Development of a PPRV challenge model in goats and its use to assess the efficacy of a PPR vaccine

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A B S T R A C T

Peste des Petits Ruminants (PPR) is a severe disease of small ruminants and has high economic impacts in developing countries. Endemic in Africa, the Middle East and Asia, the disease is currently progressing with occurrences reported in North Africa, Turkey and in Georgia, and now threatens Europe. Much remains unknown about the infection dynamics, the virulence of the different strains and species/breed susceptibility. Robust experimental challenge models are needed to explore these fields and to confirm the efficacy of currently sold vaccines. We first assessed virulence of two PPR virus strains (CI89 and MA08) in Saanen goats. Whereas the MA08 strain led to classical severe clinical signs of PPR, the CI89 strain appeared to cause a mild disease in Saanen goats, highlighting the difference in virulence between strains in this animal model. We further demonstrated the importance of the inoculation route in the appearance of clinical signs and that ocular excretion is a better choice than blood for viral detection. After developing a robust challenge model, we assessed the efficacy of a vaccine (PPR-VAC®, BVI Botswana) against the MA08 strain and demonstrated that this vaccine blocked viral excretion and significantly reduced clinical signs. These results reinforce the paradigm that a strain from one lineage could protect against strains from other lineages.

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1. Introduction

Peste des Petits Ruminants (PPR), a disease of small ruminants which is currently endemic in Africa, the Middle East and Asia, is one of the most devastating infectious diseases of domestic small ruminants (sheep and goats). It is characterised by hyperthermia, mucopurulent ocular and nasal discharges, erosion of the mucosa, apathy, anorexia, acute diarrhoea [1] and death. PPR affects sheep, goats and a large number of species in the order Artiodactyla, both wild and captive, with a mortality rate of 50–100% in susceptible populations [2–8]. The susceptibility and virulence depend both on the infected species [1] and the PPR virus (PPRV) strain [9]. In endemic regions, small ruminants develop lifelong immunity following natural infection. But the infection of naïve animals (e.g. the young-of-the-year) allows continuous circulation of PPRV.

PPR outbreaks may have serious economic impacts and are a threat to food security where small ruminants are the main livestock resource [10–12]. The disease is still progressing with occurrences in Tunisia (2006), Morocco (2008 and 2015), Algeria (2011 and 2016), Turkey (2012) and in Georgia (2016), and is becoming a threat to Europe [13].

The causative agent, PPRV, belongs to the family Paramyxoviridae, genus Morbillivirus, which also includes measles and rinderpest (RPV) viruses [3]. Although PPRV strains circulate under four genetic lineages (I–IV) [14], there is only one serotype. The most widely used vaccine strains, Nigeria 75/1 (lineage II) and Sungri 96 (lineage IV), provide complete protection across genetic lineages [15]. The infection dynamics of PPRV is poorly understood, notably the shedding kinetic patterns, the virulence of the different strains and species susceptibility. Although the efficacy of the vaccine strain Nigeria 75/1 has been well established empirically in the field, little information is available on the efficacy of the many currently sold PPR vaccines based on this strain. Feedbacks from the field are not always identical for the different vaccines.

This may relate to the way vaccines are stored, produced, including the virus titre or even perhaps to the number of passages between the master seed virus and the final product. Robust experimental infection models are required to tackle these issues, which are indispensable for the ongoing global PPR eradication programme [16].

We first evaluated the susceptibility of Saanen goats to two different PPRV strains, one from the lineage I isolated in Ivory Cost in 1989 (CI89) and one from the lineage IV isolated in Morocco in 2008 (MA08). Next, we assessed the efficacy of an attenuated vaccine (PPR-VAC®, BVI, Gaborone, Botswana) to protect Saanen goats against a MA08 virulent challenge.

2. Materiel and methods

2.1. Animals

All experiments were conducted in biosafety level 3 conditions with Saanen goats (aged 9–11 months) split into groups which were balanced for bodyweight. To compare two PPR pathogenic strains (referred hereafter as virulence experiment), 16 goats were allocated to four groups (4/group). For the efficacy assessment of PPR-VAC® (referred hereafter as vaccine experiment), 20 goats were allocated to two groups (10/group). All the animals were healthy and seronegative on D0 (tested by competitive ELISA, see below).

Animal handling was performed in strict accordance with good animal practice, as defined by the European relevant regulation, and was approved by the Ethical Committee of Merial. Pain management was performed by daily intramuscular administration of 6 µg/kg of buprenorphine, when hyperthermia (over 41 °C), apathy, hyporexia/anorexia and/or diarrhoea were observed. Animals reaching a severe clinical state were euthanized on ethical grounds.

2.2. Virus and challenge

For the virulence experiment, CI89 and MA08 strains were compared. The CI89 strain was passaged three times on primary cells from sheep skin explant and four times on VERO cells. The MA08 strain was passaged twice on SLAM-dog VERO and three times on VERO cells. The goats were inoculated with CI89 and MA08 strains on D0 with a total of 4 log10 TCID$_{50}$ per animal, either by the intravenous route (IV, 2 ml) or by the intranasal route (IN, 1 ml/nostril) using a LMA® MAD NASAL™ specific device. Based on the results of the virulence experiment and because it likely mimics natural infection, the IN inoculation was selected in the vaccine experiment, in which the goats were challenged 21 days after vaccination.

