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Evaluation of the virulence of some strains of peste-des-petits-ruminants virus (PPRV) in experimentally infected West African dwarf goats

E. Couacy-Hymann [a,*], C. Bodjo [a,c], T. Danho [a], G. Libeau [b], A. Diallo [c]

[a] *LANADA/Laboratoire Central de Pathologie Animale, BP 206, Bingerville, Côte d'Ivoire*
[b] *CIRAD, Programme Santé Animale, TA 30/G, Campus International de Baillarguet, 34398 Montpellier, Cedex 5, France*
[c] *Animal Production Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, IAEA Laboratories, A-2444 Seibersdorf, Austria*

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Abstract

Different isolates of peste-des-petits-ruminants virus (PPRV) from outbreaks in Africa and India were investigated for virulence in West African dwarf goats in the Ivory Coast. Six groups of five animals received a virulent suspension of various strains of virus at a concentration of 10^3 $TCID_{50}$/mL and the goats were observed for 15 days after infection. The Côte-d'Ivoire 89 (CI89), Guinea Conakry and Bissau Guinea PPRV strains caused a peracute disease; the India-Calcutta strain caused acute disease; the Sudan-Sennar strain produced an acute to mild disease, while the Nigeria 75/1 wild-type strain caused a mild disease and the animals recovered. The viruses studied contained examples of PPRV from specific lineage groups based on their nucleoprotein PPRV gene. This experiment indicated that virulence characteristics might be a useful marker to help classify PPRV isolates.

Keywords: Peste-des-petits-ruminants; PPR; Morbillivirus; Virulence; Goats

1. Introduction

Peste-des-petits-ruminants (PPR) is a viral disease of sheep and goats with high morbidity and mortality. It has a widespread distribution across Sub-Saharan Africa, the Middle-East and Asia, where it continues to cause serious economic losses (Taylor, 1984; Shaila et al., 1989; Lefevre and Diallo, 1990; Nanda et al., 1996). The causative agent is PPR virus (PPRV), a member of the Morbillivirus genus in the family Paramyxoviridae (Gibbs et al., 1979). PPRV is closely related to rinderpest virus (RPV), which infects cattle and other large ruminants and can cause disease in small ruminants (Anderson et al., 1990; Couacy-Hymann et al., 1995; Diallo, 2003).

PPR is characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis and pneumonia. These signs are not always present and symptomless infections have been observed (unpublished data). This is also the case for rinderpest (RP) in cattle (Taylor, 1986; Wamwayi et al., 1995) with a wide variation in the virulence of RPV strains ranging from the hypervirulent Saudi Arabia strain to the mild Egyptian isolate (Taylor, 1986).

The local goat breed in the Ivory Coast is the West African dwarf breed, which is highly susceptible to PPRV. PPR is enzootic in the country with outbreaks reported mainly during the rainy season and a national prevalence rate estimated at 19% (Couacy-Hymann, 1994). The present investigation evaluated the virulence of some PPRV isolates of domestic origin and from

* Corresponding author. Tel.: +225 22 403 136/138; fax: +225 22 403 644.
E-mail address: e.couacy-hymann@globeaccess.net (E. Couacy-Hymann).

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different geographical areas in local, susceptible West African dwarf goats.

2. Materials and methods

2.1. Animals

West African dwarf goats (35, including five uninfected control goats), aged 2–3 years, which tested negative for the presence of antibodies against RP and PPR by competitive ELISA (c-ELISA) (Anderson et al., 1991; Libeau et al., 1995), were used for the study. They were purchased from villages located in the rain forest region of the Ivory Coast and were housed in boxes at the animal house of the Central Laboratory for Animal Diseases, Bingerville, with separate feeding and drinking tanks. Each animal was treated with the anthelmintic albendazole (300 mg/kg) during the acclimatisation period.

The study was approved by the Ethics Committee of CIRAD/EMVT, Montpellier (France), the principal Coordinator of the project, and La Commission Nationale de BioSécurité et de BioEthique, Côte-d'Ivoire.

