Outbreak of Peste des Petits Ruminants Virus among Critically Endangered Mongolian Saiga and Other Wild Ungulates, Mongolia, 2016–2017


The 2016–2017 introduction of peste des petits ruminants virus (PPRV) into livestock in Mongolia was followed by mass mortality of the critically endangered Mongolian saiga antelope and other rare wild ungulates. To assess the nature and population effects of this outbreak among wild ungulates, we collected clinical, histopathologic, epidemiologic, and ecological evidence. Molecular characterization confirmed that the causative agent was PPRV lineage IV. The spatiotemporal patterns of cases among wildlife were similar to those among livestock affected by the PPRV outbreak, suggesting spillover of virus from livestock at multiple locations and time points and subsequent spread among wild ungulates. Estimates of saiga abundance suggested a population decline of 80%, raising substantial concerns for the species' survival. Consideration of the entire ungulate community (wild and domestic) is essential for elucidating the epidemiology of PPRV in Mongolia, addressing the threats to wild ungulate conservation, and achieving global PPRV eradication.

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Peste des petits ruminants virus (PPRV; family Paramyxoviridae, genus Morbillivirus) causes an acute and highly contagious infection in domestic sheep and goats (1) and multiple species of wild ungulates (2). The resultant clinical disease, peste des petits ruminants (PPR), can lead to high morbidity and mortality rates (3) and is recognized as an economically important transboundary disease (4). The substantial effect of PPR on household-level livelihoods, well-being, food security, rural communities, and national economies have made PPR a priority for eradication (5–8). PPR is reported to affect several species of free-ranging or captive wild ruminants (2,9,10) but is rarely expressed clinically in wildlife populations (11), which were therefore thought to play a negligible epidemiologic role (6,10). Nonetheless, losses among several threatened species of wild mountain ungulates (12–14) and susceptibility of many other captive endangered ungulates (15–17) make this virus a major threat to wild ungulate conservation (2,9,18,19).

In the fall of 2016, an outbreak of PPRV among domestic sheep and goats in western Mongolia was confirmed, probably originating from uncontrolled transboundary livestock movements (20,21). In total, 83,899 small ruminants from 1,081 households were reportedly affected by PPR in 14 soums (districts) of 3 aimags (provinces), of which 12,976 small ruminants died (overall case-fatality risk 15.5%) (22). After this initial outbreak, control measures included vaccination of 4,632,200 sheep and 5,800,318 goats in and around the outbreak area in October 2016. Although the vaccination campaign successfully curbed the
epidemic in livestock, on December 27, 2016, deaths among the Mongolian saiga antelope (subspecies *Saiga tatarica mongolica*) from PPRV infection were confirmed; later, deaths from PPRV infection of Siberian ibex (*Capra sibirica*) and goitered gazelle (*Gazella subgutturosa*) were also confirmed (22). In the following months, thousands of critically endangered Mongolian saiga died.

The Mongolian saiga antelope (hereafter saiga) is a nomadic antelope that now occupies <20% of its historic range in 2 provinces of Mongolia (Khovd and Gobi-Altai), representing 36,000 km² of desert steppe bordered by high mountain ranges, lakes, and sand dunes (23). The saiga range partially overlaps that of mountain ungulates, including Siberian ibex, Argali sheep (*Ovis ammon*), and other plains ungulates such as goitered gazelle and Mongolian gazelle (*Procapra gutturosa*). It is also dominated by livestock; >1.5 million sheep and goats in the 8 soums overlapping the saiga range (24) are seasonally grazed over both mountain and desert steppe areas (25).

To describe the PPRV epizootic in the wild ungulate community of Mongolia, we gathered all available evidence from field missions, histopathology examinations, government records, wildlife population monitoring efforts, and laboratory testing (including molecular characterization of the causative agent). We describe the significance of our findings for the global PPR eradication program and the conservation of wild ungulate species (Figure 1).

