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Pathogenicity of attenuated *peste des petits ruminants* virus in sheep and goats

ADU (F.D.), JOANNIS (T.), NWOSUH (E.), ABEGUNDE (A.). Pathogénicité du virus atténué de la peste des petits ruminants chez des moutons et des chèvres. *Revue Elev. Méd. vét. Pays trop.*, 1990, 43 (1) : 23-26.

Avec les passages du virus PPR sur les cellules Vero, on a noté une diminution progressive de sa virulence vis-à-vis des chevreaux. Les chèvres inoculées avec le virus au 60^e passage ont présenté la maladie clinique avec des cas de mortalité alors que celles ayant reçu le 80^e passage n'ont montré aucun signe morbide. Si la diminution progressive de la virulence du virus par passages sur cellules se poursuit, un vaccin homologue anti PPR sera obtenu rapidement à l'Institut National de Recherche Vétérinaire de Vom, Nigéria. *Mots clés* : Ovin - Caprin - Peste des petits ruminants - Pathogénie - Vaccin - Nigéria.

INTRODUCTION

Peste des petits ruminants (PPR) is a rinderpest like disease of sheep and goats characterized by fever, diarrhoea, nasal and ocular discharge, ulceration of the buccal cavity and pneumonia. The disease is caused by the PPR virus, a fourth member of the Morbillivirus genus of the Paramyxoviridae family (5).

The disease is at present controlled by vaccination, using the tissue culture rinderpest vaccine (TCRV). While satisfactory results have been observed in some animals vaccinated with TCRV (1, 4, 9), other authors (6) observed that goats incubating the virus often came down with the frank disease when vaccinated with TCRV. To this group of animals, it is thought that homologous PPR vaccine will be of better choice. Attempts to develop cell culture attenuated PPR vaccine have been made at the National Veterinary Research Institute, Vom. This paper therefore reports the progress so far made in the process of developing a homologous PPR vaccine in Vom.

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MATERIALS AND METHODS

Virus

PPR virus designated PPR 75/1 was first isolated from a sick goat in Vom (8). The virus was received in freeze drying form from the Animal Virus Research Institute, Pirbright where it had undergone forty serial passages in Vero cells (TAYLOR, personal communication).

Passage in Vero cells

Virus passage was done in Vero cells by inoculating 0.2 ml of the virus into confluent cultures containing approximately 5×10^6 viable cells per ml. This procedure was continued serially until the 60th passage. Further passages were done by the limiting dilution method. Briefly described, 0.1 ml of tenfold dilutions (10^{-1} - 10^{-7}) of the 60th passage was inoculated into each of five tissue culture tubes containing approximately 750,000 viable Vero cells in medium E.199 supplemented with 2 % calf serum. Tubes were incubated at 37 °C in roller drums and examined daily for cytotoxic effect (CTE). The dilution showing CTE in one or two tubes was used to inoculate the next confluent cell culture. This procedure was continued until the 80th passage.

Preparation of stock virus

The 60th, 70th and 80th passages were grown in large roller bottles seeded with 50 ml of medium containing 10^7 cells per ml, to which 0.5 ml of the virus was added. Cells were rolled at 37 °C and examined daily for CTE. Uninoculated cells were used as control. When 75 % CTE were observed cells were harvested, clarified by low speed centrifugation and distributed in 1 ml aliquots in ampoules. The ampoules were freeze dried and stored at - 20 °C after titration.

Pathogenicity test in goats

The degree of attenuation of the 60th, 70th and 80th passages was evaluated by pathogenicity tests using

susceptible 12-month old goats. In each test, between 6 to 9 goats were used, out of which between 3 and 5 were inoculated with a suspension of attenuated virus ($10^{4.5}$ CTID₅₀/0.2 ml/ animal), 2 or 3 with a 20 % suspension of virulent PPR virus and 1 or 2 with PBS. In the last two groups, each goat received 1 ml of the inoculum. Goats were housed separately and monitored daily for PPR symptoms.

Serology

Dead goats were posted and anatomo-pathological examination was performed. Organ suspensions were made for virus isolation, and lymph node extracts were tested in the Agar gel precipitation test (AGPT) and counter immunoelectrophoresis (CIEOP) against known positive PPR antibodies in order to detect PPR.