2.2. Virus isolates used in challenge

Six virus isolates were obtained from the virus bank of CIRAD/EMVT representing viruses from different geographical regions and belonging to different lineages based on the sequences of their nucleoprotein (NP) gene (Roeder and Obi, 2000) as listed in Table 1.

2.3. Virulent challenge

Thirty goats were divided at random into six groups (five animals per group). The seventh group comprised five control goats. Each animal (except controls) was infected subcutaneously with 1 mL of the various challenge viral suspensions, at a concentration of 10^3 $TCID_{50}$/mL. Animals were kept separately in boxes and an attendant was assigned to each box to feed and water the infected and control animals. Animals were examined daily for classical signs of PPR and body temperatures were recorded.

Table 1
Geographic origin and lineage grouping of PPRV isolates

Isolates	Country of origin	Lineage group[a]
Côte d'Ivoire 89 (CI 89)	Côte-d'Ivoire	I
Guinea Conakry V5 (GC)	Guinea Conakry	I
Bissau Guinea RM (BG)	Bissau Guinea	I
Nigeria 75/1, Virulent strain (Nig 75/1)	Nigeria	II
Sudan-Sennar (SS)	Sudan	III
India-Calcutta (IC)	India	IV

[a] PPRV isolates lineage according to Roeder and Obi (2000).

2.4. Sample collection

Blood samples were collected from all challenged animals from days 1 to 15 after infection. The control animals were sampled at days 7 and 15 after the start dates for the infections. Serum was separated and samples stored at −20 °C until examined. Using sterile precautions, mesenteric and tracheo-bronchial lymph nodes and lung tissue were collected from the challenged animals at death and placed in labelled and chilled screw-capped bottles. Lymph nodes from individual animal were pooled and treated later as one sample. The control animals were sampled as indicated above immediately after they were slaughtered on day 15 (at the end of the experiment). Collected samples were stored at −80 °C until examined.

2.5. Virus isolation

A 10% homogenate of the lymph nodes or lung tissue was made by ultrasonication in serum-free minimum essential medium (MEM). One millilitre of the fluid was added to 3×10^6 monolayer Vero cells in 9 mL of MEM in a 25-cm^2 flask (NUNC), supplemented with antibiotics and 10% fetal calf serum. Two 25-cm^2 flasks were inoculated from each sample. The medium was changed next day and then every two days up to two weeks, or until the appearance of any cytopathic effects (CPE). Where there were no CPE, a blind passage was made using the freeze–thawing method (three times) to disrupt the intact cells and 1 mL of the suspension was added to a new 25-cm^2 flask of monolayer Vero cells as above. Virus isolation was attempted in all cases of mortality.

2.6. Serological tests

A competitive ELISA (cELISA) kit (CIRAD-EMVT), based on a recombinant NP was used to detect specific antibodies against PPR (Libeau et al., 1995) following recommended protocols. Fifty microlitres were used throughout. Maxisorp 96-wells plates were coated with the recombinant NP antigen diluted 1/1600 in PBS (0.01 M, pH 7.2–7.4) and incubated at 37 °C for 1 h on an orbital shaker. After a cycle of three washes in phosphate buffered saline (PBS; 1/5, 0.05% Tween 20), test serum (5 μL), was added to 45 μL of blocking buffer (PBS 0.01 M. pH 7.2–7.4; 0.05% Tween 20 (v/v); 0.5% negative sheep serum (v/v)) followed immediately by the addition of 50 μL of the specific monoclonal antibody (MAb) against the PPRV NP at a dilution of 1/100 in blocking buffer. Control sera included were, strong positive, weak positive, negative and a MAb

control (0% competition). The plates were incubated and washed as above. Anti-mouse horse radish peroxidase enzyme conjugate (DAKO A/S), diluted 1/1000 in blocking buffer, was added and plates incubated as before. The plates were washed and 50 μL of substrate/chromogen (H_2O_2/OPD) were added and the colour allowed to develop for 10 min, after which time any reaction was stopped by the addition of 50 μL of sulphuric acid (1 M.). Plates were read on an ELISA reader (Multiskan MK II) at an absorbance of 492 nm. Optical density (OD) readings were converted to percentage inhibition (PI) values using the following formula:

$$PI\% = 100(\text{OD in test well}/\text{OD in 0\% control well}) \times 100.$$

PI% values greater than or equal to 50% were considered positive.

