Research Article

Peste des Petits Ruminants Virus Transmission by a Resistant Local Breeds of Sheep, Morocco

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Abstract | Peste des Petits Ruminants virus (PPRV) has caused a serious outbreak of disease in small ruminants recently in 2008 in Morocco. The field data obtained during the outbreak indicated that there were marked differences in breed susceptibilities to PPRV infection. In the field exotic breeds of Alpine goats were observed to be highly susceptible to the disease, with mortality rates approaching 100%, contrary to local breeds which were less susceptible to the disease. In the present study, animals of local breed of Moroccan sheep (Timahdid) were experimentally infected using the protocol previously validated for Alpine goats. The infected animals showed mild or no clinical signs of disease, with low levels of viral RNA detectable in swabs and fecal samples taken at the peak of infection. The transmission of the disease to animals (Timahdid breeds) brought into contact without manifestation of clinical signs, may indicate that local breeds of sheep might play an important role in the prevention of dissemination of PPR by virtue of its natural immunity.

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Introduction

Peste des petits ruminants (PPR) is a contagious and often fatal disease of sheep and goats caused by the Morbillivirus, PPR virus (PPRV). Farmers in the developing world rely heavily on sheep and goats to provide vital resources (nutritional, economic and cultural) in many poor communities around the world. The severe economic consequences resulting from outbreaks of PPR cause significant obstacles to the development and maintenance of sheep and goat farms within the developing world. Infection with PPRV can be asymptomatic or produce a wide spectrum of clinical manifestations ranging from mild and short-lived to lethal. This depends on many factors including the strain of virus, the sensitivity of the host species and individual animal susceptibility (Lefèvre and Diallo, 1990; Taylor and Barrett, 2007). In some cases PPR manifests itself mainly in the subclinical form, as recently observed in the Sahel zone of Africa (Kwiatek et al., 2011). PPRV is known to exhibit different levels of virulence in sheep and goats under field conditions (Abubakar et al., 2009). Indeed, Singh et al. (2004) reported differential susceptibility of breeds of small ruminants against PPR.

The recent northward expansion of PPR into North African countries such as Morocco and Algeria, and

its southward expansion into Tanzania, has been the subject of much attention and concern. The ability of the disease virus to spread so rapidly highlights the threat it poses to naïve small ruminant populations in both northern and southern Africa and increases the risk of its future introduction into Europe. PPR was first detected in Morocco in June 2008, (FAO, 2008), where the disease spread rapidly throughout the entire country. The outbreak reached a peak in August 2008 and no new outbreaks were reported after November 2008. Interestingly, during the PPR outbreak in Morocco, the overall rates of mortality in naïve local breeds of sheep and goats remained low (5.6% and 6.2% respectively) compared to what is typically reported in India (high morbidity (up to 100%) in goats and up to 90% mortality in sheep (Shaila et al., 1989; Karim et al., 2016).

This was surprising considering that the sheep and goat population in Morocco was naïve having never previously been exposed to the virus. The morbidity and mortality rates seen in exotic breeds of Alpine goats were however high, with a high percentage of this breed of goats dying from the disease. Experimental studies have recently shown that the Alpine breed of goat is highly susceptible to infection with the Moroccan field strain of PPRV (Hammouchi et al., 2012) and a reliable and reproducible experimental challenge model, through the experimental infection of alpine goats with the Moroccan PPRV strain, has been reported (El-Harrak et al., 2012).

Hence, considering the resistance shown by local sheep breed against PPRV during above outbreak, the present work was undertaken to ascertain the breed susceptibility or resistance against PPRV in sheep reared in Morocco as well as their role in transmission and perpetuation of PPRV in affected areas.

Materials and Methods

Virus strain

PPRV Maroc/2008 virus, belonging to PPRV lineage IV under the GenBank accession number: KC594074 (Muniraju et al., 2013) was generated by passaging the virus four times on VeroDogSLAM cells as previously described (Hammouchi et al., 2012). Briefly, lumphnode tissues were ground with glass beads and were used to infect a monolayer of cells according to the Reed and Munch method (1938). The titer of the infected dose was 5.1 log 50% tissue culture infective doses (TCID50/ml).

Animals and experimental protocols

Animals: A total of 8 sheep (Timahdit Moroccan local breed (TD)) aged 6-8 months were used in the study and were housed in the experimental animal unit of Biopharma, Rabat, Morocco. The infection studies were approved by the local ethics committee and were carried out following strict welfare guidelines. Experiments were conducted under the standard procedures of biosecurity level P3. Prior to experimental infection, all animals were clinically normal and were screened negative for PPRV antibodies by a serum neutralization test (SNT) as described in the manual of the WOAH (2008).