**Materials and Methods**

**Emergency Field Mission**

On January 20, 2017, shortly after the first confirmation of PPRV infection in saiga, the Crisis Management Center–Animal Health, led by the Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health, and the Mongolia government organized an investigation of PPR among wild and domestic animals (22). Reports of cases before the first official confirmation were collected from multiple sources in the field (Appendix section 1.1, https://wwwnc.cdc.gov/EID/article/26/1/18-1998-App1.pdf). In addition, samples were obtained from 27 animals that were found...
sick (2 saiga) or dead (2 ibex, 2 goitered gazelle, 21 saiga) during direct field observations. For dead animals, full necropsy examinations were performed when possible (2 ibex, 2 goitered gazelle, 9 saiga); necropsy reports were available for 2 ibex, 1 goitered gazelle, and 4 saiga. Heads were collected from the rest of the animals (12 saiga). Tissues were available for histologic examination from 17 animals (2 ibex, 2 goitered gazelle, 13 saiga) (Appendix section 1.2). We used the lateral flow device Peste-Test (The Pirbright Institute, https://www.pirbright.ac.uk) as a rapid penside field test for PPRV (26) on eye swab samples from 20 animals (2 ibex, 1 goitered gazelle, 17 saiga), except for 1 live saiga from which we noninvasively obtained feces and saliva. We sent samples to the State Central Veterinary Laboratory (SCVL) for PPRV confirmation by use of gel-based reverse transcription PCR (RT-PCR) (Appendix section 1.3) (27). For 2 of the saiga samples, the Pirbright Institute sequenced the C-terminus portion of the N-gene (Appendix section 1.4).

**Phylogenetic Analysis**
We retrieved and used the partial N-gene sequences of PPRV (n = 56) available in GenBank for southern, central, and eastern Asia through September 2018 for constructing a neighborhood-joining phylogenetic tree. Sequences included the 2 partial N-gene sequences obtained from saiga from Mongolia in this study (Appendix section 1.4).

**Reporting to SCVL**
All wildlife samples submitted to SCVL in 2016–2017 were compiled in a dataset, which included georeferences and PPR diagnostic results when available. Most samples were tested by using the RT-PCR procedure mentioned above, and a subset of samples was also tested by using ID Screen PPR Competition and ID Screen PPR Antigen Capture (IDvet, https://www.id-vet.com). Cases were considered positive when ≥1 of the 3 test results was positive. When georeferences were missing, we used the location description to determine the approximate geographic coordinates and mapped it by using ArcGIS 10.2 (ESRI, https://www.esri.com). These coordinates were used to trace the spatiotemporal progression of the PPR outbreak in wildlife, including identifying potentially undiagnosed wildlife illness and deaths that may have been part of the same outbreak.

**Government Carcass Disposal**
From January 8 through February 28, 2017, as part of the Mongolia government emergency response (Appendix section 2) in the Khovd and Gobi-Altai Provinces, livestock movements were restricted and saiga carcasses were collected and destroyed. Carcass disposal was conducted at 8 sites where records were kept of the total number of carcasses and sex of the animal (when available). In some soums, at the initiative of the soum-level government, the count and collection of carcasses was maintained until June 30, 2017.

**Saiga Population Surveys**
In 2010, distance sampling (28) was first applied to the saiga population as a way to improve population abundance estimates (23). Thereafter, it was implemented as part of a routine monitoring program; local saiga rangers conducted distance sampling surveys along 40 transects ranging from 2 to 99 km, for a total of 1,505 km of survey effort. Each survey was conducted by 4 trained teams, who drove vehicles along transects and recorded for each group of saiga seen the radial distance, angle from the transect line, and group size. To better monitor the population-level effect of the outbreak, we repeated the surveys in January, March, and May 2017, and April 2018.

Following systematic data cleaning steps (Appendix section 3.1; datasets, https://doi.org/10.6084/m9.figshare.7502252.v1; R code available upon request), we used Distance 7.2 software (29) to fit detection function models to the distance sampling data. Models were fitted separately for each survey and, when sample size was sufficient, were stratified by the 3 regions within the home range (Durgun Steppe, Khuisiin Gobi, and Sharga Gobi). In addition, to estimate population density and abundance, we assessed group size bias (e.g., when smaller groups farther from the transect line tend to be missed) and corrected when necessary (Appendix sections 3.2).