3. Results

3.1. Clinical response of goats to infection with PPRV isolates

The PPRV isolates, CI 89, Guinea Conakry, Bissau Guinea, Nigeria 75/1, Sudan-Sennar, India-Calcutta, representing the PPRV four lineages, showed variations in the onset of clinical signs, ranging from mild infection to severe disease with resultant mortality (Table 2). Whichever strain was used, the infected goats developed pyrexia after an incubation period of 2–7 days, with rectal temperatures ranging from 39 to 41 °C. The hyperthermia lasted for 4–10 days. Ocular and nasal discharges developed at day 6 post-infection and lasted no more than three days except for those animals infected with the CI 89 strain, where they were observed on day 4 for 3–7 days post-infection. Oral ulceration and necrotic lesions appeared at day 5 with CI 89 strain

Table 2
Occurrence and duration of the different clinical signs

Animal identification	Pyrexia[a]	Nasal/ocular discharges	Oral ulceration[b]	Diarrhoea	Date of death[b]	Mortality (%)	Mean survival time	Score of virulence
Côte d'Ivoire 89 1	2–8*[4.6]	4–10[4.4]	–	4–10[3.6]	11	100	9	++++
Côte d'Ivoire 89 2			5		10			
Côte d'Ivoire 89 3			8		9			
Côte d'Ivoire 89 4			7		9			
Côte d'Ivoire 89 5			5		7			
Guinea Conakry 1	2–9[6.4]	6–9[3.0]	8	7–10[2.4]	9	100	9.6	++++
Guinea Conakry 2			–		10			
Guinea Conakry 3			–		10			
Guinea Conakry 4			9		9			
Guinea Conakry 5			–		11			
Bissau Guinea 1	3–9[5.0]	7–10[2.7]	6	7–9[2.0]	9	100	9	++++
Bissau Guinea 2			7		10			
Bissau Guinea 3			–		8			
Bissau Guinea 4			9		10			
Bissau Guinea 5			8		8			
Nigeria 75/1 1	4–10[5.4]	6–9[3.0]	–	9–12[3.3]	–	0	–	+
Nigeria 75/1 2			–		–			
Nigeria 75/1 3			–		–			
Nigeria 75/1 4			–		–			
Nigeria 75/1 5			–		–			
Sudan-Sennar 1	3–13[6.8]	7–9[2.0]	–	8–10[1.6]	8	60	9.6	++
Sudan-Sennar 2			7		11			
Sudan-Sennar 3			–		–			
Sudan-Sennar 4			–		10			
Sudan-Sennar 5			–		–			
India-Calcutta 1	5–10[3.6]	7–9[2.3]	8	8–10[1.7]	10	100	10.2	+++
India-Calcutta 2			–		9			
India-Calcutta 3			7		10			
India-Calcutta 4			9		12			
India-Calcutta 5			–		10			

–, No sign; +, mean of the duration of clinical signs.
[a] Pyrexia 39–41 °C.
[b] Date signs appeared.
* Range of days post-infection.

and day 6 with Bissau Guinea strain. Diarrhoea was recorded in 25/30 infected animals followed by mortality in 23/30. However, goats infected with the Nigeria 75/1 strain did not develop mouth lesions although two animals showed a nasal discharge and three developed diarrhoea. No deaths were recorded and the symptoms observed were notably mild. In contrast, the clinical signs observed with the Guinea Conakry, Bissau Guinea and mainly with the CI 89 strains were severe.

The mortality rate was 100% with CI 89, Guinea Conakry, Bissau Guinea and India-Calcutta; 60% with Sudan-Sennar and 0% with Nigeria 75/1. Post-mortem examination revealed typical PPR lesions with zebra markings in the abomasum and large intestine, congestion of lungs and bronchopneumonia when associated with bacterial infection, along with erosive lesions observed in the mouth and diarrhoea.