Six out of eight sheep were each infected with 5 ml of inoculum (i.e. a total dose of $10^{5.18}$ TCID50 of PPRV inoculums). Of these, 3 sheep were infected by the intravenous (i/v) route and three by the intranasal (i/n) route. 2 sheep were as uninfected in-contact control animals. The routes of infection and infection protocol have been previously optimized in Alpine goats (El-Harrak et al., 2012). Briefly, sheep were housed together and observed twice daily and clinical signs were recorded for each animal throughout the study. The rectal temperature for each animal was recorded two days before infection and daily from 1-14 day post infection (dpi). Oro-pharyngeal (Ph), ocular (Oc) and nasal (N) swabs, as well as vacutainer-whole bloods were collected prior to infection and at 5; 6, 7, 8, 11, 12, 13 and 14 dpi for RT-PCR and serology analyses respectively. All animals were euthanized at 15 dpi and post-mortem examinations were carried out.

The severity of clinical signs was assessed for all animals, using the established scoring system. A ranking score from 0 to 3 based on the severity of clinical signs and symptoms such rectal temperature (pyrexia), anorexia, behaviour, nasal discharge, salivation, acute respiratory symptoms, including dyspnoea, coughing, sneezing, and diarrhea was used (El-Harrak et al., 2012). 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. When animals reach a daily clinical score of 20 or greater, they should be euthanized for animal welfare reasons.

Biological endpoints

Detection of PPRV antibodies in serum was carried out using the kit ID Screen PPR Competition (PPRC-4P ID-VET, ID. Vet, Grabel, France).

The levels of viral RNA were evaluated by the real-time reverse transcription-quantitative PCR (RT-qPCR) as described by Batten et al. (2011). The total RNA from the different samples was extracted using the NucleoSpin RNA Virus Kit (Germany).

The forward primer matched positions 483 > 508 in the N-terminus of the N gene -AGAGTTCAATATGTTRTTAGCCTCCAT-3); the TaqMan® probe positions 551 > 576 (FAM-5-CACCGGAYACKGCAGCTGACTCAGAA-3-Tamra) and the reverse primer was located at positions 603 < 624 (5-TTCCCCARTCACTCTYCTTTGT-3).

Real time RT-qPCR amplification and detection were performed by a Smartcycler (Cepheidex, USA) real time PCR machine with the Superscript III/Platinum Taq one step RT-PCR kit (Invitrogen, Paisley, UK). Briefly, each 25 µl reaction contained 3 µl extracted RNA; 12.5 µl SuperscriptIII/Platinum Taq, One-step RT-PCR reaction mix; 0.5 µl SuperscriptIII/Platinum Taq, One-step RT-PCR enzyme mix 0.5 µl; ROX reference dye; 0.5 µl probe (5 pmol/l); 1 µl (10 pmol/l) forward and reverse primer and 6 µl nuclease free water. The cycling conditions were as follows: Reverse transcription 50 °C for 30 min; reverse transcription inactivation and DNA polymerase activation 95 °C for 10 min; followed by 45 cycles of amplification (95 °C for 15 s and 60 °C for 1 min). Samples were considered positive if they had a cycle threshold (Ct). Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level of fluorescence).

Results and Discussion

Clinical scores were recorded for up to 15 dpi and were used to evaluate the severity of clinical signs. They are shown in Table 1. One of the sheep (number 1) showed a clinical score of 6 at 6 dpi, which was the highest clinical score. This animal exhibited a raised body temperature (41.4°C) and a moderate degree of nasal discharge. A second sheep (number 5) registered a clinical score of 5 showing moderate levels of nasal discharge and raised body temperature (40.6°C) at 5 dpi. The remaining four infected sheep showed no clinical signs, with the majority of clinical scores being at a low level of 1-2 for the length of the study. For these animals, no increase in the body temperature was noted. The cumulative clinical scores observed in the sheep were considerably lower than those observed in a previous experimental infection study in which Alpine goats were infected with the same titre and strain of PPRV (El-Harrak et al., 2012).

Table 1: *Levels of seroconversion (measured by ELISA) and threshold cycle (Ct) values (measured by real-time RT-PCR) in ocular (Oc), nasal (N) and pharyngeal (Ph) swabs taken from six sheep experimentally infected with a Moroccan strain of PPRV and two in-contact sheep.*

Infection route	Animal number	Samples	Day post infection							
			5	6	7	8	11	12	13	14
I/V		Swabs								
	2	N	-	28	-					
		serum (Ab)	-	P	P					
		Oc	-	-	27					
		Ph	28	-	-					
		serum (Ab)	-	P	P					
		serum (Ab)	-	P	P					
		N	-	29	-	-				
		serum (Ab)	-	-	P	P				
		serum (Ab)	-	-	P	P				
In-contact sheep	7	Oc	-	-	-	-	28	27	ND	-
		N	-	-	-	-	33	28	28	-
		serum (Ab)	-	-	-	-	-	P	P	P
		serum (Ab)	-	-	-	-	-	P	P	P

N: negative; P: positive; ND: not determined. I/V: intravenous; I/N: intranasal; Ab: antibody. The sampling dates begin 2 pays before infection and every day post infection until 14 dpi. Only samples at days when RNA loads are supposed to be high were analyzed.

