

This is the peer reviewed version of the following article:

Kock, R. A., Orynbayev, M. B., Sultankulova, K. T., Stochkov, V. M., Omarova, Z. D., Shalgynbayev, E. K., Rametov, N. M., Sansyzbay, A. R. and Parida, S. (2015), Detection and Genetic Characterization of Lineage IV Peste Des Petits Ruminant Virus in Kazakhstan. *Transboundary and Emerging Diseases*, 62: 470–479. doi: 10.1111/tbed.12398

which has been published in final form at <http://dx.doi.org/10.1111/tbed.12398>.

This article may be used for non-commercial purposes in accordance with [Wiley Terms and Conditions for Self-Archiving](#)."

The full details of the published version of the article are as follows:

TITLE: Detection and Genetic Characterization of Lineage IV Peste Des Petits Ruminant Virus in Kazakhstan

AUTHORS: Kock, R. A., Orynbayev, M. B., Sultankulova, K. T., Stochkov, V. M., Omarova, Z. D., Shalgynbayev, E. K., Rametov, N. M., Sansyzbay, A. R. and Parida, S.

JOURNAL TITLE: *Transboundary and Emerging Diseases*

VOLUME/EDITION: 62/5

PUBLISHER: Wiley

PUBLICATION DATE: 11 August 2015 (online)

DOI: 10.1111/tbed.12398

Detection and genetic characterization of lineage IV peste des petits ruminants virus in Kazakhstan

R.A.Kock^{a*}, M.B.Orynbayev^b, K.T.Sultankulova^b, V.M.Strochkov^b, Z.D.Omarova^b, E.K.Shalgynbayev^b, N.M.Rametov^b, A.R.Sansyzbay^b, S. Parida^c

^a Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts, AL9 7TA United Kingdom

^b RGE "Research Institute for Biological Safety Problems" Committee of Science, The Ministry of Education and Science of the Republic of Kazakhstan 080409, Gvardeiskiy, Kordaiskiy rayon, Zhambylskaya oblast, Republic of Kazakhstan.

^c The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, United Kingdom

*Correspondence at rkock@rvc.ac.uk

Abstract

Peste des petits ruminants (PPR) is endemic in many Asian countries with expansion of the range in recent years including across China during 2013-14 (OIE, 2014). Till the end of 2014 no cases of PPR virus (PPRV) were officially reported to the Office Internationale des Epizooties (OIE) from Kazakhstan. The present study describes for the first time clinicopathological, epidemiological and genetic characterization of PPRV in 3 farm level outbreaks reported for the first time in Zhambyl region (oblast), southern Kazakhstan. Phylogenetic analysis based on partial N-gene sequence data confirms the lineage IV PPRV circulation, similar to the virus that recently circulated in China. The isolated viruses are 99.5-99.7% identical to the PPRV isolated in 2014 from Heilongjiang Province in China and therefore providing evidence of transboundary spread of PPRV. There is a risk of further maintenance of virus in young stock despite vaccination of adult sheep and goats, along livestock trade and pastoral routes, threatening both small livestock and endangered susceptible wildlife populations throughout Kazakhstan.

Keywords: Small ruminants, wildlife, PPR, emerging disease, Lineage IV PPRV, genetic characterization, Kazakhstan.

Introduction

Peste des petits ruminants (PPR), an acute viral infectious disease of domestic goats, sheep and some wild ruminants is characterized by fever, ocular and nasal discharges, necrotic stomatitis,

1 diarrhoea, pneumonia and death with significant mortality in naïve outbreaks (Kwiattek et al, 2007;;
2 Wang et al, 2015; Soltan et al, 2014; Parida et al, 2015). Species of the family Caprinae are
3 particularly susceptible to disease including several endangered mountain and desert ungulates
4 (Abubakar et al, 2011; Abu-Elzein et al 2004; Hoffmann et al, 2012; Munir, 2014; Sharawi et al,
5 2010). Some large ruminants including wildlife and cattle show sero-conversion for antibodies
6 specific to PPR virus (Kock, 2006; Lembo et al, 2013), virus has been isolated from water buffalo
7 (Govindarajan et al., 1997), experimentally infected cattle (Sen et al, 2014) and disease has been
8 reported in camels (Ibu et al, 2008; Khalafalla et al, 2010).

9
10 PPR virus (PPRV) is a negative strand RNA virus, belongs to the genus Morbillivirus in the family
11 Paramyxoviridae (Gibbs et al, 1979). PPR was first described as an infection of sheep and goats in
12 Cote-d'Ivoire (Gargadennec et al, 1942) and since then the virus has been confirmed across Africa,
13 Asia and the European part of Turkey (Kwiattek et al, 2007; Wang et al, 2015; Soltan et al, 2014;
14 Ozkul et al, 2002; Banyard et al, 2010; Anees et al, 2013; Balamurugan et al, 2012, Muniraju et al.,
15 2014). PPRV exists as a single serotype (Shaila et al, 1996) and on the basis of studying partial F
16 and N gene sequences, PPR viruses are classified into four lineages (I, II, III and IV) that differ
17 genetically (Kwiattek et al, 2007; Ozkul et al, 2002). In the countries of Africa all four lineages
18 circulate, while only lineage IV apparently circulates across Asia (Al-Majali et al, 2008; Banyard et
19 al, 2010, Libeau et al, 2014; Kumar et al, 2014).

20
21 PPR was reported in Tajikistan and Afghanistan annually between 2005 and 2014. In 2005 samples
22 from sick and dead goats from farms in Tajikistan demonstrated for the first time the occurrence of
23 PPR in Central Asia (Orynbayev et al, 2005). In 2006 seroprevalence of PPR in small ruminants
24 was reported in Tajikistan and Kyrgyzstan in samples taken from livestock before the vaccination
25 campaign started in the Central Asian region, however no virus was isolated (Orynbayev et al, 2006).
26 Later on in 2007 circulation of lineage IV PPRV in Tajikistan was confirmed (Kwiattek et al, 2007). In
27 China a lineage IV PPR outbreak was first recorded in Tibet in 2007 and during 2013-2014 a
28 separate incursion of lineage IV PPRV was seen in the major part of the country apparently starting
29 in the northwestern provinces with viruses closely associated with viruses in Pakistan and Tajikistan
30 (Banyard et al, 2014; Bao et al, 2014; Wu et al, 2015).

31
32 Kazakhstan has produced and practiced PPR vaccination in small livestock since 2006 using a live
33 attenuated cell culture vaccine strain «G-45» RSE RIBSP MES RK that belongs to lineage II PPRV.
34 In Kazakhstan almost all susceptible animals are vaccinated against PPR in the border regions of
35 Zhambyl and South Kazakhstan oblast, and partially in the border regions of Almaty and Kyzylorda
36 oblast. Despite this vaccination strategy outbreaks were recorded in three organized farms, mainly

1 in young animals. Therefore, the main objectives of this study were to a) study the epidemiology of
2 PPRV circulation in the face of vaccination in southern Kazakhstan to define the epidemiological
3 risk for both small livestock and wildlife populations in the region and to b) characterize the
4 circulating PPRV genetically.