Goats that survived the inoculation were bled 21 days

post inoculation (p.i.) and serum was tested for specific PPR antibodies in the AGPT and CIEOP tests.

RESULTS

Results of pathogenecity tests of the 60th, 70th and 80th passages are shown in Tables 1-3. Three of the four goats inoculated with the 60th passage developed pyrexia between the 4th and 5th day p.i. while diarrhoea, ocular and nasal discharge was noticed from day 6 p.i. Two goats died while two recovered after classical symptoms of PPR.

The three goats inoculated with a 20 % PPR virus suspension developed the disease, and died between day 7 and day 15 p.i.

TABLE I Daily observation of experimental animals inoculated with the PPR virus 75/1 60th passage.

Goat n°	Inoculum	Days post inoculation											
		1	2	3	4	5	6	7	8	9	10	11	12
6562	PPR 75/1 60th passage	38.2*	39.2	38.4	42.0	S	Dr	OD	OD/NC	D	—	—	—
6592		38.6	38.4	39.2	38.2	38.2	Dr	OD/ND	OC	37.5	37.0	D	—
6587		38.4	38.1	39.0	39.6	40.1	40.2	38.2	38.2	38.5	38.2	38.2	38.0
6800		37.5	37.9	38.8	39.0	41.2	40.4	Dr	ND	ND	OC	38.0	38.1
6801	20 % PPR suspension	38.6	38.4	38.2	42.4	42.3	42.3	Dr	Dr	Dr	ND	D	—
6802		37.0	37.4	39.3	39.9	42.0	42.4	D	—	—	—	—	—
6803		37.3	38.4	39.8	40.6	42.0	42.0	OD	OD	Dr	36.2	36.2	D
6804	PBS	38.2	38.9	39.4	38.5	38.5	37.2	38.0	38.0	37.8	37.6	37.5	37.5
6805		39.4	39.6	39.2	38.5	39.0	38.0	38.4	38.0	38.0	38.0	38.6	38.0

* Daily temperature. S : Sick. Dr : Diarrhoea. OD : Ocular discharge. NC : Nasal discharge. D : Dead.

TABLE II Daily observation of goats inoculated with the 70th passage of PPR virus.

Goat n°	Inoculum	Days post inoculation														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
892	PPR 75/1 70th passage	37.5	38.3	37.7	38.3	38.4	37.7	38.2	38.2	38.3	38.0	37.7	38.2	37.0	37.9	38.0
893		37.8	37.6	38.6	38.5	38.6	37.7	38.6	38.3	38.7	38.4	37.5	37.9	38.2	38.2	38.4
894*		37.5	37.4	38.2	38.2	38.3	37.7	38.6	38.6	38.4	37.3	38.3	38.4	38.3	38.6	38.3
896		37.8	38.2	38.1	38.4	38.3	37.7	38.6	37.5	38.1	38.3	37.4	37.4	38.4	37.7	38.0
897		37.7	38.9	38.3	38.5	38.5	37.7	38.6	37.3	38.4	38.2	38.2	38.7	38.3	38.4	37.8
898	20 % PPR suspension	37.5	38.4	38.6	40.9	40.5	OD	Dr	OD/ND	D	—	—	—	—	—	—
895		37.7	37.5	39.9	39.8	40.6	40.5	Dr	Dr	ND/OD	36.5	36.5	D	—	—	—
890		37.5	38.3	38.4	37.7	37.5	37.5	38.3	38.3	38.0	37.5	37.6	38.0	37.6	38.0	38.0

* Died after 25 days after showing symptoms of PPR.

TABLE III Daily observation of goats inoculated with the 80th passage of PPR virus 75/1.

