JCM
Journals.ASM.org

A Reliable and Reproducible Experimental Challenge Model for Peste des Petits Ruminants Virus

Mehdi El Harrak,[a] Nadia Touil,[b] Chafiqua Loutfi,[a] Mustapha Hammouchi,[a] Satya Parida,[c] Gizlane Sebbar,[a] Nadia Chaffai,[a] Bachir Harif,[a] Nezha Messoudi,[d] Carrie Batten,[c] and Chris A. L. Oura[c,e]

Société de Productions Pharmaceutiques et Vétérinaires, Laboratoire de Virologie, Rabat, Morocco[a]; Hôpital Militaire d'Instruction Med V, Service de Virologie, Faculté de Médecine et de Pharmacie, Rabat, Morocco[b]; Institute for Animal Health, Pirbright, Woking, Surrey, United Kingdom[c]; Hôpital Militaire d'Instruction Med V, Service d'Hématologie, Faculté de Médecine et de Pharmacie, Rabat, Morocco[d]; and School of Veterinary Medicine, University of the West Indies, St. Augustine, Trinidad and Tobago[e]

Experimental challenge protocols that consistently reproduce clinical signs of peste des petits ruminants in Alpine goats infected with a tissue culture-passaged peste des petits ruminants virus are described. The protocols can be used to carry out quality-controlled vaccine efficacy and pathogenesis studies under experimental conditions.

Peste des petits ruminants (PPR) is an acute and highly contagious disease of small ruminants caused by peste des petits ruminants virus (PPRV) (2). Infection with any of the four lineages of PPRV can be asymptomatic or produce a wide spectrum of clinical manifestations, ranging from mild and short lived to lethal, depending on many factors, including the strain of virus, sensitivity of the host species, and individual animal susceptibility (6). Goats are considered to be more susceptible than sheep to PPR (7).

In the past, it has proved extremely difficult to reproduce, under experimental conditions, the clinical signs of PPR that are observed in sheep and goats during field outbreaks. The reasons for this are currently unknown; however, it could be due to a reduced susceptibility of the sheep or goat breeds used in the studies or reduced virulence of the infecting virus, due to storage or passage of the virus in the laboratory. Many of the experimental studies that have been carried out up to now have focused on testing and developing vaccines. In these studies, in order to reproduce consistent clinical signs of PPR under experimental conditions, it was necessary to use a challenge virus in the form of splenic suspension serially passaged in sheep or in goats (4, 5). Under these conditions, it was not possible to titrate the virus, which made it difficult to repeat the experiments using the same titer of virus, thus affecting the reproducibility of results between experiments. The aim of this study was to develop an effective experimental challenge protocol, using a tissue-culture-passaged PPRV, which reliably and consistently produced clinical signs of PPR.

A total of 12 Alpine goats, of ages between 4 and 6 months, were used in the study. French Alpine goats were purchased from the Royal Douiet Domains (Fès, Centre of Morocco). Prior to inoculation, all animals were clinically normal and were negative for PPRV antibodies. Groups of 4 goats were infected intravenously (i.v.), intranasally (i.n.), and subcutaneously (s.c.). All animals were infected with a total dose of 5.1 log 50% tissue culture infective dose ($TCID_{50}$) of a Vero cell-passaged (5 passages) Moroccan field strain of PPRV (Maroc/2008) (3). Oropharyngeal (PH), ocular (OC), and nasal (NS) swab samples were collected prior to infection and then daily for up to 9 days postinfection (dpi). EDTA-blood was collected daily. Samples were processed as described by Hammouchi et al. (3), and the levels of viral RNA in the different swabs, the blood, and in the lung and mesenteric lymph nodes were evaluated by real-time reverse transcription-quantitative PCR (RT-qPCR) as described by Batten et al. (1). The tissues were collected from the goats when they were euthanized, which was dependent on when/if they reached the clinical endpoint. Goats that did not reach a clinical endpoint were euthanized at the end of the study (14 days postinfection [dpi]). Detection of PPRV antibodies in serum was carried out using a hemagglutinin protein enzyme-linked immunosorbent assay (H-ELISA) (IAH, Pirbright, United Kingdom).