**Ethics Considerations**
No ethics approval was required for the outbreak response because the investigation was a response to an emergency situation, and no live animal handling was required to obtain the samples (samples obtained from dead animals or from environmental recovery of excreted/secreted material). The driving transect survey technique for estimating the population of Mongolian saiga was reviewed by the wildlife research advisory committee of the Mongolian Academy of Sciences. Members of the Mongolian Academy of Sciences act as the main scientific advisors to the Ministry of Environment in issuing of permits related to wildlife research in Mongolia.
Results

Clinical Manifestations of PPRV Infection in Wild Ungulates

One live clinically ill saiga (confirmed positive for PPRV by Peste-Test) could be approached and displayed the following clinical signs: lethargy with tachypnea and dyspnea, seropurulent ocular discharge, diarrhea, and weakness. Of the 20 animals tested with Peste-Test, results were positive for 17 (1 ibex, 1 goitered gazelle, and 15 saiga); of the 17 animals tested by RT-PCR, results were positive for 16. Of the 22 total tested animals, 20 were positive by either Peste-Test or RT-PCR. For the 6 animals PPRV positive by RT-PCR that underwent necropsy (4 saiga, 1 goitered gazelle, 1 ibex), notable gross pathology findings included emaciation (n = 4), red nasal mucosa or discharge (n = 3), erosive to ulcerative lesions of the oral mucosa (n = 3), red or consolidated portions of lungs (n = 6), and red discoloration of the intestinal mucosa or presumptive enteritis (n = 4). Tissues available for histology from PPRV-infected animals (positive test result, histologic evidence, or both) showed acute cellular degeneration and necrosis, with varying degrees of associated acute inflammation, that affected the oral/pharyngeal mucosa, hepatocytes, cholangiolar epithelium of bile ductules, bronchiolar epithelium, and intestinal crypt epithelium (Table 1; Figure 2). We observed viral inclusion bodies and a few viral syncytia to varying degrees in oral/pharyngeal, liver, lung, and intestinal lesions. In some cases, postmortem artifacts hindered intestinal evaluation. Concurrent diseases in PPRV-infected animals were found in 1 saiga with stomatitis typical of parapoxviral infection (contagious ecthyma) and 1 goitered gazelle with bacterial sepsis. Atrophy of adipose tissue and lymphoid depletion were identified in animals with and without evidence of PPRV infection (Appendix section 1.2; individual animal data, https://doi.org/10.6084/m9.figshare.7502258.v1).

Phylogenetic Analysis

We obtained partial N-gene sequences from 2 PPRV-infected saiga. The phylogenetic analysis, conducted by using 58 partial N-gene sequences (Figure 3), confirmed that the PPRV sequences were of PPRV lineage IV and formed 1 cluster with sequences from livestock in Mongolia in 2016 (20) and from outbreaks in China in 2013–2016 (Figure 3). In addition, these sequences are genetically close to sequences from...
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central Asia (i.e., Iran [GenBank accession no. KY550670] and Tajikistan [accession no. DQ840198]).

Mapping of Confirmed and Unconfirmed Cases
From the onset of the outbreak in livestock to December 2018, samples from 30 georeferenced individual animals of 4 species (23 saiga, 5 ibex, 1 argali sheep, and 1 goitered gazelle) were submitted to and confirmed PPRV positive by the SCVL (Figure 4; case mapping data, https://doi.org/10.6084/m9.figshare.7502264.v1). Most of the early cases in saiga (until January 2017) were detected in the Durgun Steppe and the Khuisin Gobi portions of the saiga home range (particularly in Chandmani Soum, Khovd Province); the cases in the Sharga Gobi portion of the saiga range and the rest of the Gobi-Altai Province first appeared in February 2017 (Figure 4). After the last reported saiga case in May 2017, all subsequent confirmed cases were in ibex that died in the Gobi-Altai Province through January 2018.