6 **Materials and Methods**

8 All ethics, field and laboratory studies were reviewed and approved by the appropriate committees
9 of the Research Institute for Biological Safety Problems (RIBSP), Ministry of Education & Science in
10 Gvardeiskiy, Zhambylskaya oblast, Republic of Kazakhstan.

12 *a. Clinical examination, sampling, and specimen delivery*

14 As part of routine epidemiosurveillance, sick small livestock in each of three farms of Zhambyl oblast
15 (Republic of Kazakhstan) were examined by government veterinarians between July and October
16 2014. Suspected PPR clinical cases were reported and biological samples were taken from dead
17 sheep and goats for diagnosis. Vaccination history and total susceptible population in each location
18 were recorded. The samples taken from each animal were placed separately in indelibly marked
19 plastic bags with the species name, animal i.d. and tissue name. These were packed in iced
20 refrigerator bags and delivered frozen to the RIBSP. Detailed information on the farms, susceptible
21 population, clinical signs and samples taken is shown in Table 1.

23 {Table 1}

28 *b. Laboratory studies*

30 The tissues collected from the infected animals were stored at -80° C. These frozen tissues were
31 used to prepare a 10% suspension in saline for RNA extraction. Briefly, 1 gram of tissue was ground
32 in a homogenizer or in a porcelain mortar with sterile sand and PBS to get a slurry with a
33 concentration of 10%. The cell suspension was centrifuged at 1000 rpm for 10 minutes to get rid of
34 the cellular debris. The resulting supernatant was used for RNA extraction.

1 The N gene of PPRV was amplified by Reverse Transcriptase Polymerase Chain Reaction – (RT-
2 PCR) using the forward primer NP_01F – 5' GGA GTA AAG ATC CTA CTG TCG GG 3' at the
3 position 57-79 and the Reverse primer NP_01R– 5' TCG ATG TGT GCT TGC TTG GA 3' at the
4 position 1758-1777 designed using the Primer-BLAST software. RT-PCR was carried out using
5 SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase. Each 25 ml
6 amplification reaction mixture comprised: 2X Reaction Mix (containing 0.4 mM of each dNTP, 3.2
7 mM MgSO₄) - 12.5 ul, primer F - 0.5 ul, primer R - 0.5 ul, RNA - 1.5 ul, Taq DNA Polymerase –
8 0.5 ul and water – 9.5 ul.

9 The RT-PCR was carried out as follows: cDNA synthesis at 45 °C for 60 min followed by PCR
10 with an initial heating step at 94 °C for 2 min, 31 cycles of denaturation at 94 °C for 15 sec,
11 annealing at 55 °C for 30 sec and the extension at 68 °C for 3 min followed by a final extension at
12 68 °C for 7 min.

13
14
15 This resulted in the production of a PCR amplicon of the expected size (1721 bp) that was confirmed
16 by agarose gel electrophoresis. The PCR products were purified using QIAquick PCR purification
17 kit (Qiagen) following the manufacturer's protocols. The purified products were then sequenced
18 using Big Dye-Terminator® v3.1 Cycle Sequencing Reaction Kit on ABI-3730 DNA Analyzer
19 (Applied Biosystems) following the manufacturer's instructions.

20
21 Partial N gene sequences of PPRV (nucleotide positions 1253 to 1507) that have a detailed history
22 of collection date and place were retrieved from the GenBank. These partial sequences along with
23 the data generated in this study were aligned using the ClustalW algorithm incorporated in BioEdit
24 software v7.2.0. (Hall, 1999) and edited to remove unreliable sequences/regions. Neighbour-joining
25 trees were then constructed using the distance matrices in MEGA 5.2 with 1000 bootstrap replicates
26 to test the robustness of the tree topology (Tamura et al., 2011).

27 28 *c. Epidemiological analysis*

29
30 To ascertain likely provenance and progress of reported disease in small livestock in three farms in
31 southern Kazakhstan, data were collected on population demography, veterinary reports of
32 outbreaks in the region and vaccination records. The risk of spread through Kazakhstan and in
33 particular, to the regions occupied by the highly endangered and iconic saiga antelope (*Saiga*

1 *tatarica tatarica*) were assessed by geospatial analysis of likely animal movements from known
2 infected sites.

4 **Results and Discussion**

6 *Clinical, pathological, laboratory, demographic and epidemiological assessment*

8 The 3 locations where disease was reported and samples taken in Zhambyl oblast in 2014 are
9 shown in Fig. 1.

11 {Figure 1}

13 a. Beybitshilik"farm, Zhylybulak village, Zhualy district

15 In 2014 the farm had an estimated 3,500 sheep and goats which were divided into 5 flocks with
16 about 600-800 animals in each flock. The animals were grazing locally in the area. The distance
17 between the pastures where flocks of sheep and goats graze was about 2-3 km. Animal disease
18 was noted in two flocks (comprised of 1,470 sheep (768 adults and 702 young) and 130 goats (67
19 adults and 63 young). The disease was first observed on 21st June 2014. There were no reported
20 market purchases of animals in the month prior to this outbreak. Affected sheep and goats (all sick
21 animals were lambs and kids aged between 3 and 4 months) showed fever, depression, and loss of
22 appetite, coughing and shortness of breath, diarrhea, and discharge from eyes and nose, oral
23 mucosal lesions. Of 765 clinically-affected lambs and kids, 282 were found dead. According to the
24 farmer, the flocks had not been vaccinated prior to the outbreak in 2014 but the adult sheep and
25 goats on the farm were vaccinated in autumn 2013. Young animals born in April to early May would
26 normally be vaccinated at 3 months of age but given the high temperatures vaccine efficacy can be
27 reduced and some farmers take their flocks to high pasture without the young being vaccinated in
28 that year. On 9th July 2014 a dead lamb and a dead kid were necropsied and samples were brought
29 to RIBSP for diagnosis.

31 b. Karasu rural district of Kordai district (rayon)

33 PPR-like disease was reported from 26th August 2014 affecting only sheep in the Karasu rural
34 district which is located adjacent to the National border with The Republic of Kyrgyzstan. Pasture
35 land for sheep grazing is shared between the countries alongside the Shu River. There were no

1 reports of market bought animals introduced to these flocks during the previous month. Small
2 livestock were examined and clinical symptoms reported were fever, marked depression, loss of
3 appetite, coughing and shortness of breath, diarrhea, discharge from eyes and nose and oral
4 mucosal lesions and signs of exhaustion. From a population of 488 adult and 412 young animals,
5 65 lambs died. On 4th September 2014 one case was necropsied. The pathology included serous
6 conjunctivitis, rhinitis and acute catarrhal pneumonia. Samples were taken from a dead lamb and
7 delivered to RIBSP for diagnosis. The adult animals were vaccinated during autumn 2013 and the
8 new born were not vaccinated until the outbreak was seen.

