First Evidence of Peste des Petits Ruminants (PPR) Virus Circulation in Algeria (Sahrawi Territories): Outbreak Investigation and Virus Lineage Identification

M. De Nardi1, S. M. Lamin Saleh2, C. Batten3, C. Oura3, A. Di Nardo3 and D. Rossi4

1 Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy
2 Sahrawi Veterinary Services, Ministry of Public Health, Sahrawi Arab Democratic Republic, Rabouni, Algeria
3 Institute for Animal Health, Pirbright, UK
4 Faculty of Veterinary Medicine, University of Bologna, Bologna, Italy

Introduction

Peste des petits ruminants (PPR) is one of the most economically relevant diseases of small ruminants in the regions where it occurs (Rossiter, 2004a; Diallo et al., 2007) and economic losses can be aggravated by the control measures imposed, which often involve livestock movement and animal by-products trade restrictions.

Peste des petits ruminants is widespread in Africa, Arabia, the Middle East and in some geographical areas of Asia, including much of the Indian subcontinent (CFSPH, 2008). Since 2007, more than one billion small ruminants in Africa and Asia have been considered at risk of being infected with the peste des petits ruminants virus (PPRV) (FAO, 2009). The disease is mostly present in developing countries which often rely heavily on subsistence farming of small ruminants for trade and food supply. As a consequence, the economic impact of PPR, especially in a naïve population with mortality rates as high as 50–80%, can be devastating (Anderson, 1995; Rossiter, 2004a; Diallo et al., 2007; Banyard et al., 2010). In countries where PPR outbreaks occur, annual economic losses are in the range of millions of USD (Kaukarbayevich, 2009; Banyard et al., 2010).

Peste des petits ruminants is caused by a highly contagious RNA virus (family Paramyxoviridae, genus Morbillivirus) affecting small ruminants such as sheep, goats and taxonomically related species (CFSPH, 2008). Peste des petits ruminants virus is genetically similar to other members of the Morbillivirus genus such as measles virus, rinderpest (RP) virus, canine distemper virus and other viruses affecting aquatic mammals (Banyard et al., 2010). Historically, PPR and RP have often been confused because of their similar clinical, pathological and immunological signs (Kaukarbayevich, 2009), even though RP is a disease of large ruminants and other artiodactyls (Rossiter, 2004b). Four genetically distinct lineages (namely, I to IV) of PPRV have been recognized, and molecular studies have shown that an inherited differentiation exists between non-African and African isolates.

Keywords: peste des petits ruminants; outbreak investigation; lineage IV; Algeria; Sahrawi; contingency plan

Correspondence: M. De Nardi. Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell’Università 10, 32050 Legnaro (PD), Italy.
Tel.: +39 347 1376774; Fax: +39 049 8084360;
E-mail: mdenardi@izsvenezie.it

Received for publication April 30, 2011

Summary

Following reports of increased mortality in the small ruminant population of the Sahrawi territories, western Algeria, between January and May 2010, local veterinary authorities suspected an outbreak of peste des petits ruminants (PPR). An investigation was implemented in May 2010 and followed up in October 2010 in the Sahrawi refugee camps, Tindouf province, with the objective of confirming the circulation of the peste des petits ruminants virus (PPRV). Laboratory results confirmed the presence of PPRV in 33.3% of the samples. Sequence analysis revealed that the virus belonged to Lineage IV and phylogenetic analysis indicated a close relationship (99.3%) with the PPRV isolated during the Moroccan outbreak in 2008.
It is still unclear whether differences between lineages are merely reflecting geographical speciation or if they are also correlated to pathogenicity variability between isolates (Banyard et al., 2010). The virus is transmitted through direct contact between infected and susceptible populations: the presence of mixed populations (i.e. flock of sheep and goats) and the introduction of new animals into a flock/ herd are major risk factors for the PPR spread (CFSPH, 2008). In pastoral areas, livestock trade, nomadic herding (FAO, 2009) and the congregation of susceptible populations close to watering points during dry seasons and/or in livestock markets play an important role in spreading the disease.