Goat n°	Inoculum	Days post inoculation														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
6568	PPR 75/1 80th passage	37.7	37.3	38.4	37.2	37.1	37.0	38.5	38.0	38.5	37.4	37.3	37.9	38.0	38.1	37.0
3822		38.8	38.4	37.0	37.3	38.0	38.0	37.9	39.9	40.4	37.3	38.0	38.0	38.3	37.5	37.4
4615		38.3	38.0	37.7	37.7	38.0	37.5	37.9	37.3	37.4	37.3	37.4	38.0	38.0	38.3	37.4
4832	20 % PPR suspension	37.3	37.4	37.0	39.4	39.4	40.4	Dr	Dr	OC/ND	Dr/OC/ ND	36.0	D	—	—	—
5224		37.6	37.6	40.3	41.2	40.9	40.3	40.0	40.3	40.4	40.0	Dr	37.0	37.4	37.3	37.8
5512	PBS	37.5	38.0	37.4	38.3	38.4	37.3	37.6	38.2	37.3	37.4	37.3	38.0	38.4	38.0	37.5

No pyrexia was observed in goats inoculated with the 70th passage within the experimental period. However, goat No. 894 developed the disease later on and died on day 25 p.i.

A mild pyrexia that subsided within 2 days (39.9-40.4 °C) was noticed in one of the three goats (3822) inoculated with the 80th passage. No other clinical signs of PPR or mortality was noticed.

In both cases, all the goats inoculated with the 20 per cent PPR virus suspension developed PPR, out of which three died. Goats inoculated with PBS did not show any sign of the disease.

PPR virus was isolated from the target organs of the goats, while lymph node extracts gave positive precipitin line in the AGPT and CIEOP tests when tested with specific PPR and Rinderpest hyper-immune serum. Likewise, the goats that survived challenge developed PPR antibodies that were detected in both tests.

DISCUSSION

Our results indicate a progressive loss of virulence of the wild PPR 75/1. An earlier report indicated that the

42nd passage was still very pathogenic for goats (2). The 60th passage was also very pathogenic for goats as shown in this study. This finding is in agreement with the previous findings of BENAZET (3) who found the virus still virulent after the 65th passage in susceptible goat kids.

Results obtained in this study showed a progressive loss of virulence in goats from the 70th passage. Only very mild pyrexia was noticed while mortality only occurred outside the observation period. The progressive loss of virulence may be due to the limiting dilution technique adopted from the 60th passage.

An interesting phenomenon was noticed during the process of virus passaging in Vero cells. When cells were inoculated with the virus, CTE normally appeared from day 6 post-inoculation. There was an eclipse for the next two days during which the CTE noticed earlier disappeared, while reappearing only on day 9 post-inoculation. The CTE persisted until all the cells were destroyed between day 11 and day 13 p.i. This may well be a result of the continued passage of PPR virus in cells which had then been fully adapted to the virus.

If the present decline in virulence observed in this study continues, a homologous PPR vaccine will soon be produced in Vom.

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Progressive loss of virulence for goat kids was noticed when *peste des petits ruminants* (PPR) virus was passaged in Vero cells. While goats inoculated with the 60th passage suffered from the clinical PPR disease and mortality, goats inoculated with the 80th passage did not show any sign of the disease. If the progressive loss of virulence of the virus with passage continues, it will not be long before a homologous PPR vaccine will be obtained at the National Veterinary Institute, Vom. *Key words* : Sheep - Goat - *Peste des petits ruminants* - Pathogenicity - Vaccine - Nigeria -

ADU (F.D.), JOANNIS (T.), NWOSUH (E.), ABEGUNDE (A.). Patogenicidad del virus atenuado de la peste de los pequeños rumiantes en cabras y ovejas. *Revue Elev. Méd. vét. Pays trop.*, 1990, 43 (1) : 23-26.

Una pérdida de virulencia progresiva fue observada en cabritos, cuando el virus de la *peste des petits ruminants* (PPR) fue sometido a pasajes en células Vero. Las cabras inoculadas con virus de « 60 pasajes » sufrieron clínicamente la enfermedad de PPR y mortalidad, mientras que las cabras inoculadas con virus de « 80 pasajes » no mostraron ningún signo de enfermedad. Si la pérdida progresiva de virulencia del virus continúa conforme se realizan los pasajes, no pasará mucho tiempo antes de que se obtenga una vacuna homóloga de PPR en el Instituto Nacional Veterinario, en Vom, Nigeria. *Palabras claves* : Oveja - Cabra - Peste de los pequeños rumiantes - Patogenicidad - Vacuna - Nigeria.

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