9
10 c. Merke sheep farm, Merke district (rayon)

11
12 In 2014 the Merke sheep farm had about 20,000 South Kazakh Merino sheep breed, comprised of
13 18 breeding flocks and 3 male flocks (approximately 800 animals in each flock with a total estimated
14 15,000 adults) and 8 nursery flocks (3-4 months of age 5000 young stock, approximately 600
15 animals per flock) . During the summer period (May 20th to September 10th) small ruminants were
16 on pastures in the mountains on the border with Kyrgyzstan. Around September 10, 2014 sheep
17 and goats began to leave to winter pastures in desert lowlands to the North. During the pastoral
18 movements animals were detained for some time near the Akermen village. Adult sheep in this
19 population were vaccinated against PPRV with the vaccine G-45KM in the autumn of 2013, whereas
20 newly borned young animals were not vaccinated. . On September 30th 2014 the disease was
21 reported among the young sheep in three flocks (approximately 1800 lambs were infected) located
22 near the village. There were no market-bought animals introduced into these flocks as they are a
23 specialist breeding farm for the production of fine-wool. Affected 3 and 4 months old sheep and
24 goats showed fever, depression, loss of appetite, coughing and shortness of breath, diarrhea,
25 discharge from eyes and nose and oral mucosal lesions.

26
27 The recorded mortality amongst unvaccinated lambs was 170 which was approximately 9.4% of the
28 total young stock. On October 3rd two dead lambs were necropsied and samples were delivered to
29 RIBSP for diagnosis. The pathology included: serous conjunctivitis and rhinitis, acute catarrhal
30 pneumonia, inflammation of all lymph nodes, patchy hemorrhage under the serous membrane of
31 the lungs and acute catarrhal enteritis. After the disease was observed, 3 clinically affected flocks,
32 were isolated, and were placed officially under quarantine. All apparently healthy animals in the
33 remaining flocks were vaccinated against PPR.

34
35 As part of the National disease control regulations, in order to prevent the spread of the disease, all
36 the sheep and goats in all farms of Zhambyl region were then vaccinated.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

Laboratory results

Diagnostic samples from these 3 farms were analyzed. From these samples suitably prepared materials were assayed in the RT-PCR for presence of PPRV. The results have shown that all samples of lymph nodes, spleen and lungs tested from the 3 different disease loci were positive by RT-PCR specific for PPR virus. In all samples PCR-products of 1721 bp in length were observed. Phylogenetic analysis of PPR virus fragment length of 255 nucleotides showed that the viruses isolated in Kazakhstan, were similar to viruses isolated in China in 2013-2014 (Fig. 2). Viruses isolated in Iraq: Sirwan-Sulaimani, Kurdistan in 2013 and Pakistan, were considerably different in the nucleotide sequences from that of the Kazakhstan viruses. Similarly, the vaccine strain used in the vaccination campaign in Kazakhstan was shown to be close to the Nigeria 75/1 vaccine (Fig. 2).

N-gene sequences of PPRV strains isolated from different animals in the farms of Zhambyl oblast have shown high nucleotide homology level (99.8-99.9%). Nucleotide sequence analysis has demonstrated that these Kazakhstan viruses are 99.8-100% identical to PPRV isolated in 2013 from the outbreak in Yili region of Xinjiang province in China (Bao et al., 2014) and 99.5-99.7% identical to the virus isolated in 2014 from the outbreak in Heilongjiang Province, China (Wang et al., 2015) (Fig. 2).

{Figure 2}

Demography and epidemiology

Small livestock demography, distribution, movement, marketing and trading structures within the area were as follows: In the farms of Zhambyl region in 2014 there were 2,088,631 sheep and 267,205 goats, including 197,468 sheep and 6467 goats in the Merke district, 140,419 sheep and 21,841 goats in the Zhualinskiy district and 338,017 sheep and 17,096 goats in the Kordai district. Within the oblast animal trade is organized through a number of local livestock markets located in the city of Taraz, Merke village, Kulan village and Kordai village of Zhambyl region. Some of these animals are subsequently or directly driven to the markets in Uzynagash City of Almaty region and Almaty City. A small number of animals from Zhambyl region enter the markets of South Kazakhstan region. Animals destined for regional markets are transported by road on the highway between Shymkent and Almaty, along which there are dozens of settlements. The livestock at risk along the highway are about 2 million head of sheep and goats and the overall at-risk population from exposure to animals from the markets in South Kazakhstan is 5 million heads of sheep and goats.

1
2 Improvement of living standards in Kazakhstan in recent years has brought increased demand for
3 livestock products, higher prices and increased imports into the southern regions from other
4 countries of Central Asia (Kyrgyzstan, Uzbekistan and Tajikistan). Animals from Central Asia enter
5 the markets of the southern regions of Kazakhstan both legally, where they are monitored and
6 recorded or, illegally through smuggling across the border on foot or in vehicles where they likely
7 join local flocks, very often owned by relatives of the same Kyrgyz and Uzbek ethnic groups that live
8 in these countries and in southern Kazakhstan. According to local tradition, they give gifts in the
9 form of livestock to each other.

10
11 A map showing the general pattern of trade movements of small ruminants (arrows) is provided (Fig.
12 1) showing potential epidemic spread in unvaccinated stock to the North East and West of the
13 region.

14
15 Wild ruminant populations: Saiga

16
17 The historic range and calving of saiga antelope in Kazakhstan is shown in Fig. 3. Since the collapse
18 of the population in 1999 there has been range contraction and a gradual shifting of calving sites to
19 the North and this might reflect rising average temperatures associated with climate change (Singh
20 et al, 2010). The central (Bet-pak Dala) population is recovering from a low of approximately 100,000
21 individuals in 2000 to an estimated 250,000 individuals in 2014. 285 sera from saiga in Kazakhstan
22 were tested for PPR virus antibodies (ID-Vet France) between 2012 and 2014 with negative results.
23 To emphasise the risk of epidemic disease to this critically endangered species, a recent
24 catastrophic disease outbreak has reduced the central population by an estimated 50% and its
25 cause continues to be investigated (New Scientist 2015).

26
27 {Figure 3}

28
29 Rapid spread of PPR in Asia and Africa requires urgent attention. The first steps should be aimed
30 at effective control of the disease and then ultimately eradication but before this can be achieved a
31 better understanding of the epidemiology of emergent PPR in the region is required.