The disease is widespread in western, central, eastern and northern Africa (Banyard et al., 2010; OIE, 2011a), and the four genetic lineages are all present in different regions of the continent. In northern Africa, only Libya has not reported the disease to date (Banyard et al., 2010; OIE, 2011b). In 2008, an extensive epidemic of the PPRV lineage IV occurred in Morocco. This finding was particularly relevant as it marked further geographical spread of this lineage outside eastern and western Africa and its first detection in northern Africa. Furthermore, because of these outbreaks in Morocco and the existing commercial trade between Morocco and both Algeria and Spain, the situation raised huge concern owing to the increased risk of introduction of the disease into free zones in northern Africa and into Europe (FAO, 2009; Khalafalla et al., 2010).

In March 2011, the Director of the Algerian Veterinary Services officially notified the World Organization for Animal Health (OIE) about serologically confirmed cases of PPR in five Algerian provinces, even though samples tested negative by reverse transcription – polymerase chain reaction (RT-PCR) and no clinical signs were observed. The source of the outbreak was declared unknown (OIE, 2011b). This was the first official notification of PPR by the Algerian authorities to the OIE, although serological reactors had previously been detected in western Algeria in 2005 and 2008 (A. Broglia, unpublished observation; Di Nardo A, Rossi D, Lamin Saleh S.M, Lejlifa S.M, Hamdi S.J, Sabatini M, Teodori L, Savini G, Thrusfield M.V, unpublished observation).

In the Tindouf province (western Algeria), a geographical area inhabited by the Sahrawi community, the local veterinary authorities (Sahrawi Veterinary Services) were alerted between January and May 2010 by a significant increase in livestock deaths (MoPH, 2010). Members of the Sahrawi community live in six refugee camps, named El Aaiun, Awserd, Smara, Dakhla, 27 Febrero and Rabouni, and, with the exception of Dakhla, they were all severely affected. Furthermore, signs of severe respiratory disorders associated with enteritis, fever and depression in sheep and goats were reported in all refugee camps, again with the exception of Dakhla. Young animals seemed to be more affected (S.M. Lamin Saleh, personal communication). Despite the clinical picture suggesting a presumptive diagnosis of pasteurellosis, a disease frequently reported in the livestock population in the camps because of the rearing condition, the suspicion of an underlying viral disease was strengthened by the high and unusual mortality rate. Peste des petits ruminants virus was suspected as a possible pathogen causing the reported increased mortality. The suspicion was supported by the fact that: a) outbreaks of PPR occurred in 2008 in Morocco in the areas close to the border with northwestern Algeria; b) PPR antibodies were detected in small ruminants in 2005 and 2008 in the camps (A. Di Nardo et al., unpublished observation; A. Broglia, unpublished observation); c) small ruminants reared in the camps are normally purchased in Mauritania and Mali, where the disease is endemic (FAO, 2008); d) local breeds are considered to be more resistant to PPR and have been associated with sub-clinical or mild forms of the disease, which are insufficiently severe to attract veterinary attention (Rossiter, 2004a). As a result, an outbreak investigation was implemented in the Sahrawi refugee camps in May 2010 and followed up in October 2010 with the objective of confirming the suspicion of PPRV circulation. This paper presents the first report of PPRV lineage 4 circulation in Algeria.

Materials and Methods

Study area

The Sahrawi community

Sahrawi, literally ‘people from the desert’, are nomadic and pastoral tribes who inhabited the Western Sahara (Fig. 1) (Ramondino, 1997; Broglia and Volpato, 2008). In 1975, as a consequence of the military occupation of Western Sahara by Mauritanian and Moroccan military forces, about 70 000 Sahrawi fled into Algeria (Spiegel and Qassim, 2003; Loewenberg, 2005) where they gathered in refugee camps in the Tindouf province. Subsequently, a long political process led to the establishment of the Sahrawi Arab Democratic Republic (SADR). Beside the refugees camps, the SADR has also political control over the eastern part of Western Sahara (the so called ‘liberated territories’ or ‘free zone’), which was liberated from Moroccan occupation through guerrilla fighting. The western portion of the Western Sahara is under Moroccan control and is referred as Moroccan Southern Province, separated from the ‘liberated territories’ by an earthen wall (the ‘Berm’) and protected by land mines.
(San Martín, 2004; Loewenberg, 2005; Broglia and Volpato, 2008). Overall, the Sahrawi territories encompass the refugee camps and the liberated territories in Western Sahara.