Suspected but unconfirmed cases were documented through interviews conducted during the Crisis Management Center–Animal Health field mission, review of provincial and central laboratory records of undiagnosed wildlife deaths, and interviews with local herders and rangers patrolling the saiga range (Figure 4). Unconfirmed cases revealed 2 clusters, 1 in the southwestern part of Khovd Province close to the border with China and 1 between Khar-Us and Durgun Lake north of the saiga range. The first cluster involved 10 ibex with severe diarrhea that led to death at 3 locations in July and August 2016. At that time, PPR was not suspected because the outbreak in livestock was not confirmed and declared until September 2016 (although retrospective serologic evidence indicates that PPRV was probably circulating in livestock as early as November 2015 [22]). The second cluster was reported by herders, who indicated substantial saiga deaths in December 2016, before the first PPR diagnosis in saiga was confirmed on December 27, 2016. Saiga rangers confirmed the death of at least 27 animals in these locations. This pattern mirrors quite closely the PPR outbreak observed in livestock; initial cases in livestock clustered at the southwestern Mongolia–China border and at a secondary outbreak focus in the Khar-Us Lake area (22).

Government Carcass Disposal
From January through February 2017, the emergency response team collected and destroyed 4,202 saiga carcasses. Adding the soums for which collection continued until June 2017, the collection efforts totaled 5,425 saiga, 41 goitered gazelles, and 24 ibex. Among soums for which information on animal sex was recorded (Bayan-Uul, Darvi, Khukhmorit and Sharga),
Figure 3. Neighbor-joining tree constructed on the basis of partial N-gene sequences of peste des petits ruminants virus (PPRV), showing relationships among the PPRV isolates. The Kimura 2-parameter model was used to calculate percentages (indicated by numbers beside branches) of replicate trees in which the associated taxa clustered together in 1,000 bootstrap replicates. Red rectangle outlines the 2 PPRV sequences from saiga obtained from this study (BankIt2279588 MOG/saiga5-2017, GenBank accession no. MN648447; BankIt2279588 MOG/saiga8.1-2017, accession no. MN648448). GenBank numbers are indicated. Scale bar indicates nucleotide substitutions per site.
sex ratios ranged from 2 to 6 females for 1 male. The absence of information on exact carcass locations, search routes, and search efforts prevented further assessment of the comprehensiveness of carcass collection and of the spatial distribution of the carcasses.

**Saiga Population Surveys**

Surveys conducted in January, March, and May 2017 and April 2018 indicated a steep decline in direct saiga observations along transects, from 2,130 saiga in 328 groups in January 2017 to 369 saiga in 46 groups in April 2018, despite similar survey efforts (Table 2). Abundance estimates provided by the best model for each period (Appendix section 3.3) confirmed the saiga population decline from 25,699 (95% CI 19,249–34,310) in January 2017 to 8,806 (95% CI 6,095–12,721) by May 2017, the last month of reported saiga deaths. However, the saiga population continued declining after May 2017; the last survey in April 2018, almost a year after the outbreak, showed an estimated abundance of 5,142 (95% CI 2,929–9,028), 20% of the January 2017 population size (Figure 5). The average probability of detecting live animals in the surveyed area ranged from 0.38 to 0.59.

**Discussion**

Epidemiologic, pathology, and ancillary test findings in this PPR outbreak in Mongolia support the

![Figure 4. Spatiotemporal distribution of confirmed and unconfirmed cases of peste des petits ruminants (PPR) in several wild ungulate species in Mongolia. Each panel illustrates cases that occurred during the panel-specific time period (incident cases) and cases that occurred during previous periods (past cases). In the 2 periods before the first laboratory confirmation of PPR in saiga in December 2016, 2 clusters of unconfirmed cases (open shapes) were documented and matched the pattern of livestock PPR case distribution. In January 2017, the outbreak spread rapidly through the saiga population and expanded southward and northward from February 2017 through May 2017, when the last confirmed saiga cases were reported. Subsequent PPR cases involved ibex until at least January 2018. Maps at bottom show location of study area in Mongolia and specific location names.](image-url)
diagnosis of PPRV infection causing wild ungulate deaths across both desert steppe and mountain ecosystems. Saiga antelope were confirmed to be susceptible and capable of spreading PPRV infection within their population over a short time, suggesting high viral excretion loads and contact rates over the period of the epidemic. PPR in 3 other species of antelope in a semicaptive private collection in the United Arab Emirates has been previously reported (30), and our report indicates that free-ranging antelope exhibit the same level of susceptibility. The multiple clusters of ibex deaths suggest multiple spillovers from livestock followed by effective intraspecies transmission. The occurrence of apparently sporadic deaths among goitered gazelle and Argali sheep confirms the susceptibility of these species but raises questions about their ability to spread infection.