32
33 The laboratory diagnosis confirmed the presence of Lineage IV PPRV in the sheep and goat
34 populations in the Zhambyl region of southern Kazakhstan between June and September 2014. The
35 timing of the outbreaks first in Zualy, then Kordai and finally Merke is not consistent with a lateral
36 spread across the region from the West to East and/or between these farms. There was no clear

1 trade or animal movement connection between these locations, which are over 100 kilometers apart.
2 None of these flocks affected with PPR were linked to a market purchase in the previous month.
3 Flocks are generally vaccinated in the autumn of each year in the Southern border regions and the
4 infections were restricted to unvaccinated young stock born after autumn 2013 and in 2014
5 confirming recent circulation. Most likely these infections are a result of transboundary movement
6 of virus between neighbouring countries. Sequence data of circulating viruses in Kyrgyzstan and
7 other neighboring countries are not available except from China. As Kazakhstan and Kyrgyzstan
8 livestock share pasture lands between them, incursion from infected regions in Kyrgyzstan, through
9 contact between flocks at pasture in border areas might be possible. However, since this movement
10 pattern is not new and there was monitoring for 7-8 years by RIBSP of small ruminant outbreaks in
11 this area, this circulation appears to be novel. This provenance seems highly likely for the Merke
12 farm outbreak based on the history of movement but is not entirely clear for the other two outbreaks.
13 In relation to this, it seems likely that the infection is now widespread in the border areas and infection
14 might be present along the southern regions of Kazakhstan beyond the foci currently identified.
15 Given the spread of this strain of virus across China between 2013 and 2014 the possibility of similar
16 spread in Kazakhstan cannot be ruled out, given the practical difficulties of vaccinating young stock
17 at pasture and where high summer temperatures can affect vaccine efficacy.

18
19 The study of genetic affinities between the viruses isolated in different geographic areas is vital to
20 determine spatial and temporal aspects of the viruses spread and persistence.

21 Data show that until recently only PPR viruses of lineage IV had been isolated in Asian countries
22 (Kwiatek et al, 2007; Balamurugan et al, 2012; Kerur et al, 2008; Bao et al, 2012; Abubakar et al,
23 2014; Bao et al, 2014; Kumar et al, 2014). However, lineage IV and II viruses were isolated from the
24 outbreak in Heilongjiang province of China in spring of 2014 (Wang et al, 2015). The lineage II China
25 viruses were 99% identical to their vaccine strain used, i.e., Nigeria75/1. This might be explained by
26 the detection of vaccine product although this possibility was not reported by the researchers but
27 similar out-of-range isolates have been reported recently from sheep and goats in Tandahimba,
28 Tanzania 2011 (Misinzio et al, 2015) and in sheep, goats and wildlife in Ngorongoro District Tanzania
29 2014 (Mahapatra et al, submitted) but the Tanzanian viruses are not suggested to be close to the
30 vaccinal strain although of the same genotype. The PPR viruses isolated in Kazakhstan could not
31 have been derived from the vaccine strain since the vaccine virus belongs to Lineage II and field
32 isolates to Lineage IV.

33
34 PPRV classification based on the analysis of N-gene sequence is most suitable for phylogenetic
35 distinction of close viruses and provides a comprehensive view of PPR molecular epidemiology
36 (Kwiatek et al, 2007; Kerur et al, 2008; Munir et al, 2012; Kumar et al, 2014). The findings reported

1 here showing >99.8% affinity with recent isolates in China provide evidence of transboundary
2 circulation of PPRV between China and Kazakhstan. Long distances between outbreaks in China
3 and those reported here suggest cases occurred in Kyrgyzstan and warrants further investigation to
4 establish the full extent of the epidemic.

5
6 Despite high vaccination coverage these data suggest persistence of the virus at sub-clinical level in
7 pastoral systems with peaks of virus transmission in unvaccinated young stock each year. Therefore, further
8 studies of possible endemic PPRV in Kazakhstan is also urgently needed with both, large
9 populations of unvaccinated small ruminant livestock and endangered wild saiga antelope, a small
10 ruminant species which are probably susceptible based on recent serology and therefore the
11 population is most probably at risk and a good indicator of cryptic circulation in livestock under
12 vaccination programmes.

13
14 Critical control points to prevent infection entering unvaccinated livestock and saiga populations are
15 on the northern borders of the Zhambyl region, which touch the southern-most migration of the Bet-
16 pak Dala population of saiga. This is the largest remaining concentration of this species globally.
17 Since the collapse of the saiga population from over a million to <200,000 animals in the late 1990s,
18 the range contracted and calving sites had shifted northwards (Singh et al, 2010), which is perhaps
19 fortunate given the recent PPR incursion. The calving period (in May each year) sees mass
20 aggregation of saiga. For example, in June 2014 approximately 250,000 animals were spread over
21 a few square kilometers for 2 weeks, a period of high risk for directly transmissible infections like
22 PPR virus. This is also a time when livestock begin to be pastured more extensively, increasing
23 likelihood of contact with saiga antelope. Buffer zone vaccination for small livestock adjacent to this
24 population might be warranted

25
26 Tighter control of animal movements and imports across the borders is critical together with
27 regional control through regular vaccination to ensure that all stock at pasture are immune. Mass
28 vaccination for small ruminants is recommended to increase the herd immunity to more than 80%.
29 In addition to this mass vaccination for adult sheep and goats, regular revisits for vaccination of
30 new born lambs and kids are advocated at the completion of three months age to to exclude the
31 virus from the population..

32 **Acknowledgements**

33
34 The research leading to these results has received funding from the Committee of Science of the
35 Ministry of Education and Science of the Republic of Kazakhstan. We

1 thank the Biotechnology and Biological Sciences Research Council, the European Commission
2 ANIHWA ERA fund for providing support for PPR epidemiological research under the IUEPPR
3 project led by the Pirbright Institute (BB/L013657/1) and the Royal Veterinary College
4 (BB/L013592/1). The study was undertaken under appropriate ethical review at RIBSP and did not
5 involve the use of experimental animals. The activities of the project have been ethically approved
6 through the ANIHWA ERA process, BBSRC, RVC (2015 1326) and with the Kazakhstan
7 Government departments responsible for research on wildlife and livestock mentioned above.
8

9 **Conflict of interest**

10
11 The authors affirm that no financial or personal relationship existed that could have
12 inappropriately influenced the content of this manuscript or the opinions expressed.
13 The authors include R. Kock of the Royal Veterinary College, S. Parida of the Pirbright Institute,
14 UK, collaborating globally on the emergence and epidemiological understanding of PPR virus
15 emergence. M. Orynbayev, K.Sultankulova^b, V.Strochkov^b, Z.Omarova^b, E.Shalgynbayev^b,
16 N.Rametov^b, A.Sansyzbay of the RIBSP, who undertook the core research activities in the field
17 and laboratory. The present article is published under the sole responsibility of the authors.
18