Since 1975, the Sahrawi people have lived as refugees in camps (El Aaiun, Awserd, Smara, Dakhla, 27 Febrero and Rabouni) which are located in the desert plateau of Hamada in close proximity to the Algerian town of Tindouf, in the Tindouf province (Fig. 2). The closest camps are located at a distance ranging between 15 and 50 km from Tindouf; the distance between the six camps is variable, spanning from a minimum of 12 km (Rabouni-27 Febrero) to a maximum of about 160 km (Rabouni-Dakhla). The camps currently host about 165 000 people whose livelihood (in terms of trade and source of proteins) relies primarily on the livestock kept within the camps. Livestock breeding represents one of the main income generating activities for Sahrawi. About 60 000 sheep and goats and 2000 camels are reared in semi-intensive conditions in the camps: animals move freely during the day, but are kept in rudimental holdings during the night. Small ruminants normally do not move between camps. Semi-nomadic pastoralism is also practiced: livestock keepers in the camps move to grazing areas within the liberated territories in Western Sahara during times of low rainfall (September/October and March/April). Transhumance for grazing purposes is important to fatten animals and to increase the size of the herd/flock before returning to the camps. In addition, these semi-nomadic practices allow Sahrawi tribes to preserve their nomadic traditions and cultures (Broglia and Volpato, 2008).

The Sahrawi Veterinary Services (SVS), under the framework of the SADR Ministry of Public Health and in collaboration with the Algerian veterinary authorities,
despite being still embryonic, makes huge efforts to perform animal health surveillance and to carry out disease control in the Sahrawi territories. The surveillance system currently in place relies predominantly on the recording of livestock mortality rates and on clinical inspections at the frontier posts and at the check points close to the camps. Surveillance strategies and contingency plans for the control of major transboundary animal diseases (TADs) have not been developed and only basic laboratory facilities are available.

Data on livestock mortality events
A database with livestock mortality events reported in the refugee camps since April 2009 was provided by the SVS. The database was cleaned; and the mean of mortality events in the periods between April and December 2009, January and May 2010, and June and August 2010 was estimated. The recording of the mortality rates is being implemented by SVS in collaboration and under the coordination of the international Non-Governmental Organization (NGO) ‘Movimiento por la Paz, el Desarme y la Libertad’ funded by the Spanish Cooperation. Carcasses of livestock are routinely collected from the camps to be adequately disposed of. This system, primarily established to improve the hygienic condition within the camps, also guarantees the efficient recording of mortality events in the livestock population and represents, therefore, an ‘embryonic’ early warning system to detect unusual events. A strong deviation from the average mortality rate normally triggers further investigations.

Survey area
In May 2010, SVS were still issuing, although at reducing levels, reports of small ruminant deaths in the camps of Smara, Awserd, Rabouni and El Aaiun. A targeted survey was therefore implemented in Smara, Rabouni and Awserd in May 2010 with the aim of sampling diseased animals, as per case definition, to confirm the PPRV circulation. The target population was sheep and goats reared within the camps, and the study population was represented by diseased animals. All field activities were supported by SVS and the ‘Africa ‘70’ International NGO.

Case definition
A clinical suspicion of PPR was based on the identification of animals presenting at least two of the following clinical signs: high fever (>41°C, with inappetence and marked depression), signs of pneumonia (respiratory distress and/or coughing), nasal and/or ocular discharges (serous or mucopurulent discharges), mouth lesions (necrotic foci and erosions) and diarrhoea. The PPRV infection was confirmed by laboratory diagnosis using real-time and conventional RT-PCR in the World Reference Laboratory for PPR, Institute for Animal Health, Pirbright, UK. The location of suspected cases of PPR within the targeted camps relied on the SVS reports and interviews with pastoralists.