The pathologic lesions found in this outbreak were largely consistent with typically reported PPR lesions in domestic species (31) (Appendix section 1.2). The prominence of liver lesions and involvement of biliary epithelium were unusual, although they have been reported for other morbillivirus infections, including infection of wild lesser kudu (Tragelaphus imberbis) by the closely related rinderpest virus (32). Pathologic findings in the examined animals were not consistent with hemorrhagic septicemia caused by Pasteurella multocida, which caused large-scale saiga mortality in Kazakhstan (33,34). Diminished fat reserves and lymphoid depletion in PPR-positive and PPR-negative saiga was a nonspecific finding and could indicate the existence of environmental stressors, possibly explained by midwinter conditions. Further research is required to assess whether poor body condition and potential immunosuppression could contribute to virulent expression of PPRV in these animals. The presence of at least 2 pathogens of livestock in the examined saiga also suggested a high permeability of this livestock–wildlife interface to infectious diseases, which may have contributed to the overall mortality rate.

The 2 PPRV N-gene sequences obtained from infected saiga were similar to sequences obtained from livestock during outbreaks in Mongolia in 2016 and China in 2013–2016. This similarity is consistent with a spillover of PPRV from infected domesticated sheep and goats to wild ungulates. In addition, these sequences were genetically close to sequences from central Asia (i.e., Iran [GenBank accession no. KY550670] and Tajikistan [accession no. DQ840198]) but different from sequences from China in 2007 (accession nos. EU340363 and JF939201) (Figure 3), which suggests recent spread of PPRV from central Asia to China and then to livestock and wild ungulates in Mongolia.

PPRV outbreak mapping suggested that wildlife may have been infected earlier (possibly July 2016) than the first confirmed case (December 2016) and that wildlife infections closely followed the timing of the livestock outbreak. The absence of laboratory confirmation of PPRV infection for these initially unconfirmed clusters warrants cautious interpretation, but strong epidemiologic evidence indicates that these suspected cases were part of the same PPRV outbreak. The apparent spatial discontinuity between the 2 outbreak foci supports the hypothesis that the spread of PPR was mainly driven by livestock movement, because the wild mountain ungulates (ibex in the first putative outbreak focus) are relatively resident and unlikely to move long distances across multiple ecotypes. This spatial discontinuity also suggests multiple spillover events from livestock to different wildlife populations, which will require further analysis based on genetic data.
The early onset of PPRV in ibex and the lower and more prolonged incidence of cases in this species (at least until January 2018) are in contrast with the rapid transmission through the saiga population (apparently ceased by June 2017). This contrast in incidence suggests different dynamics of PPRV transmission in the 2 species, influenced by population structure, habitat, and interspecies–intraspecies interactions. Further work, including identification of shared resources between species (e.g., watering points, residual snow patches, and mineral licks), contact rates, and modeling should be conducted to better determine the most likely transmission routes and the respective roles of these wild and domestic ungulates in this multihost system. The probable 5-month delay between the first unconfirmed cases documented and the first confirmation in saiga underscores the value of maintaining operational wildlife health surveillance systems for early detection of wildlife illness and deaths.

The initial mortality estimates, obtained from carcass collection and disposal efforts, were probably underestimated because of imperfect detection (35–38) (Appendix section 3.3). If systematic carcass removal is determined to be cost-effective, adopting standard ecological monitoring methods to ensure reliable and unbiased characterization of mortality patterns is imperative (39). The most compelling estimate of the population-level effect of the PPRV outbreak in saiga was, therefore, derived from the population monitoring efforts by using distance sampling methods (which account for imperfect detection), indicating a saiga population decline of >80%. These estimates depict a serious situation for the Mongolian saiga population and a substantial setback after >10 years of conservation efforts to secure saiga population recovery after a historical low in the early 2000s (40,41). The significance of this event to saiga goes beyond the Mongolian subspecies because other unrelated mass mortality events have recently affected the species and are threatening its global conservation (33). Although saiga have shown great potential for recovery (42), in part because of fertility and frequently giving birth to twins or triplets (43,44), the population estimates a year after the outbreak showed little evidence of recovery. The timing of the outbreak just after rut season (which may have facilitated transmission because of congregation of animals) and during gestation probably delayed recovery through effects on recruitment. In addition, very cold temperatures with exceptionally heavy snowfall (known as dzud) also resulted in saiga deaths during winter 2018 and probably contributed to the additional population decline from June 2017 through April 2018 (Figure 5). We cannot exclude as potential causes for the sustained population decline the cumulative effects of multiple factors, other concurrent conditions, and undetected PPRV circulation. The lack of similarly detailed data for the other species of ungulates prevented assessment of the full conservation effect of the outbreak, but deaths across the ungulate community suggest broader effects on these ecosystems.