19 **References**

- 20
21 Abubakar, M., M. Munir, 2014: Peste des petits ruminants virus: an emerging threat to goat farming
22 in Pakistan. *Transbound. Emerg. Dis.* 61 (Suppl. 1), 7–10.
- 23 Abubakar, M., Z. I. Rajput, & Q. Ali, 2011: Evidence of peste des petits ruminants virus (PPRV)
24 infection in Sindh Ibex (*Capra aegagrus blythi*) in Pakistan as confirmed by detection of antigen
25 and antibody. *Health (San Francisco)* (July 2009), 745–747. doi:10.1007/s11250-010-9776-y
- 26 Abu-Elzein E.M.E., F.M.T. Housawi, Y. Bashareek, A.A. Gameel, A.I. Al-Afaleq, E.C. Anderson,
27 2004: Severe PPR infection in gazelles kept under semi-free range conditions in Saudi Arabia.
28 *J. Vet. Microbio. B* 51 (2), 68-71.
- 29 Al-Majali A.M., N.O. Hussain, N.M. Amarin, A.A. Majok, 2008: Seroprevalence of, and risk factors
30 for peste des petits ruminants in sheep and goats in Northern Jordan. *Prev. Vet. Med.* 85, (1–
31 2):1–8.
- 32 Anees M., M.Z. Shabbir, K. Muhammad, J. Nazir, M.A. Shabbir, J.J. Wensman, M. Munir, 2013:
33 Genetic analysis of peste des petits ruminants virus from Pakistan *BMC Vet. Res.* 9, 60.
34 doi:10.1186/1746-6148-9-60

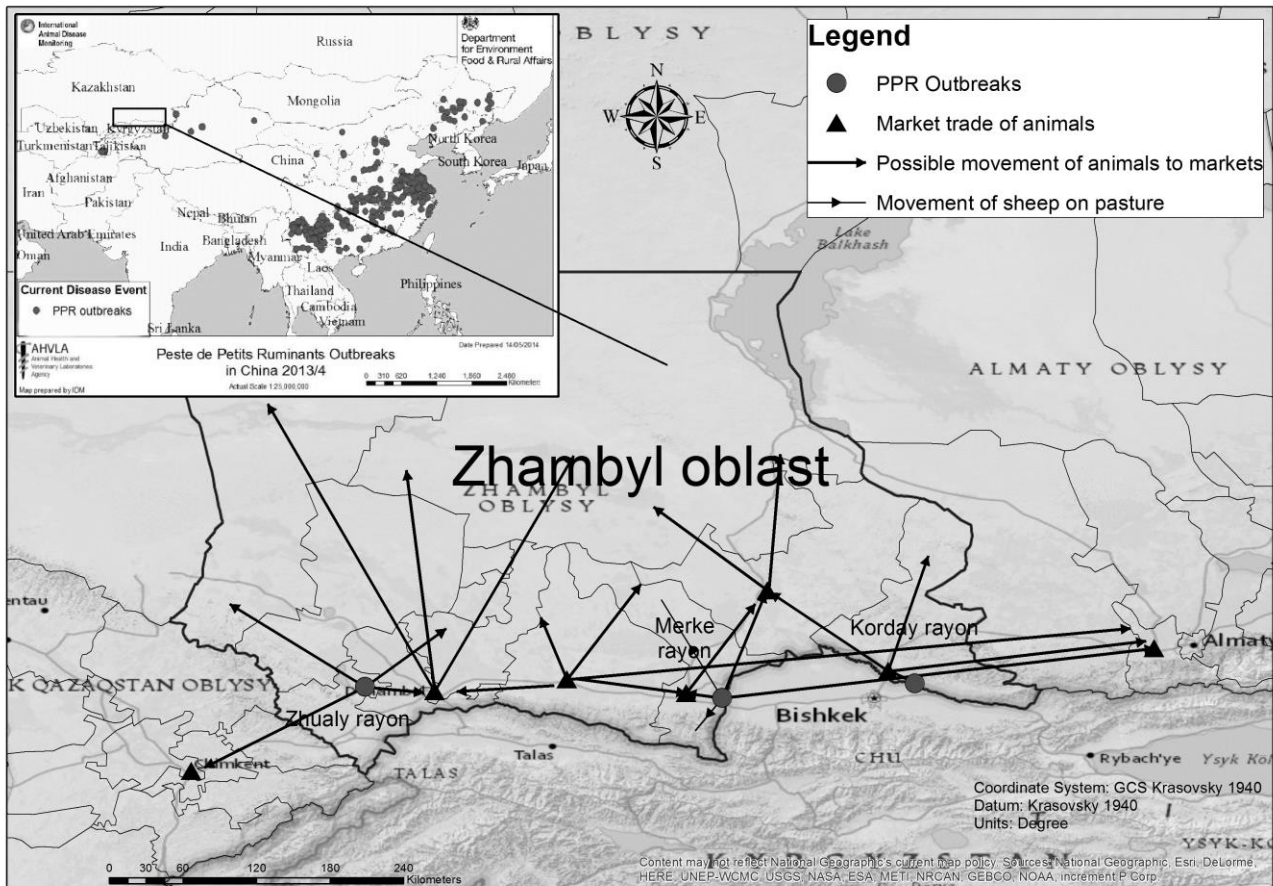
- 1 Balamurugan V., P. Saravanan, A. Sen, K.K. Rajak, G. Venkatesan, P. Krishnamoorthy, V.
2 Bhanuprakash, R.K. Singh, 2012: Prevalence of peste des petits ruminants among sheep and
3 goats in India. *J. Vet. Sci. (India)* 13(3), 279-285.
- 4 Banyard, A.C., S. Parida, C. Batten, C. Oura, O. Kwiatak, G. Libeau, 2010: Global distribution of
5 peste des petits ruminants virus and prospects for improved diagnosis and control. *J. Gen.*
6 *Virol.* 91, 2885–2897.
- 7 Banyard A C, Z. Wang, S. Parida, 2014: Rapid emergence of peste des petits ruminants virus in
8 East Asia. *Emerg. Infect. Dis.* 20(12), 2176-8. doi: 10.3201/eid2012.140907
- 9 Bao, J., Q. Wang, S. Parida, C. Liu, L. Zhang, W. Zhao and Z. Wang, 2012: Complete genome
10 sequence of a peste des petits ruminants virus recovered from wild Bharal in Tibet, China. *J.*
11 *Virol.* 86 (19), 10885–10886.
- 12 Bao J., Q. Wang, Y. Zhang, C. Liu, L. Li, Z. Wang, 2014: Complete genome sequence of a novel
13 variant strain of peste des petits ruminants virus, China/XJYL/2013. *Genome Announcements*
14 2(5), e00762-14. doi:10.1128/genomeA.00762-14.
- 15 Gargadenec, L., A. Lalanne, 1942: La peste des petits ruminants. *Bulletin des Services Zoo*
16 *techniques, et des Epizooties de l’Afrique Occidentale Francaise* 5, 16–21.
- 17 Gibbs E.P., W.P. Taylor, M.J. Lawman, J. Bryant, 1979: Classification of peste des petits ruminants
18 virus as the fourth member of the genus Morbillivirus. *Intervirol.* 11, 268–74.
- 19 Govindarajan, R., A. Koteeswaran, A.T. Venugopalan, G. Shyam, S. Shaguna, M.S. Shaila, and S.
20 Ramachandran, 1997: Isolation of peste des petits ruminants virus from an outbreak in Indian
21 buffalo (*Bubalus bubalis*). *Vet. Rec.* 141, 573–574.
- 22 Hall T.A. 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program
23 for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41, 95-8.
- 24 Hoffmann, B., H. Wiesner, J. Maltzan, R. Mustefa, M. Eschbaumer, F. A. Arif, M. Beer, (2012).
25 Fatalities in wild goats in kurdistan associated with peste des petits ruminants virus.
26 *Transbound. Emerg. Dis.* 59(2), 173–6. doi:10.1111/j.1865-1682.2011.01270.x
- 27 Ibu, O., S. Salihu, J. Luther, K. Suraj, A. Ceaser, A. Abechi, E. Aba-Adulugba, D. Shamaki, 2008:
28 Evaluation of peste des petits ruminant and rinderpest virus infection of camels in Borno and
29 Kano states of Nigeria. *Niger Vet. J.* 29, 76–77.
- 30 Kerur, N., M.K. Jhala, C.G. Joshi, 2008: Genetic characterization of Indian peste des petits
31 ruminants virus (PPRV) by sequencing and phylogenetic analysis of fusion protein and
32 nucleoprotein gene segments. *Res. Vet. Sci.* 85, 176–183.
- 33 Khalafalla A.I., I.K. Saeed, Y.H. Ali, M.B. Abdurrahman, O. Kwiatak, G. Libeau, A.A. Obeida, Z.
34 Abbas 2010: An outbreak of peste des petits ruminants (PPR) in camels in the Sudan. *Acta*
35 *Trop.* 116(2), 161-5. doi: 10.1016/j.actatropica.2010.08.002