Sampling strategy
The sample size (n) was based on the 2007 census population data (MoPH, 2007) and was calculated to achieve a 0.95 probability (P) of detecting at least one positive case in the sample assuming an expected prevalence (d) of 30% and an infinite (N) population size (Thrusfield, 2007) as:

\[ n = \left\lfloor 1 - (1 - P)^{\frac{1}{d}} \times \left\lfloor \frac{N}{d} \right\rfloor \right\rfloor + 1 \]

The specificity and sensitivity of the real-time and conventional RT-PCR have been assumed to be equal to 100%.

The prevalence was expected to be as high as 30% based on results of a country-wide cross-sectional survey implemented in 2008, which confirmed the presence of antibodies against PPRV throughout the whole region in 28.3% of the sampled animals. In some provinces, seroprevalence was >40% (Di Nardo A, Rossi D, Lamin Saleh S.M, Lejifa S.M, Hamdi S.J, Sabatini M, Teodori L, Savini G, Thrusfield M.V, unpublished observation).

Laboratory testing
Samples collected were oral, ocular and nasal swabs, and whole unclotted blood (in EDTA). Samples were kept refrigerated at +4°C during collection procedures and until shipment to the laboratory. All samples were dispatched to the World Reference Laboratory for PPR, Institute for Animal Health, Pirbright (UK), where the initial screening was performed by real-time RT-PCR (Batten et al., 2011) and positive samples were confirmed by conventional RT-PCR assay using a set of primers specific for the F gene of the PPRV. Briefly, RNA was extracted from 1/10 tissue suspensions through the Universal (Qiagen, Crawley, UK) extraction robot using the ‘One for All’ protocol. RNA was then used as template in real-time RT-PCR (Batten et al., 2011).

Sequencing and phylogenetic analysis
Sequence analysis of the F gene was performed on PCR-positive samples to identify the genetic lineage of the virus. The evolutionary history was investigated using the neighbour-joining method with distance matrices being
Epidemiological data collection
Supplementary epidemiological data (such as animals age and sex, flock structure in term of species and breed, geographical origin of the flock, flock movement patterns, recent introduction of new animals in the flock) and clinical signs were collected at the time of the survey through semi-structured interviews conducted with animal owners and clinical inspection of suspected animals. For this purpose, a specific questionnaire was developed and an ad hoc database was created in Excel® 2007 (Microsoft Corporation, Redmont, WA, USA).

Statistical analysis
The chosen statistical approach reflects the small number of sampled animals.
For each refugee camp, the mean of deaths in the periods April–December 2009 and June–August 2010 were compared with the mean of deaths in the January–May 2010 period by using the Student’s t-test (considering unequal standard deviations) and the one-way analysis of variance (when comparing simultaneously the means for the three periods). In the latter, the assumption of similar population standard deviations was assessed by the Bartlett’s Test. (Kirkwood and Sterne, 2003). The calculation of the Confidence Intervals for the mean was based on the t-distribution, whereas the calculation of exact Confidence Intervals for proportion was based on probabilities derived from the binomial distribution (Dohoo et al., 2010).
Univariable analysis was performed using the Fisher’s exact test to identify potential risk factors and clinical signs associated to PPR cases and to investigate the association between clinical signs identified in PPR positive and, overall, in sampled animals. Statistical analysis and the design of the graph were performed in Stata® 10.1 SE (StataCorp LP, College Station, TX, USA). A statistically significant threshold was set at a P-value of 0.05. A P-value >0.05 and <0.10 was considered indicative of a weak association.