One of the in-contact control sheep (number 7) started to show clinical signs of PPR at around 10 days after the six sheep were infected, and five days later presented with a clinical score of 6. This animal initially presented with a minor degree of nasal discharge without any increase in body temperature; however, at the peak of clinical disease, when the clinical score reached 6, the animal showed a pronounced nasal discharge with some coughing. PPRV RNAs was detected in nasal swabs taken from this in-contact sheep from 11-13 days after the commencement of the experiment (Ct values of 28-33 respectively), indicating that this animal had been infected through direct contact with the experimentally infected sheep. The second in-contact control sheep (number 8) however did not

show any clinical signs of PPR and no increase in body temperature was observed. In addition, no viral RNA was detected in any of the swabs taken from the animal. Both in-contact control sheep seroconverted by 12 days after the in-contact sheep were experimentally infected, indicating that they were likely to have been infected at around 5-6 days after the experimental infection. The levels of RNA were detected only at times when RNA loads were expected to be high based on previous experiments (Hammouchi et al., 2012; El-Harrak et al., 2012) in Ph, Oc and N swabs. The data are summarized in Table 1. PPRV RNA levels in the swabs were both transient and low. Out of a total of 16 swabs taken between 4 and 7 dpi, when the highest levels of viral RNA would be expected, only two swabs were RT-PCR positive in one sheep (number 2), one swab was positive in a further two sheep (numbers 1 and 4) and no swabs were RT-PCR positive in the remaining 3 sheep (numbers 3, 5 and 6). The three sheep that were infected through intravenous (i/v) route seroconverted at 6 dpi, one day earlier than the sheep that were infected through the intranasal (i/n) route, seroconverted at 7 dpi (Figure 1). On post-mortem examination no lesions that could be attributed to PPR infection were seen in all animals euthanized at 15 dpi.

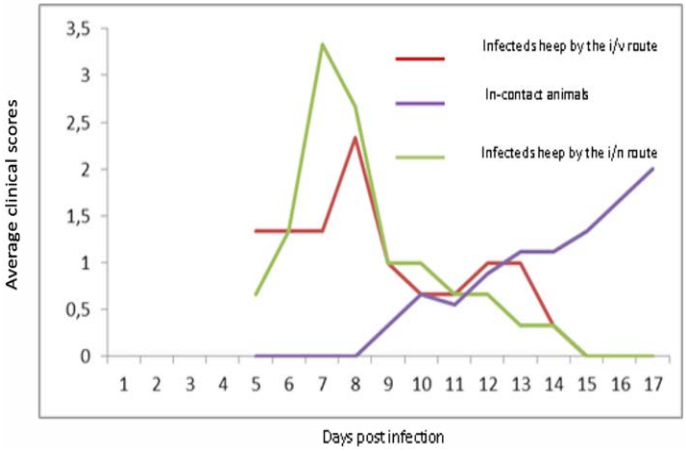

Figure 1: *Average clinical scores (CS) of 3 sheep infected by a virulent strain of PPRV through intravenous (i/v) and 3 animals infected by the intranasal (i/n) routes. The average CS from 2 infected animals by direct contact is also reported.*

The local breeds of Moroccan sheep exhibited a low level of susceptibility / higher degree of resistance to PPRV infection which was in marked contrast to alpine goats shown to be highly susceptible to PPRV infection, showing severe clinical signs of disease and high levels of mortality (Hammouchi et al., 2012; El-Harrak et al., 2012). It is however important to note however that the susceptibility of other breeds of sheep from across the world may vary. This may explain the low rate of morbidity and mortality seen in sheep during the 2008 Moroccan PPR outbreak. The lack of clinical signs of PPR seen in local breeds of sheep compared to alpine goats emphasizes the possibility of introducing sentinel alpine goats into herds/ compartments/ regions containing high numbers of local sheep, in order to detect virus circulation or to demonstrate freedom from disease. Despite the lower susceptibility, reduced levels of clinical signs and low levels of virus excreted, the local sheep did transmit the virus to in-contact sheep, thus proving that local breeds of sheep do play an important role in the onward transmission of the virus. These results emphasise the importance of vaccinating sheep as well as goats against PPRV when carrying out control/ eradication programs.

Author's Contributions

FFO, HM and EM conceived the study. HM and LC performed the experiments and participated in the design and the follow up of the study. NT and MY analysed and drafted the manuscript. SG carried out PCR testing. MY made critical review of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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