2.3. Vaccine and vaccination

PPR-VAC® is produced by the Botswana Veterinary Institute (BVI, Gaborone, Botswana) and is a live vaccine based on Nigeria 75/1 attenuated strain (lineage II). To assess the efficacy of this vaccine, a low payload (2.2 log10 TCID$_{50}$/dose of 1 ml) was used in the vaccine experiment: 10 goats received a 1-ml subcutaneous injection on D0 after disinfection of the injection site and 10 control animals remained unvaccinated.

2.4. Clinical monitoring

During both experiments, individual clinical monitoring was implemented daily for 14 days after challenge. For all animals, the clinical signs recorded were general behaviour, lesions of mucous membranes, respiratory and ocular disorders, diarrhoea, hypertrophy of the lymph nodes and hyperthermia. These signs were scored as described in Table 1. The presence of any other clinical sign was also documented and scored 1 if associated with PPR. In addition, goats were weighed before the challenge and at the end of the study (or the day of euthanasia).

2.5. PPR genome detection in blood and ocular shedding

During the virulence experiment, ocular swabbing was performed daily on all animals. On the same occasion, blood was collected by jugular puncture with EDTA tubes. During the vaccine experiment, ocular swabbing was performed during the vaccination phase on D0, D2, D4, D7, D9, D11, D14 and D17 on all vaccinated animals to evaluate excretion of the vaccine virus. After which all the animals (vaccinates and controls) were swabbed every two days from the challenge (D21) to the end of the study (D35). On D0 and D21, the blood and ocular samples were collected before vaccination (D0) and challenge (D21).

Total RNA was extracted from blood and swab samples using the NucleoSpin® 96 Virus core kit (Macherey-Nagel, Hoerdt, France) and a BIOMEK automated extractor (Beckman Coulter, Villepinte, France). PPRV specific RNA was quantified in blood and ocular samples by real time RT-PCR (RT-qPCR), by amplifying the partial end of the N protein gene using a one-step method [17]

Table 1
Scoring of recorded clinical signs.

Clinical signs		Score
General behaviour		**Score**
Apathetic		2
Anorexia	Partial	2
	Total	3
Prostrated		6
Lying down		15
Lesions of mucous membranes		**Score**
Labial lesions	Light	1
	Moderate	2
	Severe	3
Perinea/vulva	Congestion	1
	Purpura	2
	Necrosis	3
Nasal crusts and pustules		2
Ocular disorders		**Score**
Lacrimation	Light	1
	High	2
Respiratory disorders		**Score**
Nasal discharge	Light	1
	High	2
	Very High	3
Cough	Sporadic	1
	Bout	3
Dyspnea		5
Digestive disorders		**Score**
Diarrhoea	Aqueous	3
	Haemorrhagic	5
Hypertrophy of lymph nodes		**Score**
Right submaxillary		+ = 0
Left submaxillary		++ = 1
Right prescapular		+++ or ++++ = 2
Left prescapular		
Right precrural		
Left precrural		
Hyperthermia		**Score**
T°C ≤ 40.0		0
40.0 < T°C < 41.0		1
41.0 ≤ T°C		2
Maximum daily clinical score		**58**

Any other clinical sign associated with PPR was scored 1.

with Qscript XLT kit one-step RT-qPCR ToughMix (Quantabio, VWR, Fontenay-sous-Bois, France).

2.6. PPR antibody detection

Blood was collected from all goats using plain tubes on D-3, D1, D4, D8, D11 and D14 during the virulence experiment, and on D0, D9, D21 and D35 during the vaccine experiment. Sera were tested using the IDScreen® PPR competition ELISA kit (IDvet, Grabels, France).

2.7. Data transformation and statistical analyses

Global RNA viral load: the area under the curve (AUC) in log10 RNA copies/μl × day over the challenge period was determined for each animal, as follows:

$$AUC = log10\left(1 + \sum \frac{Vd_i + Vd_j}{2} \times (d_j - d_i)\right)$$

where d_i and d_j are two adjacent dates, Vd_i (or Vd_j) is the viral detection on d_i (or d_j) from which the limit of quantification was subtracted and ($d_j - d_i$) is the interval (in days) between dates d_i and d_j.

Maximum rectal temperature (MRT) was determined as the maximum of rectal temperatures of each animal recorded during the challenge period. The daily clinical score was the sum of all scores attributed in one day per animal. The total clinical score (TCS) was the sum of all the daily clinical scores per animal. Individual average daily gain (ADG) was calculated as follows:

$$ADG = (final\ weight(kg) - initial\ weight(kg))/number\ of\ days$$

between initial and final weighing

Statistical analyses were performed with StatGraphics® Centurion XV (Version 15.2.14). The significant threshold was set at $\alpha = 2.5\%$. The efficacy of the vaccine was assessed by comparing the following parameters: viral AUC, MRT, TCS and ADG, with those of the control group. When normality of the distributions was assumed, the comparisons were performed using a one-sided Student's t test (assuming homoscedasticity or not) or using a one-sided Mann-Whitney W test otherwise. Analysis of results from the virulence experiment was only descriptive because of the small number of animals in the group (n = 4).