Survey data and laboratory analysis
Considering the expected prevalence and the sampling strategy, it was necessary to sample nine animals to detect at least one positive case with a 95% certainty. However, to increase the chance of detecting the PPRV circulation in a timely and cost-effective manner, only suspected animals (as per case definition) were sampled and more than one diagnostic sample was taken from each animal.
In Smara, Rabouni and Awserd, a total of 21 samples (four ocular swabs, seven nasal swabs, three oral swabs and seven whole blood samples) were collected from nine animals (eight sheep and one goat) suspected of having been infected with PPRV. The real-time and conventional RT-PCR test conducted on swabs and whole blood detected the presence of PPRV genetic material in two sheep and one goat reared respectively in Smara and Awserd, confirming the circulation of the virus in the refugee camps. With reference to the samples, seven of 21 samples tested positive (three nasal swabs, two ocular swabs and two whole blood). The two PPR-positive sheep were purchased in Mauritania and then brought to the
Smara livestock market; the PPR-positive goat was purchased in the Zemur location (Western Sahara) and moved into the Awserd camp.

According to the livestock owners, the flocks from which the positive animals originated had resided in Mauritania and Western Sahara for the previous 12 months, during which time the flocks were not moved from the origin villages. Both sheep were kept in a small flock (five sheep) and no mortality events were recorded in the previous 2 weeks, whereas the goat was part of a larger mixed flock (20 sheep and 25 goats) with a history of about 20 deaths in the previous 2 weeks. No statistical association between animal status (PPR positive or negative) and selected variables such as species ($P = 0.33$), age ($P = 1.00$) and origin and/or location of the flock in the previous 12 months ($P = 0.21$) was detected. Furthermore, no animals were introduced in the original flocks in that same period.

The clinical picture observed during the inspection reflected a very generalized clinical situation. Clinical signs detected in the PPR-positive animals are shown in Table 1. With the exception of nasal discharge, no other clinical signs were common within positive animals and PPRV infections were only weakly associated with signs of emaciation ($P = 0.083$). No mouth lesions were detected in any of the positive animals.

Most of the sampled animals (Table 2) presented nasal discharge (77.8%) varying from serous to mucopurulent, fever (up to 41.5°C) and depression (77.8%) and respiratory syndrome with dyspnoea and/or hyperpnoea (66.7%). Few animals showed mucous ocular discharge (33.3%), diarrhoea (33.3%), cough (11.1%) and signs of emaciations (22.2%). One-third of the animals (33.3%)...
presented oral exudates and/or foam, but none of the sampled animals presented oral lesions. Most of the animals with fever and depression (7/9) were also affected by respiratory signs ($P = 0.083$) but only one had diarrhoea ($P = 0.083$).

Sequencing of the viral $F$ gene performed on samples from positive animals indicated that the PPRV belonged to lineage IV. Neighbour-joining analysis indicated a 99.3% percentage of identity with the PPRV isolated in Morocco in 2008–09 (Fig. 4).

Participatory appraisal

The retrospective participatory survey showed an unusual increase in mortality in small ruminant flocks kept in the grazing areas of the Western Sahara in the period December 2009–April 2010. Of 29 interviewed pastoralists, 10.3% (3/29) reported a mortality rate of ≥50% among their animals (reaching a peak of 70% in one case), 48.3% (14/29) pastoralists reported rates between 20% and 49% while 41.4% (12/29) pastoralists recorded rates of <20%. Although it was not possible to substantiate these data through field investigations and laboratory analysis, they seemed to be indicative of an unusual situation consistent with the outbreak of an infectious disease such as PPR.

Discussion

The survey implemented in the Sahrawi refugee camps (western Algeria) in May 2010 resulted in the detection of PPRV genetic material in three of nine sampled animals. Despite the recently reported serological evidence for PPR circulation in different regions of Algeria, this is the first time that the presence of the PPRV has been confirmed to be circulating in Algeria. Molecular typing and phylogenetic analysis characterized the strain discovered as belonging to lineage IV, a lineage that is widespread across the Arabian Peninsula, in southern Asia, in the Middle East and recently across different African coun-
tries (Banyard et al., 2010; Khalafalla et al., 2010). The phylogenetic analysis indicated a close relationship with the PPRV isolated during the Moroccan PPR outbreak in 2008. To notify the OIE of the first evidence of PPRV circulation in Algeria and the identification of the genetic lineage, the Director of the Sahrawi Veterinary Services communicated these findings to the Director of the Algerian Veterinary Services in August 2010.