- 1 Kwiatek, O., C. Minet, C. Grillet, C. Hurard, E. Carlsson, B. Karimov, E. Albina, A. Diallo, G. Libeau,
2 2007: Peste des petits ruminants (PPR) outbreak in Tajikistan. *J. Comp. Path.* 136, 111–119.
- 3 Kock R.A., 2006: Rinderpest and wildlife. In: Barrett, T., Pastoret, P-P. and Taylor. W. (eds),
4 Rinderpest and Peste des Petits Ruminants Virus. *Plagues of large and small ruminants.*
5 *Biology of Animal Infections.* pp. 144-162, Elsevier publications, Academic Press, London.
- 6 Lembo, T., C. Oura, S. Parida, R. Hoare, L. Frost, R. Fyumagwa, F. Kivaria, C. Chubwa, R. Kock,
7 S. Cleaveland, C. Batten, 2013: Peste des petits ruminants infection among cattle and wildlife
8 in northern Tanzania. *Emerg. Inf. Dis.* 19(12), 2037–2040.
- 9 Libeau, G., A. Diallo, S. Parida, 2014: Evolutionary genetics underlying the spread of peste des
10 petits ruminants virus. *Animale Frontiere* 4, 14–20.
- 11 Mahapatra, M., K., Sayelel, M. Muniraju, E. Eblate, R. Fyumagwa, S. Shilinde, M. Maulid Mdaki, J.
12 Keyyu, S. Parida, and R. Kock, 2015: Spill-over of peste des petits ruminants virus from
13 domestic sheep and goats to wild ruminants in the Serengeti ecosystem, Northern Tanzania',
14 (submitted: *Emerg. Infect. Dis.* – under review).
- 15 Misinzo G., T. Kgotlele, E.A. Muse, J.V. Doorselaere, M. Berg, & M. Munir, 2015: Peste des petits
16 ruminants virus Lineage II and IV from goats in Southern Tanzania during an outbreak in 2011.
17 *Brit. J. Virol.* 2(1), 1-4.
- 18 Munir M., S. Zohari, A. Saeed, Q.M. Khan, M. Abubakar, N. LeBlanc, M. Berg 2012: Detection and
19 phylogenetic analysis of peste des petits ruminants virus Isolated from outbreaks in Punjab,
20 Pakistan. *Transbound. Emerg. Dis.* 59, 85–93.
- 21 Munir M. 2014: Role of wild small ruminants in the epidemiology of peste des petits ruminants
22 *Transbound. Emerg. Dis.* 61 (5), 411–424.
- 23 Muniraju, M., M. Munir, A. R. Parthiban, A. C. Banyard, J. Bao, Z. Wang, S. Parida, 2014: Molecular
24 Evolution of Peste des Petits Ruminants Virus. *Emerg. Infect. Dis.* 20(12), 2023–2033.
25 doi:10.3201/eid2012.140684
- 26 Orynbayev M.B., S.M. Mamadaliyev, Zh.K. Koshemetov, S.Sh. Nurabayev, 2005: Peste des petits
27 ruminants in the Republic of Tajikistan. *Urgent problems of veterinary medicine and agricultural*
28 *biotechnology. Materials of the International Science and Practice Conference.* May 19-20,
29 2005. Pavlodar. 66-71 (in Russian).
- 30 Orynbayev M.B., S.M. Mamadaliyev, B.M. Khairullin, Zh.K. Koshemetov, V.M. Matveyeva, S.Sh.
31 Nurabayev, A.Zh. Azhibayev, B.S. Katubayeva, Y. Myktybekova, 2006: Serological monitoring
32 of PPR among animals in Central Asia. 3rd Science and Practice Conference "Problems of
33 infectious pathology in the regions of Siberia, Far East and Extreme North". Novosibirsk.
34 September 27-29, 2006, Novosibirsk, 176-177 (in Russian).