The levels of RNA detected by RT-PCR in swabs, in blood, and from the organs of the euthanized animals are summarized in Table 1. PPRV RNA was detected in blood samples as early as 2 dpi in 2 out of the 4 goats infected through the i.v. route, in 3 out of 4 goats infected by the s.c. route, and in only 1 out of the 4 goats infected by the i.n. route. These data indicate that viremia may be detected earlier through the i.v. and s.c. routes. Regardless of the route of inoculation, PPRV was excreted through nasal, oropharyngeal, and ocular routes (measured by the presence of viral RNA in swabs) from as early as 2 or 3 dpi as previously observed (3). All infected animals exhibited lower threshold cycle (C_T) values (higher levels of viral RNA) between 6 and 8 dpi, and viral RNA levels peaked at around 6 to 7 dpi. Early in infection (2 to 4 dpi), higher C_T values (lower levels of viral RNA) were seen in the swabs taken from the s.c. infected goats than were observed in the i.v. and i.n. infected goats (Table 1), indicating a reduced level of viral replication. This may have been the reason for the less-severe clinical signs observed in the s.c. infected as opposed to i.v. and i.n. infected goats. All infected animals exhibited a marked immunosuppression, with white blood cell counts (WBCs) decreasing significantly (from 12,575 to 4,725 cell/μl) between 1 and 4 dpi in the three groups of goats. The WBCs then plateaued at a low level between 4 and 8 dpi before increasing in the 4 s.c. infected goats that survived the infection (data not shown). These results corroborate those obtained by Jagtap and collaborators (8), who demonstrated the immunosuppressive nature of PPR in goats challenged with PPRV (Izatnagar/94 strain). Interestingly, in the present

Received 7 July 2012 **Accepted** 9 August 2012
Published ahead of print 22 August 2012
Address correspondence to Nadia Touil, ntouil2003@gmail.com.

doi:10.1128/JCM.01785-12

TABLE 1 C_T values for swabs, whole blood (viremia), and organs from experimentally infected goats[a]

