affecting mainly small ruminants should arouse suspicion of PPR (Obi *et al.*, 1988). The case fatality rate observed in this study is within the range of that reported by Opasina and Putt (1985) during different outbreaks among goats in the humid zone of Southern Nigeria. All the animals that showed the frank clinical signs in this report were young (6-18 months). PPR is known to affect kids than adults (Nduaka and Ihemelandu, 1973; Ahmad *et al.*, 2005). It was also observed that there was a gradual increase in percentage seroprevalence with increase in age of the goats. Similar observations were reported of Al-Afaleq *et al.* (2004) and Ahmad *et al.* (2005). One interesting finding in this report is that only few of the infected animals showed alimentary tract involvement, both in the live animals and at post mortem. This is similar to the report by Aruni *et al.* (1989). None of the incontact sheep presented any clinical sign or death during this study, but 45% of them appeared to have protective PPR antibodies (titre \geq 1:10). This is similar to the

reports by Reoder *et al.* (1994) and Ahmad *et al.* (2005), who also observed some sheep grazing with PPR infected goats to be PPR seropositive and not presenting with the disease. The high seroprevalence noticed in this study compliments the findings of other workers (Taylor 1979; Shamaki *et al.*, 2004). Since the animals were never vaccinated against PPR, the antibodies could only have come as a result of subclinical infection as earlier observed by Diop *et al.* (2005). The PPR virus PPRV/Nig 75/1 belonging to lineage II is reported to cause mild or inapparent disease among goats (Couacy-hymann *et al.*, 2007a). The high seroprevalence with high virus neutralizing antibody titres, but low mortality rates observed in this study could be due to differences in the relative sensitivity of the different breeds of goats to PPRV infection. It is known that the West African dwarf breeds are more susceptible than to PPRV infection than the long-legged Sahel breeds (Diop *et al.*, 2005). Only the swabs (nasal and ocular) from the sick goats were positive both with AGID and virus isolation. Diop *et al.* (2005) had earlier reported 85-100% of nasal secretions to be positive for PPRV among infected goats and negative among the uninfected.

Based on the clinical signs, post mortem findings, virus isolation and serology; we concluded that the offending pathogen is PPRV. It is suggested that more efforts be put in place to control this disease in this environment through vaccination and proper quarantine. The newly introduced homologous PPR vaccine has proven to be efficacious and safe (Awa *et al.*, 2002). The control of this disease will ensure sustained food security and alleviate poverty in areas where the disease is prevalent.

Acknowledgement

The authors wish to acknowledge with thanks the assistance of the department of animal science University of Maiduguri.

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