- 1 Ozkul, A., Y. Akca, F. Alkan, T. Barrett, T. Karaoglu, S.B. Dagalp, J. Anderson, K. Yesilbag, C.
2 Cokcaliskan, A. Gencay, I. Burgu, 2002: Prevalence, distribution, and host range of peste des
3 petits ruminants virus, Turkey. *Emerg. Infect. Dis.* 8, 708–712.
- 4 Parida, S., E. Couacy-Hymann, R.A. Pope., M. Mahapatra, M. El Harrak, J. Brownlie, A.C. Banyard,
5 2015: Pathology of peste des petits ruminants. In: Munir M (ed), *Peste des Petits Ruminants*
6 *Virus* pp. 51-67, Springer, Berlin.
- 7 Kumar, S.K, G. Sundarapandian, R. Parimal, A. Thangavelu., R. Arumugam, N.D.J.Chandran, B.
8 Aravindh, M. Muniraju, M. Mahapatra, A.C. Banyard, B. M. Manohar and S. Parida, 2014:
9 Molecular characterisation of Lineage IV Peste des petits ruminants virus using multi gene
10 sequence data. *Emerg. Infect. Dis.* 20 (12), 2176-8. doi/10.1016/j.vetmic.2014.08.031.
- 11 Sen A., P. Saravanan, V. Balamurugan, V. Bhanuprakash, G.Venkatesan, J. Sarkar, K. Rajak, A.
12 Ahuja, V. Yadav, S.B. Sudhakar, S. Parida, R.K. Singh, (2014): Detection of subclinical peste
13 des petits ruminants virus infection in experimental cattle. *Virus Disease* 05/2014; DOI:
14 10.1007/s13337-014-0213-0
- 15 Shaila, M.S., D. Shamaki, M.A. Forsyth, A. Diallo, L. Goatley, R.P. Kitching, T. Barrett, 1996:
16 Geographic distribution and epidemiology of peste des petits ruminants virus. *Virus Res.* 43,
17 149–153.
- 18 Sharawi S.S., M.R. Yousef, A.N. Al-Hofufy, M.H.Al-Blowi, 2010: Isolation, serological and real time
19 PCR diagnosis of peste des petits ruminants virus in naturally exposed Arabian Gazelle in
20 Saudi Arabia. *Veterinary World* 3(11), 489-494.
- 21 Singh, N. J., I. A. Grachev, A. B. Bekenov, & E. J. Milner-Gulland, 2010: Saiga antelope calving site
22 selection is increasingly driven by human disturbance. *Biol. Cons.*, 143(7), 1770–1779.
23 doi:10.1016/j.biocon.2010.04.026
- 24 Soltan, M.A., M.M. Abd-Eldaim 2014: Emergence of peste des petits ruminants virus lineage IV in
25 Ismailia Province, Egypt. *Infect. Genet. Evol.* 28, 44–47.
- 26 Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, 2011: MEGA5: Molecular
27 evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum
28 parsimony methods. *Molecular. Biol. Evol.* (10), 2731-9.
- 29 Wang, J., M. Wang, S. Wang, Z. Liu, N. Shen, W. Si, G. Sun, J. A. Drewe, X. Cai, 2015: Peste des
30 petits ruminants virus in Heilongjiang Province, China, 2014. *Emerg. Infect. Dis.* 21(4), 677-
31 680. doi: 10.3201/eid2104.141627
- 32 Wu X., L. Li, J. Li, C. Liu, Q. Wang, J.Y. Bao, Y. Zou, W. Ren, H. Wang, Y. Zhang, Y. Lv F. Liu, S.
33 Wang, H. Ma, Z. Wang, (2015) Peste des Petits Ruminants Viruses Re-emerging in China,
34 2013-2014. *Transbound Emerg Dis.* doi: 10.1111/tbed.12308. (Epub ahead of print)
- 35

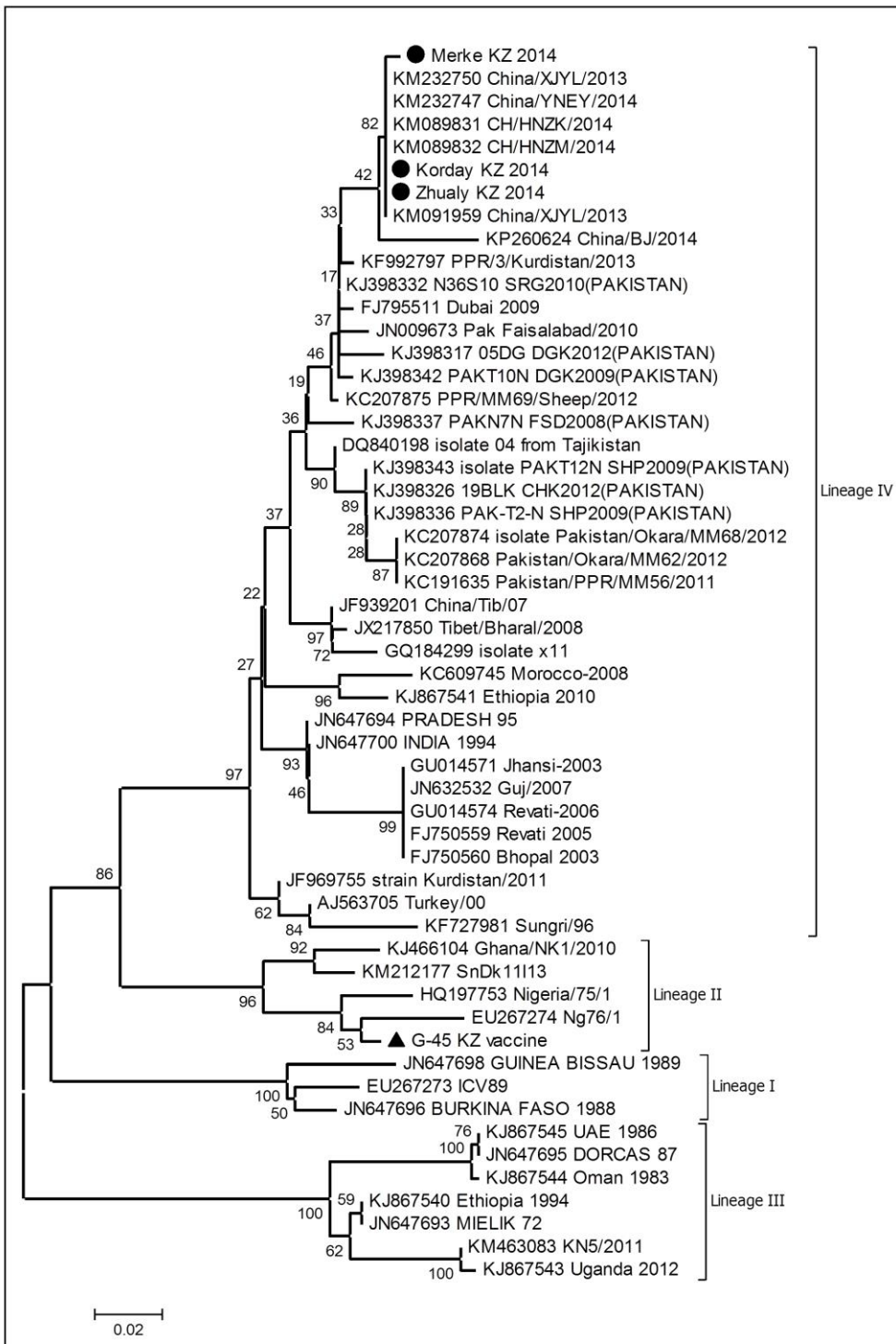
1 **Legends to figures and tables**

2 **Figure 1:** Map of recent PPR outbreaks reported in Asia and Zhambyl oblast marked by the
3 rectangular box on the continental map (AHVLA 2014). The oblast map extracted to show the three
4 outbreak sites (black dots) reported in this study in the Zhualy, Merke and Korday districts (rayon)
5 and showing small ruminant movement and trade patterns (arrows).