Animal no.	Samples	C_T value												
		On day postinfection:											For organ	
		1	2	3	4	5	6	7	8	9	12	13	Mes LN	Lung
1	OC	No C_T	No C_T	27.08	20.55	17.48	23.00	21.11					20.15	16.17
	NS	No C_T	No C_T	26.34	21.26	18.93	21.16	21.09						
	PH	No C_T	No C_T	28.63	27.89	22.69	24.92	26.08						
	Viremia	No C_T	30.9	26.22	26.1	26.43	28.4	ND						
2	OC	No C_T	28.97	27.7	29.81	29.38	25.36	25.9	26.09				29.59	No C_T
	NS	No C_T	30.23	27.1	29.55	19.53	21.9	19.81	26.15					
	PH	No C_T	No C_T	No C_T	30.0	26.9	28.78	28.22	27.02					
	Viremia	27.46	27.47	29.79	30.08	30.65	29.68	29.79	28.9	27.09	27.71			
3	OC	No C_T	No C_T	27.58	25.55	21.05							19.16	21.33
	NS	No C_T	No C_T	25.84	26.71	19.28								
	PH	No C_T	No C_T	27.38	23.83	20.22								
	Viremia	27.51	27.77	27.42	26.62	ND								
4	OC	29.56	28.26	27.50	23.68	24.34	26.28						20.1	22.29
	NS	29.35	28.21	25.33	23.65	23.5	21.38							
	PH	29.1	29.06	29.65	21.6	21.23	25.21							
	Viremia	No C_T	No C_T	26.73	27.51	25.27	24.34							
5	OC	No C_T	No C_T	28.78	23.17	23.17	24.04	24.89					20.33	19.83
	NS	No C_T	No C_T	26.13	23.68	19.54	19.61	20.28						
	PH	No C_T	No C_T	29.42	24.0	23.61	26.27	26.22						
	Viremia	No C_T	28.0	27.83	26.63	25.43	26.9	ND						
6	OC	34.82	30.19	31.46	27.67	24.18	21.4	20.17					No C_T	23.19
	NS	33.44	32.57	26.36	23.29	26.0	19.19	20.8						
	PH	31.0	30.02	28.15	21.28	28.22	21.22	22.37						
	Viremia	No C_T	No C_T	No C_T	No C_T	27.94	29.57	28.06	28.94					
7	OC	30.23	31.44	28.42	26.62	23.22	19.96	21.26	23.48				20.2	21.54
	NS	35.53	28.94	29.23	20.01	22.08	16.63	20.25	17.56					
	PH	33.25	30.25	29.18	27.43	23.98	18.82	ND	22.19					
	Viremia	No C_T	No C_T	30.47	27.94	27.32	26.25	27.45	ND					
8	OC	29.69	28.83	28.71	27.71	26.58	21.65						17.1	18.45
	NS	27.6	25.98	26.29	19.84	18.4	27.57							
	PH	27.29	30.41	29.07	27.2	25.05	25.72							
	Viremia	26.85	24.87	26.95	28.08	27.4	29.82							
9	OC	No C_T	32.56	30.32	30.09	25.61	ND	ND					29.18	31.45
	NS	No C_T	33.23	30.81	27.56	25.11	ND	ND						
	PH	33.53	34.12	30.26	29.92	31.19	ND	ND						
	Viremia	27.65	28	30.87	29.29	28.38	26.29	25.72	27.03					
10	OC	No C_T	No C_T	No C_T	28.63	26.51	26.4	22.18	20.1				No C_T	No C_T
	NS	No C_T	No C_T	No C_T	27.68	25.55	22.8	19.6	21.21					
	PH	No C_T	No C_T	No C_T	28.14	30.41	27.57	21.12	24.7					
	Viremia	27.41	30.38	27.38	29.73	26.33	30.08	28.23	26.71	26.75	28.71			
11	OC	32.56	27.4	31.13	31.03	26.34	31.07	25.98					28.35	No C_T
	NS	30.59	32.43	31.43	27.72	23.19	23.69	25.95						
	PH	30.86	30.26	31.24	29.23	29.11	23.42	21.35						
	Viremia	No C_T	No C_T	33.33	30.72	30.29	30.25	30.23	29.92	30.02	35.96			
12	OC	31.56	30.36	29.87	24.05	23.99	24.07	25.95					No C_T	29.57
	NS	30.62	31.18	26.99	25.66	22.51	22.69	21.95						
	PH	31.29	29.99	30	21.96	25.17	24.93	24.91						
	Viremia	28.4	27.5	31.69	26.48	26.86	27.06	27.62	ND	27.5	27.2			

[a] OC, ocular; NS, nasal; PH, pharyngeal; ND, not done.

TABLE 2 Clinical scores for Alpine goats experimentally infected through the i.v., i.n., and s.c. infection routes with a Moroccan strain of PPRV

Infection route	Animal no.	Total clinical score on day postinfection:												Time euthanized (dpi)
		3	4	5	6	7	8	9	10	11	12	13	14	
i.v.	1	5	8	12	17	19	20							8
	2	4	5	7	12	13	15	17	19	19	19	16	15	14[a]
	3	2	10	17	21									6
	4	1	8	14	15	18	20							8
i.n.	5	1	3	7	11	19	21							8
	6	1	3	8	11	14	19	18	19	19	19	20		13
	7	0	9	10	13	16	18	23						9
	8	6	8	14	14	17	20							8
s.c.	9	0	4	4	7	8	6	4	3	4	4	2	2	14[a]
	10	1	2	3	7	7	9	9	9	6	3	3	2	14[a]
	11	0	2	3	10	10	10	11	13	12	9	7	4	14[a]
	12	2	3	7	11	10	12	13	12	12	10	6	3	14[a]

[a] Euthanized at 14 dpi, on the last day of the study.

study, increasing WBCs were observed after 8 dpi in animals surviving the infection, as has been previously reported (8).