3. Results

3.1. PPRV genome detection in blood and ocular excretion during the virulence experiment

Following intravenous (IV) infection with MA08 strain, PPRV genome started to be detected in blood three days post infection (dpi) and all animals were positive from 6 to 8 dpi. The detection
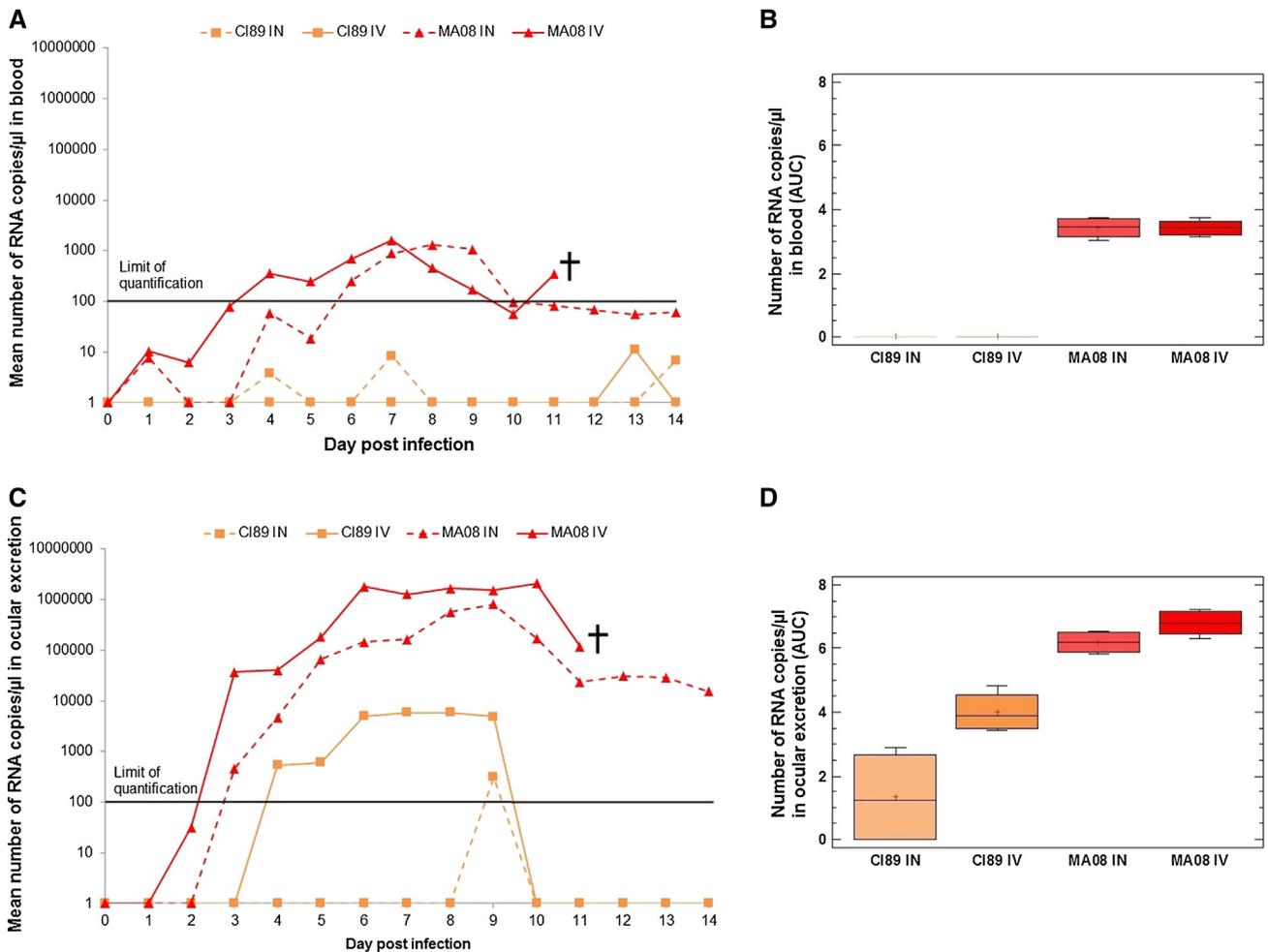

Fig. 1. PPRV detection after infection of Saanen goats with MA08 and CI89 strains. (A) Kinetics of mean number of RNA copies/μl in blood in each group over the challenge period monitored by qRT-PCR. (B) Dispersions of individual AUC of number of RNA copies/μl in blood in each group over the challenge period (expressed in log10 RNA copies/μl × day). (C) Kinetics of mean number of RNA copies/μl in ocular excretion in each group over the challenge period monitored by qRT-PCR. (D) Dispersions of individual AUC of number of RNA copies/μl in ocular excretion in each group over the challenge period. In A and C, † means that four goats from the group infected with MA08 IV were euthanized on ethical grounds on D11.

in blood increased sharply from 4 to 7 dpi, then decreased (Fig. 1A). Comparatively, detection in blood reached an equivalent peak in the group infected via the intranasal (IN) route but exhibited a one-to-two-day delay before its onset. Dispersions of individual corresponding AUC were similar in the two MA08-infected groups (Fig. 1B). Conversely, PPRV genome was not detectable in blood in CI89-infected goats whatever the inoculation route.