Peracute PPR disease was observed with the CI 89, Guinea Conakry and Bissau Guinea strains. Although the India-Calcutta virus caused 100% mortality in infected goats, clinical signs were less severe than in the above mentioned strains.

The overall mortality rate was 76.7% (23/30) with a mean survival time of 9.5 days post-infection ranging from 9 to 10.2 days. The clinical response to PPRV infection is listed in Table 2. No clinical signs were recorded in control animals.

3.2. Virus isolation

The CI 89 strain was isolated from a lymph node sample from one infected goat after a single blind passage. Two blind passages were needed to isolate the Guinea Conakry strain from a lung sample. The character of the isolates was confirmed by RT-PCR with the set of primers P1/P2 (Couacy-Hymann, 1994). No other isolations were made from any other infections, even after three blind passages.

3.3. Serological response of goats to infection with PPRV isolates

Of the total animals, 12/30 became positive at day 7; 25/28 at day 8 and 20/22 at day 9. All infected animals sero-converted, but the early appearance of antibodies did not affect the severity of the disease. The goats that died showed sero-conversion 1–5 days before death and before the antibody reached a plateau. The control animals remained negative. The mean OD values of each virus isolate and controls are presented in Fig. 1.

4. Discussion

The various strains produced a gradation of syndromes, ranging from mild infection with the Nigeria 75/1 (virulent wild) strain to acute disease with the India-Calcutta and peracute fatal disease with the Guinea Conakry, Bissau Guinea and CI 89 strains. The majority of animals infected with each of the isolates died. The mean survival time of 9.5 days can be compared to cattle where Taylor (1986) found that the mean survival time was 4.5 days after infection with the Saudi Arabia RPV strain, (known to be a hypervirulent strain) and 7.8 days with the Nigeria 1983 strain (regarded as a mild strain). In our study, mortality occurred in infected goats from days 7 to 12 whatever the PPRV isolate. However, there were differences in the severity of clinical signs as indicated in Table 2.

Virus was isolated from only two samples in tissue culture. It is known that the isolation of PPRV is more difficult than the isolation of RPV and often needs blind passages (E. Couacy-Hymann; A. Diallo; G. Libeau, personal communications). This difficulty observed in the laboratory could be explained by an earlier cessation in virus shedding than is found with RPV – a very fresh clinical sample or isolated virus could require more

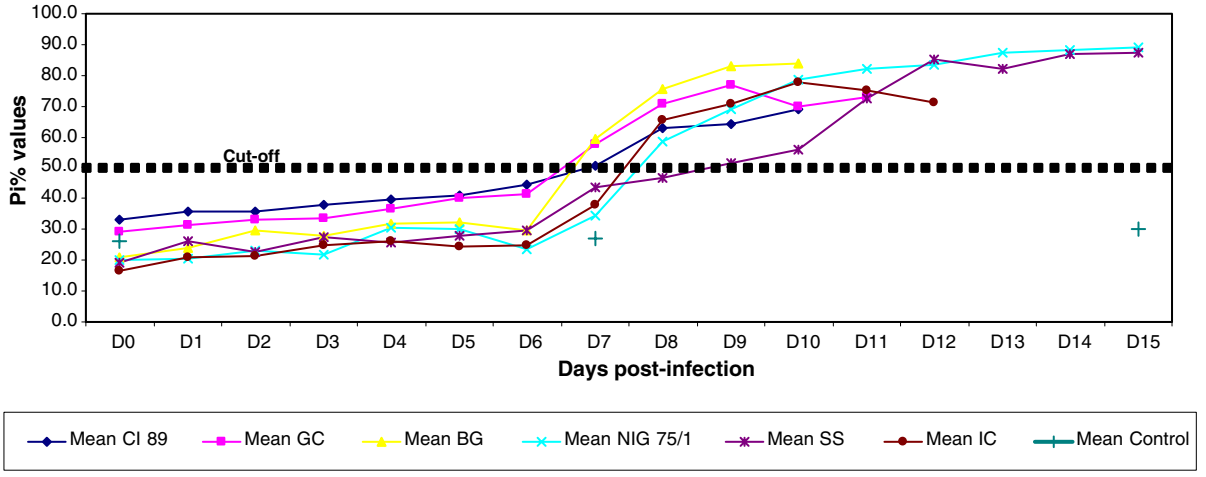

Fig. 1. Serological results with N-PPR c-ELISA (cut-off at 50%) of infected goats with various strains of PPRV.