Clinical scores were recorded up to 14 dpi and were used to evaluate the severity of clinical signs and to compare the different levels of clinical signs observed in goats infected through the i.v., i.n., and s.c. routes (Table 2). A clinical scoring system was established with a ranking from 0 to 3 based on the severity of clinical signs and symptoms; rectal temperature (pyrexia), anorexia, behavior, nasal discharge, salivation, acute respiratory symptoms, including dyspnoea, coughing, sneezing, and diarrhea. The severity of clinical disease observed per day was evaluated using the following scheme: score 0, no symptoms; score 1, mild clinical signs and symptoms; score 2, high temperature and moderate clinical signs; and score 3, severe clinical signs. A total cumulative score per animal and a score per day were measured. Animals that reached a daily clinical score of 20 or greater were euthanized for animal welfare reasons. Three of the four goats in the i.v. group were euthanized at from 6 to 8 dpi, having reached a clinical endpoint. All four goats infected by the i.n. route were euthanized at from 8 to 13 dpi on reaching a clinical endpoint. The group of 4 goats infected by the s.c. route developed mild clinical signs, with a maximum daily clinical score of 13 (Table 2), and all 4 goats in this group recovered and mounted positive antibody responses at 7 dpi. Except for animal 2, which survived infection, none of the remaining animals in the i.v. and i.n. infected groups developed significant PPRV antibodies before 9 dpi. The reduced levels of clinical signs and subsequent survival of the s.c. infected goats might be explained through an earlier and more pronounced immune response stimulated through the skin, which is a primary site of the immune system. The trends of clinical pattern with time were compared using the analysis of variance (ANOVA) test. Whatever the infection route, the clinical score increased up to 6 dpi (P value was <0.009), with no significant difference between groups. Neither significantly higher nor earlier-onset clinical scores were observed for the i.v. or i.n. infected goats compared with those for the s.c. infected goats. A *post hoc* test (Bonferroni correction) was performed in order to check if the route of infection affected the clinical scores. The results revealed significantly higher clinical scores ($P < 0.008$) for the i.v. and i.n. infected goats than for the s.c. infected goats, while there was no significant difference in clinical scores between the i.v. and i.n. infected goats.

This study set out to develop an experimental infection protocol that consistently produced clinical signs of PPR, using a susceptible breed of goat (Alpine) and a tissue culture (5th passage) PPRV. Any of the three infection routes (i.v., i.n., and s.c.) described could be used in future PPRV infection studies depending on the requirements of the study: in experimental infection studies where severe clinical signs were required, i.v. or i.n. routes of infection would be recommended. The i.n. infection route, however, may be considered to be the method of choice for immunology/pathology studies since this is considered to be the natural way that PPRV is transmitted from animal to animal in the field. In studies requiring less-severe clinical signs (to reduce the level of suffering of the animals) or in longitudinal studies where survival of the animals is a prerequisite, the s.c. route of infection would be recommended. These protocols can be used in laboratories across the world for carrying out PPRV infection and vaccine efficacy studies under experimental conditions.

ACKNOWLEDGMENT

We thank Karim Idrissi Sabai for performing the statistical analyses.

REFERENCES

1. **Batten CA, et al.** 2011. A real time RT-PCR assay for the specific detection of Peste des petits ruminants virus. J. Virol. Methods **171**:401–404.
2. **Gibbs EP, Taylor WP, Lawman MJ, Bryant J.** 1979. Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. Intervirology **11**:268–274.
3. **Hammouchi M, et al.** 17 May 2012. Experimental infection of alpine goats with a Moroccan strain of peste des petits ruminants virus (PPRV).Vet. Microbiol. [Epub ahead of print.] doi/10.1016/j.vetmic.2012.04.043.
4. **Rajak KK, et al.** 2005. Experimental studies on immunosuppressive effects of peste des petits ruminants (PPR) virus in goats. Comp. Immunol. Microbiol. Infect. Dis. **28**:287–296.
5. **Saravanan P, et al.** 2010. Comparative efficacy of peste des petits ruminants (PPR) vaccines. Biologicals **38**:479–485.
6. **Sen A, et al.** 2010. Vaccines against peste des petits ruminants virus. Expert Rev. Vaccines **9**:785–796.
7. **Singh RP, Saravanan P, Sreenivasa BP, Singh RK, Bandyopadhyay SK.** 2004. Prevalence and distribution of peste des petits ruminants virus infection in small ruminants in India. Rev. Sci. Tech. **23**:807–819.
8. **Jagtap SP, et al.** 2012. Effect of immunosuppression on pathogenesis of peste des petits ruminants (PPR) virus infection in goats. Microb. Pathog. **52**:217–226.