Higher PPRV genome amounts were excreted in ocular samples than in blood samples. In the MA08 IV-infected group, ocular excretion started 2 dpi and all the animals were positive from 3 dpi to the end of experiment. The peak of mean ocular excretion was reached 6 dpi (Fig. 1C). In the MA08 IN-infected group, similar results were found, but still with a delay. Individual ocular excretions were similar in the two MA08-infected groups (Fig. 1D). Ocular excretions were also detected in CI89-infected groups, but were weaker than in MA08-infected groups. Notably, goats infected IV with CI89 excreted more than those infected IN (Fig. 1D).

All the goats remained negative for specific PPRV-N antibodies until 4 dpi and all the goats were found to be positive from 8 dpi to the end of the study (see Fig. S1 in supplementary materials).

3.2. Clinical signs during the virulence experiment

Marked hyperthermia was observed in MA08-infected groups but not in the CI89 groups (Fig. 2A). Dispersions of individual MRT indicated that the individual levels of hyperthermia were similar in the MA08-infected groups whatever the infection route (Fig. 2B).

In MA08-infected groups, moderate to severe clinical signs were recorded and were those commonly observed during PPRV natural infection: apathy, anorexia, ocular and nasal discharge, lip and nose lesions, diarrhoea, etc... The mean daily clinical score increased strongly from 6 dpi to reach a peak (20.0) 10 dpi in the IV-infected group and increased from 7 dpi to reach a peak (12.8) 9 dpi in the IN-infected group (Fig. 2C). Goats infected IV were more affected than those infected IN (Fig. 2D). In CI89-infected groups, the clinical signs were mild and consisted principally in lip lesions and swelling of lymph nodes sometimes with lacrimation and nasal discharge. In these groups, the mean daily clinical score never exceeded 3.5 (Fig. 2C). Dispersions of individual TCS indicated that infection IV or IN of goats with the CI89 strain led to low virulence (Fig. 2D).

Regarding weight gain, mean bodyweight increased between the first day and the 14th dpi in CI89-infected groups, but decreased in MA08-infected groups (see Fig. S2A in supplementary materials). This decrease was more pronounced in IV-infected groups than in IN-infected groups. Mean weight gains were similar and positive in CI89-infected groups (+1.9 kg), but were negative in the MA08-infected groups (−1.9 kg & −5.1 kg for the IN & the IV-infected groups respectively; see Fig. S2B in supplementary materials).
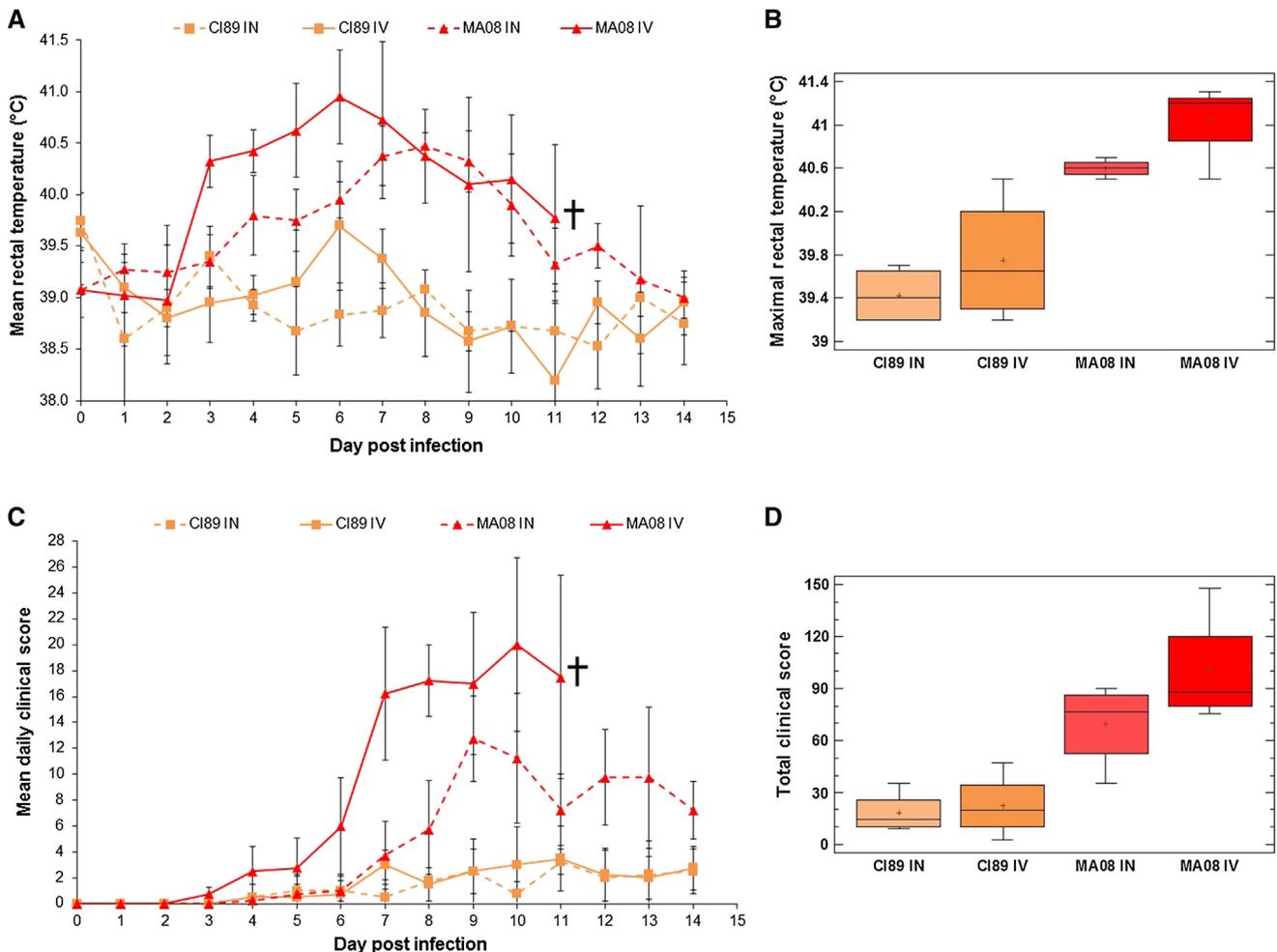