blind passages to become tissue culture adapted. This could also explain our failure to isolate the different PPRV strains from all infected animals despite the presence of PPRV antigen detected by immunocapture ELISA (Libeau et al., 1994) and the good conditions for sample collection (as the experiment was performed in the laboratory). Infected animals ideally should be euthanased and samples tested at the onset of pyrexia to increase the chance of virus isolation, but this was not done here since sick animals could recover from severe PPR disease.

Twelve out of 30 (40%) of the infected animals sero-converted positively by day 7 post-infection and this number increased along with the following days. All of the infected goats that died sero-converted 1–5 days before death demonstrating that the early production of antibodies did not affect the severity of disease and resulting mortality.

Based on the severity of PPR disease in the animal groups, the mortality rate and the survival mean time, a score of virulence ranging from one mark (+) with the Nigeria 75/1 strain to four marks (++++) with the CI 89, Guinea Conakry and Bissau Guinea strains, was allocated to each PPRV strain used in this experiment (Table 2).

This study, although undertaken using a limited number of goats, showed that the virulence varied with the lineage grouping: Lineage I (CI 89, Guinea Conakry, Bissau Guinea) caused peracute to acute PPR disease; Lineage II (Nigeria 75/1) caused mild to inapparent PPR disease; Lineage III (Sudan-Sennar) caused acute to mild PPR disease; Lineage IV (India-Calcutta) caused acute PPR disease. The mildness of the Nigeria 75/1 wild type was demonstrated again in this study. Indeed, this was used as the virus for initial attenuation by multiple passages on Vero cells to produce the current homologous PPR vaccine strain (Diallo et al., 1989).

RPV isolates have been classified according to their virulence which could be used as a genetic marker (Taylor, 1986). The present experiment showed that the virulence of PPRV can serve to classify PPRV isolates and although this too might used as a genetic marker there are possible drawbacks. For example, the storage of PPRV strains has been implicated in alterations in virulence that may cause variation in pathogenicity studies. Highly virulent strains of PPRV isolates used in another challenge experiment producing a peracute PPR disease and stored in a deep freezer at −80 °C occasionally produced mild disease in susceptible goats (E. Couacy-Hymann; A. Diallo, personal communications). Our study was not however influenced by the storage conditions of virus isolates and indeed they caused PPR disease as we expected.

There are also differences in the relative sensitivity of different goat breeds to PPRV and it is known that the West African dwarf breed from the rainy tropical region is more highly susceptible to PPRV infection than the West African long-legged goat breed from the Sahalian region, and this difference in sensitivity is related to breed and not to virus. Recently, Diop et al. (2005) also observed this finding involving both breeds during the same PPR outbreak and reported that the acute form of the disease affected West African dwarf goats while the West African long-legged goat exhibited the milder form.

We have observed from previous experiments that the challenge of goats with highly virulent PPRV isolates can lead to death in 3–5 days post-infection, before the appearance of any clinical signs of PPR, due to the inapparent reactivation of heartwater disease with typical signs and post-mortem lesions. The causative agent, *Cowdria ruminantium*, has been detected from brain samples (E. Couacy-Hymann, personal communication). This reactivation reflects deep immunosuppression caused by PPRV isolates that enhance the resurgence of latent infection as is known with Morbilliviruses. In previous studies, there was an inapparent infection of goats by *Cowdria ruminantium* before the challenge with a virulent strain of PPRV. This has been observed on a regular basis with highly virulent PPRV isolates and jeopardized some experiments where infected animals died before the end of the study.

The virulence of the PPRV isolates may be affected by a number of factors such as storage conditions, breed of goat and genetic relationship. Further investigations are now needed to understand fully these effects.

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