The origin of the outbreak remains unknown despite evidence from field data collected suggesting that the virus could have been introduced into the refugee camps in November 2009 following the importation of small ruminants from Mauritania and the liberated territories of Western Sahara for the celebration of the Aid al Adha festivity (Islamic Easter, end of November 2009). Alternatively, animals could have been illegally moved directly from Morocco or from the Moroccan Southern Province into Algeria (S.M. Lamin Saleh, personal communication). Owing to the high demand of meat in the camps, the amount of animals raised is not sufficient to satisfy the protein needs of the population. For this reason, there is a flourishing livestock trade bringing animals into the camps. Animal traders usually buy goats and sheep in Mauritania, Algeria, Mali or in the liberated territories and sell the animals in the livestock markets located in the refugee camps. Islamic festivities result in an increase in animal trade from neighbouring countries. For example, the Aid Al Adha festivity is celebrated by each family by slaughtering a ram and, as a consequence, thousands of animals are purchased and then trucked to the camps and sold in the livestock markets. In 2009, more than 7000 sheep were traded from Mauritania and Western Sahara (MoPH, 2009). Refugee camps do not only represent the final trade destination: small ruminants and camels are also exported to other destinations such as the town of Tindouf and sometimes into northern Algeria. Therefore, the refugee camps represent also a trade hub where animals are imported from neighbouring countries, sold to traders in the livestock markets in the camps and finally transported to other destinations. The recent evidence for a high seroprevalence of PPR in the Tindouf province (OIE, 2011b) may be linked to this trade network.

The high percentage of animals detected with antibodies against the PPRV (as the 2005 and 2008 surveys indicate) and the presence of local breeds of sheep and goats might have contributed to the sub-acute and, probably, to the sub-clinical spread of the virus within the camps and in the Sahrawi territories. Indeed, only generalized, non-specific clinical signs were detected in PPR-positive animals hardly referable only to PPR infection. This is not unusual as PPR infections are often confused and exacerbated by secondary infections including, among others, pasteurellosis, contagious ecthyma, contagious caprine pleuropneumonia and bluetongue (Rossiter, 2004a; Couacy-Hymann et al., 2005; Diallo et al., 2007).

In the Sahrawi territories, factors such as the onset of the cold season (January–February) associated with the emergence of secondary infections might have thereafter contributed to an increase in the mortality rates recorded between January and May 2010. Climatic stresses are well-documented triggering factors for PPR (Rossiter, 2004a).

As indicated by the mortality rates in the period June–August 2010, the spread of disease since June 2010 appeared to be drastically reduced in the camps: the reduction of the susceptible population, in a rather closed environment such as the camps, has presumably contributed to limiting the spread of the disease. In fact, the majority of the livestock trade occurs in specific periods but, overall, animals are only sporadically moved out of and between the camps during the year. As a consequence, the susceptible animal population present in late May was probably too small to efficiently maintain the virus circulation.

Although the disease appeared to be self-limiting, the risk of further re-introduction into the camps from affected regions and the dissemination to other free areas should not be underestimated. A contingency plan based on enhanced surveillance and preventive vaccination of small ruminants was therefore promoted to control the disease and to prevent the occurrence of new outbreaks.

Acknowledgements

The authors would like to thank the personnel of the Sahrawi Veterinary Services for providing invaluable contributions to the study, the Italian Society for Tropical Veterinary and International Cooperation (SIVTRO) and the International NGO ‘Africa ’70’ for the logistics. The study was implemented under the framework of the ‘Soutien à l’élevage de bétail dans les camps de réfugiés Sahraouis’ project (ONG-PVD/2006/131-812) coordinated by Africa ’70.

References

Batten, C.A., A.C. Banyard, D.P. King, M.R. Henstock, L. Edwards, A. Sanders, H. Buczkowski, C.C. Oura, and