Fig. 2. Differential virulence of PPRV MA08 and CI89 strains in Saanen goats. (A) Kinetics of mean rectal temperature per group over the challenge period. For each point, the error bar represents the mean more or less the standard deviation. (B) Dispersions of individual maximum rectal temperatures (maximum rectal temperature recorded over the challenge period for each animal) in each group. (C) Kinetics of mean daily clinical score in each group over the challenge period. For each point, the error bar represents the mean more or less the standard deviation. (D) Dispersions of individual total clinical scores (sum of daily clinical scores over the challenge period for each animal) in each group. In A and C, † means that four goats in the group infected with MA08 by IV were euthanized on ethical grounds on D11.

From the virulence experiment, the MA08 IN challenge model was selected to assess vaccine efficacy.

3.3. Impact of PPR-VAC® vaccination on clinical disease

Following challenge with MA08, mean rectal temperature increased from 4 dpi with a peak 8 dpi (40.8 °C) in the control group (Fig. 3A). Clinical signs began to appear from 3 dpi with swellings of lymph nodes for three days. Then classical clinical signs of PPR were observed: apathy, anorexia, ocular and nasal discharge, lip and nose lesions, diarrhoea, etc. The mean daily clinical score reached a peak 11 dpi (11.4) and as of this date, four goats were euthanized on ethical grounds because of their severe clinical state (Fig. 3C).

Conversely, in the vaccinated group, mean rectal temperature never exceeded 39.2 °C during the challenge period. MRT were significantly lower than in the control group (p < 0.0001) (Fig. 3B). No severe signs were observed during the challenge: swellings of the lymph nodes also appeared at 3 dpi, but were less severe, and only mild lacrimation and lip lesions were recorded. The mean daily clinical score never exceeded 0.9 and individual TCS were significantly lower than in the control group (p = 0.0002) (Fig. 3D).

Evolution of mean body weights during the vaccination phase were similar in the two groups (see Fig. S3A in supplementary materials), demonstrating that vaccination had no impact on growth. In the control group, mean bodyweight decreased during the challenge phase (−1.1 ± 1.6 kg), whereas the vaccinates continued to gain weight (+3.1 ± 1.4 kg), with a similar slope to that observed during the vaccination phase. The post challenge ADG was significantly lower in the control group than in the vaccinated group (p = 0.0024; see Fig. S3B in supplementary materials).

3.4. Impact of PPR-VAC® vaccination on viral excretion and PPRV serology

In the control group, PPRV genome was detected in five goats in ocular swabs as early as 4 dpi, while all 10 controls were positive from 6 to 12 dpi. A peak of excretion was reached 8 dpi. At the end of the study, four of the remaining six goats were still positive for RNA excretion. In the vaccinated group, PPRV was not detected in ocular excretions either during the vaccination phase (before the challenge) or after the challenge (Fig. 4A and B).

All goats were seronegative at the beginning of the study (Fig. 4C). All the goats in the vaccinated group were seropositive 9 days post vaccination. Goats in the control group remained seronegative until the challenge. At the end of the study, all the goats were seropositive.