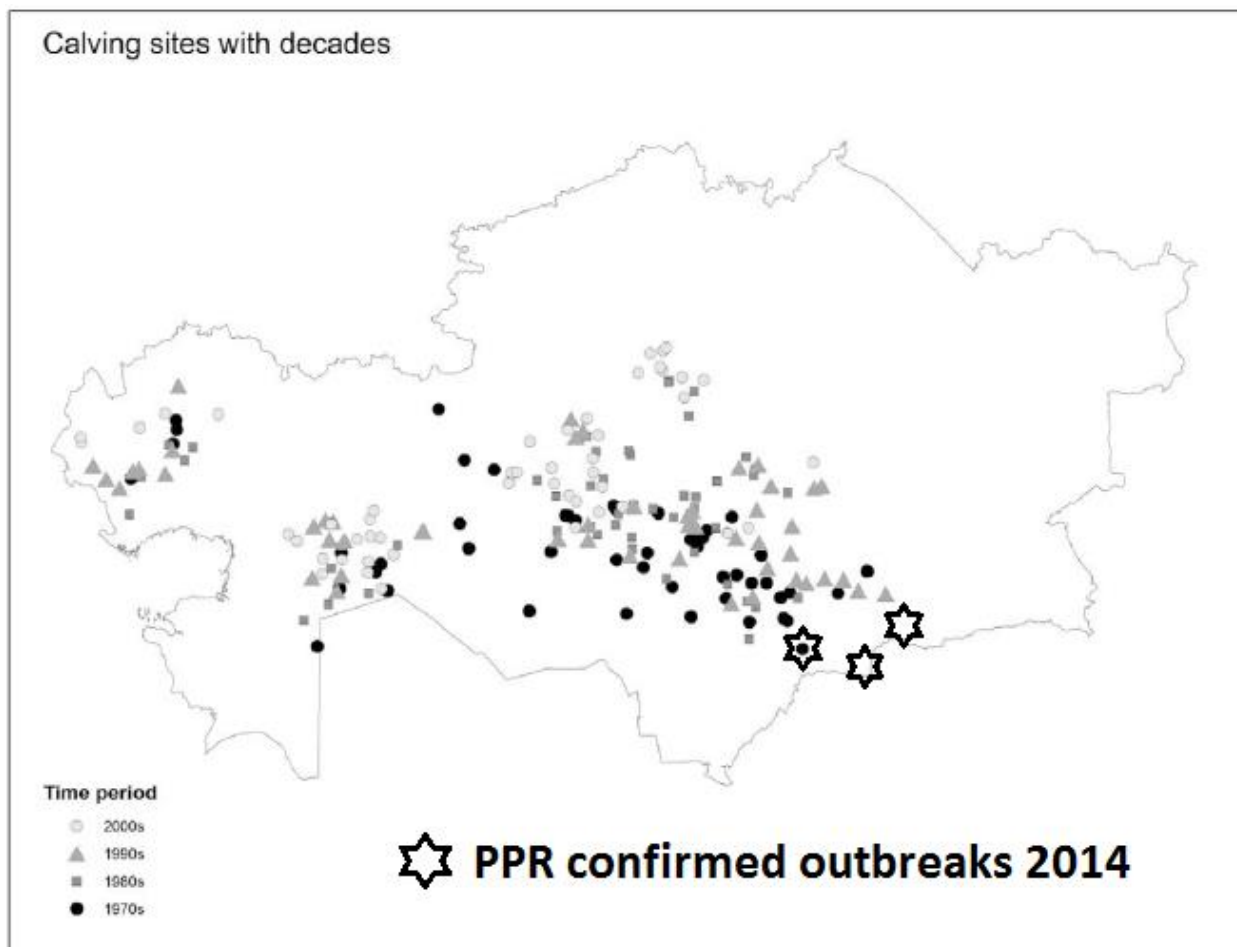
6
7
8

1 **Figure 2:**
 2 Phylogenetic analysis of circulating PPR viruses. Neighbour-joining tree was -constructed using
 3 255-nucleotide sequences of the PPRV nucleoprotein gene showing the relationships between the
 4 PPR viruses circulating in Asia, Middle East and Africa. The percentage of replicate trees in which
 5 the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the
 6 branches. Bullet points indicate the viruses isolated in this study and triangle represents the vaccine
 7 strain of Kazakhstan.



1
2
3
4
5
6

Figure 3: Map of historic saiga range and calving sites in Kazakhstan (Modified from Singh et al 2010), shown in relation to recent outbreaks of PPRV reported here.



1

2 **Table 1.** Descriptive PPR data and samples used in the study.

Outbreak locus	Clinical signs	Date of delivery of biological samples	Mortality: number of dead animals /total susceptible ¹ at time of assay	Samples delivered for assay
Zhambyl oblast , Zhualy rayon, collective farm "Beibitshilik"	Fever (temperature > 39.5°C), depression, anorexia, ocular and nasal discharges, pneumonia, mucosal necrosis and ulceration, severe diarrhea	09.07.2014	282/765 young stock	Blood, spleen, lungs, mesenteric lymph nodes of 1 dead goat and 1 dead sheep
Zhambyl oblast , Merke rayon, JSC "Merke Pedigree Farm"	Fever (temperature > 39,5°C), depression, feed cessation, cough and heavy breathing, diarrhea, ocular and nasal discharge, mouth mucosa lesion	03.10.2014	170/1800 young stock	Blood, spleen, lungs, mesenteric lymph nodes of 2 dead sheep
Zhambyl oblast , Kordai rayon, Karasu Settlement	Fever (temperature > 39.5°C), depression, feed cessation, diarrhea, ocular and nasal discharge, mouth mucosa lesion	04.09.2014	65/412 young stock	Blood, spleen, lungs, mesenteric lymph nodes of 1 dead sheep
¹ Adults vaccinated against PPRV in the autumn of 2013 acquired long lasting immunity whilst young stock aged between 3 and 4 months were unvaccinated and susceptible				

3

4