4. Discussion

Following the successful eradication of the closely-related RPV, PPR is a new target for global eradication in a programme launched
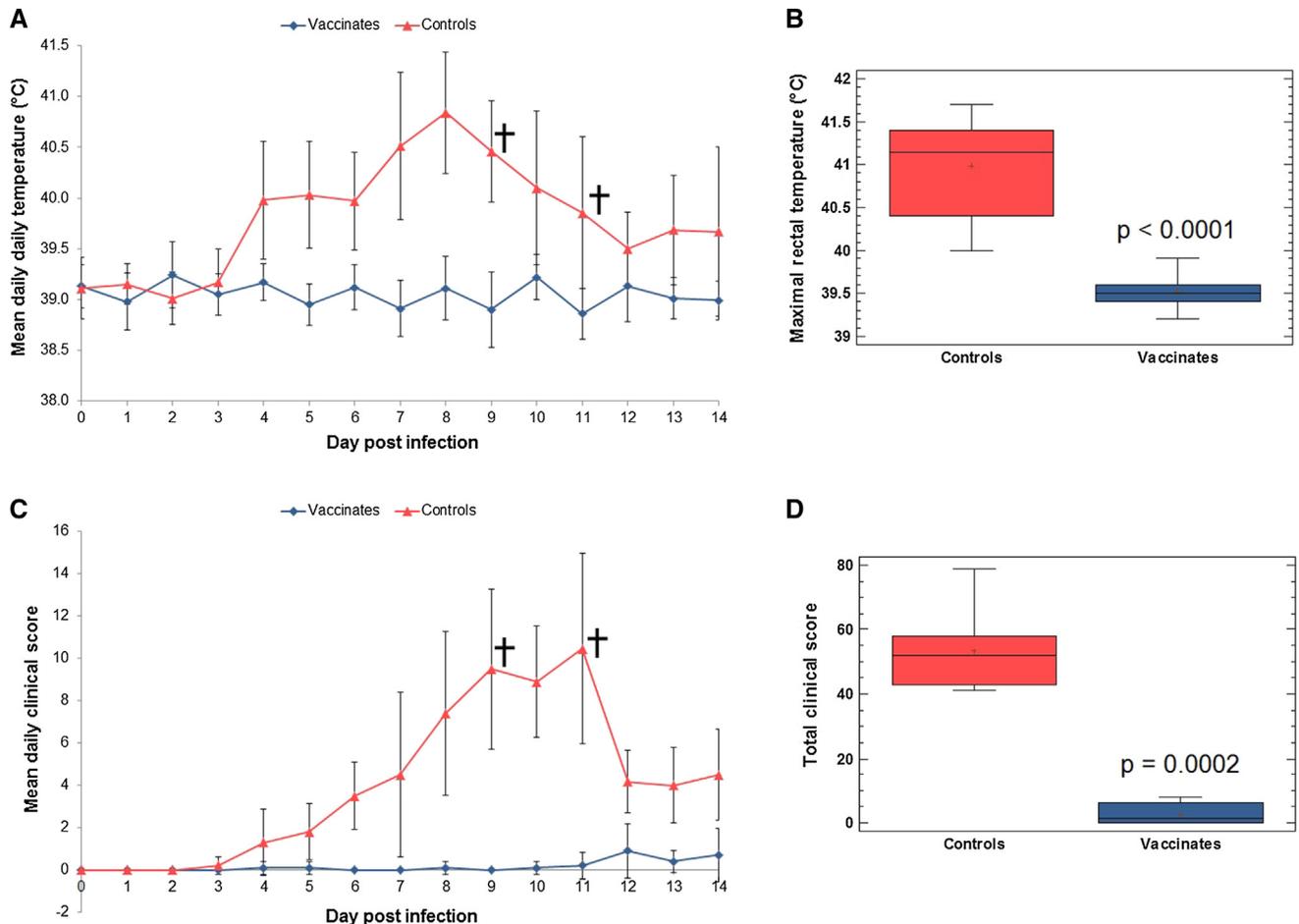

Fig. 3. PPR-VAC® vaccination led to a marked and significant decrease in clinical signs after challenge with the MA08 strain. (A) Kinetics of mean rectal temperature in each group (control and vaccinated groups infected with MA08 IN) over the challenge period. For each point, the error bar represents the mean more or less the standard deviation. (B) Dispersions of individual maximum rectal temperatures (maximum rectal temperature recorded over the challenge period in each animal) in each group. (C) Kinetics of mean daily clinical score in each group over the challenge period. For each point, the error bar represents the mean more or less the standard deviation. (D) Dispersions of individual total clinical scores (sum of daily clinical scores over the challenge period for each animal) per group. In A and C, † means that four goats from control group were euthanized on ethical grounds on D30 (one goat) and D32 (three goats).
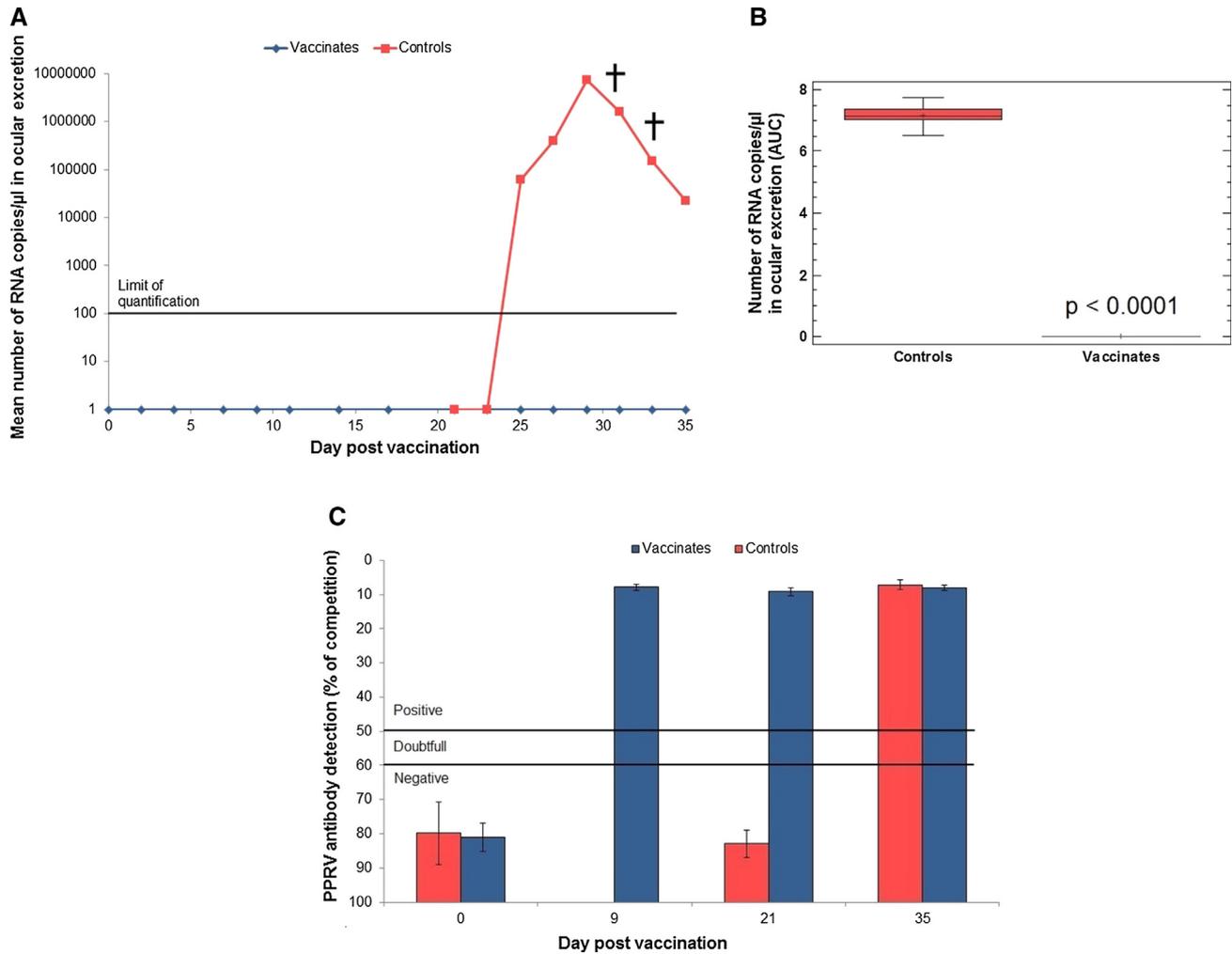
A

B

C

Fig. 4. PPR-VAC® vaccination led to rapid seroconversion in Saanen goats and completely blocked ocular viral excretion after challenge with the MA08 strain. (A) Kinetics of mean number of RNA copies/μl in ocular excretion in each group (control and vaccinated groups infected with MA08 IN) over the study period (vaccination on D0 and challenge on D21) monitored by qRT-PCR. † means that four goats in the control group were euthanized on ethical grounds on D30 (one goat) and D32 (three goats). (B) Dispersions of individual AUC of number of RNA copies/μl in ocular excretion in each group over the challenge period (expressed in log 10 RNA copies/μl × day). (C) Detection of PPRV antibodies monitored by competitive ELISA (expressed in % of competition). Thresholds are defined as follows: from 0% to 50% = positive, from 50% to 60% = doubtful and from 60% to 100% = negative. For each point, the error bar represents the mean more or less the standard deviation.

in 2015 by FAO and OIE [16]. PPRV has many characteristics that could make eradication a success [11]: vaccines already available, lifelong immunity, short excretion period, absence of an occult reservoir, etc. Current understanding of the PPRV pathology and disease progression has mainly been based on other morbillivirus infections together with information from outbreaks. However, PPRV experimental infections fail to reproduce the pathogenicity observed in the field. Few experimental models of PPRV have been designed specifically to monitor the progress of the disease, i.e. by associating the viral load and clinical scoring, and evaluate the efficacy of PPRV vaccines.

Here, we successfully reproduced PPR clinical signs in experimental conditions using Saanen goats. In agreement with previous publications [18–20], PPRV genome was detected very early (from 3 dpi in IN-infected goats), prior to the appearance of clear clinical signs and sometimes lasted more than 12 days. Viral excretion has been reported to last 26 days in goats in certain conditions [21] and PPR antigens were detected in faeces of West African Dwarf (WAD) goats until 12 weeks post recovery [22]. These data question the relative "short" excretion window of PPRV sometimes claimed in some studies [11]. This apparent discrepancy between authors might be due to the viral detection method: there are several examples in the literature where viral genome can be detected

for weeks while infectious virus is only detected for days in the same organ/sample. Interestingly, ocular swabs appear to be a better choice than whole blood samples to detect viral RNA, since higher viral RNA loads were detected in the swabs. The virulence experiment was also the occasion to generate rectal swabs. The detection of PPRV RNA over the challenge period appeared to be quite similar in ocular and rectal samples, with just an earlier detection for some animals in ocular excretions (manuscript under review). These results are in favor of ocular swabbing for an early PPRV detection.

Studies on PPR in experimental settings have suggested that the administration route does not influence disease progression [1,9,19,23–25]. However, we observed that the inoculation route does have an impact on the earliness and intensity of viral excretion and clinical signs. As the upper respiratory tract is the natural entry point for PPRV, results obtained after infection via IN may be more representative of natural infection and raise questions about the timing and severity of the disease observed with other inoculation routes [9,23]. It can be hypothesized that IV inoculation allows the virus to be directly in contact with lymphocytes in blood circulation. This hasty contact would lead to a faster viral replication and to a quicker reaching of deep target organs, such as the digestive tract, and would generate an earlier and more severe

expression of certain clinical signs as compared to the natural (IN) route of infection.

Differences in virulence between several PPRV strains were already highlighted in a previous study [9]. That study reported that CI89 (lineage I), Guinea Conakry (lineage I), Bissau Guinea (lineage I) and India-Calcutta (lineage IV) PPRV strains caused peracute or acute diseases in WAD goats, whereas Sudan-Sennar (lineage III) and Nigeria 75/1 (lineage II) strains caused milder diseases. Here, we identified differences in virulence between the CI89 and MA08 strains. Infection by CI89 resulted in mild clinical disease in Saanen goats whereas MA08 was highly virulent. Difference in virulence might also be due to the passage history of both strains. Indeed, cell passages are known to attenuate virulence and in our case, the less virulent virus (CI89) had been more passaged than the most virulent one (MA08). The results obtained in previous studies using other European goat breeds [23,25] confirm that the susceptibility of the breed and species plays an important role in the manifestation of the disease [9,26]. More information on host-specific viral shedding kinetics is clearly needed to better implement disease control in the field.

To our knowledge, this is the first time that PPR virulence is described in Saanen goats. The high virulence described in this breed in our study (with the MA08 strain), the previous descriptions of PPRV virulence in Alpine goats [23] and the large populations of these two breeds in Europe underline the current threat that PPR poses to Europe [13]. In order to eradicate the disease, here we provide further proof that good tools are already available for the control of the disease. Indeed vaccination with PPR-VAC® led to rapid seroconversion with no ocular excretion of vaccine virus. Together with the complete blockage of viral excretion and the significant reduction of clinical signs following MA08 challenge, this demonstrates both its efficacy and safety. These results also support field observations that a vaccine strain from lineage II can protect against a virulent strain from lineage IV.

According to FAO recommendations, future vaccination campaigns will need to provide an immunity of at least 70% of the target populations to block viral circulation. To reach this objective, there is a need to confirm the efficacy of currently commercialized PPRV vaccines using robust models that allow comparative evaluations, and provide clear and robust information on vaccines available to veterinary services seeking to control this disease. Here, we propose a validated method to test the efficacy of currently available vaccines and to further our understanding of host-PPR interactions. Based on the results of this study, we recommend intra-nasal challenge and use of ocular secretions to detect viral RNA. Using this method, we showed that Saanen goats are extremely susceptible to a PPRV strain circulating closed to Europe and demonstrated that PPR-VAC® makes it possible to simultaneously limit the impact of PPR on the herd performances, thus reducing economic losses, and to block the diffusion of the disease with the aim of global eradication.

Declaration of interest

FE, CH, DG, CM, HB, SG, CPR and PH are employees of Merial (a part of Boehringer Ingelheim), which collaborate with the BVI for PPR-VAC® vaccine production. OK, GL and AB declare that they have no conflict of interest.

Authors' contributions

All the authors made substantial contributions to study design, animal phase, analyses and/or interpretation data. CH, DG, CM, HB, SG, CPR and PH implemented and/or managed the studies. OK performed the serological and RT-qPCR analyses. FE did the statistical analyses and wrote the manuscript, which was then revised by CH, GL and AB. All the authors read and approved the final manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.01.057.

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