Factors that favored the eradication of rinderpest included an expectation that wildlife did not act as a reservoir of infection for domestic animals (8). This multispecies mass mortality event in Mongolia and recent similar events in eastern Asia and the Middle East (18) challenge the assumption that wildlife play a negligible role in the epidemiology and ecology of PPRV. This observation has substantial implications for the current global eradication program and efforts to outline National Strategic Plans for PPR control and eradication. The explicit integration of wildlife protection into these National Strategic Plans should be considered, and plans should include setting livestock vaccination targets that can effectively prevent spillover of virus from livestock to other susceptible wildlife (45).

The growing number of livestock on rangelands of low productivity, such as in Mongolia and much of central Asia (26,46,47), exerts increasing pressure on sympatric wild ungulates through competition for resources (48,49). Restricted access of wild ungulates to quality forage, water, and minerals may result in poor nutritional status and immune function (50), possibly reducing their resilience to livestock pathogens to which they are increasingly exposed.
Global changes in climate and expected shifts in species distributions and habitat suitability may further reduce resource availability and increase wildlife-livestock interactions. Evidence for possible dislocation of species-habitat-climate relationships leading to increased susceptibility to disease can be found in the mass mortality that occurred because of hemorrhagic septicemia in another subspecies of saiga (Saiga tatarica tatarica) in Kazakhstan (34). These combined factors could result in an increasing number of disease spillover events, followed by rapid amplification in populations already under multifactorial stresses. To ensure that objectives of rural development and biodiversity protection are compatible and jointly met, integration of livestock and wildlife management must be improved (Figure 1). Doing so proactively in the face of global climate change and increasing demands of a growing global population is a critical challenge of this century.

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Appendix

1. Emergency field mission

1.1 Interviews during field mission

During the field mission organized by Food and Agriculture Organization of the United Nations (FAO)/ World Organisation for Animal Health (OIE) / Crisis Management Center-Animal Health (CMC-AH) and the Mongolian government, meetings were held with local officials, veterinarians, herders, and local offices of international NGOs [Wildlife Conservation Society (WCS) and World Wide Fund for Nature (WWF)]. Reports of cases before the first official confirmation were collected from these sources.

1.2 Pathology

A rapid response to the wildlife mortality enabled preliminary pathologic description of PPR in saiga, goitered gazelle and ibex. Tissues were fixed in 10% neutral phosphate-buffered formalin for at least 24 hours and processed routinely for histologic examination. The number of organ tissues available for histology (from 13 saiga, 2 ibex, 2 goitered gazelle) varied from animal to animal based on ability to conduct a full or partial necropsy under field conditions. Tissues were pooled for two pairs of saiga and each pair was counted as one case in the histological result section. Some tissue types (e.g., oral/pharyngeal mucosa, lymph nodes) commonly included multiple samples per case, but were not specifically anatomically differentiated.

Gross necropsy observations were hindered by field conditions including post-mortem condition and inter-observer variation. The most significant repeated macroscopic observation
was mucosal (oral or nasal) reddening and erosion, which was corroborated by mucosal lesions identified in all of these cases histologically, though not all PPRV+ cases had histologically identified mucosal lesions in the available samples. The macroscopic observation of intestinal reddening correlated with necrosis in one of the cases (saiga) in which it was observed; the others were too autolyzed for reliable intestinal assessment (1 saiga, 1 goitered gazelle), or not examined (1 ibex). The majority of cases (4/6) in which red lung discoloration was observed macroscopically correlated with inflammatory lesions typical of aspiration pneumonia. In one saiga there were concurrent (milder) lesions of PPRV. The lung discoloration seen in the remaining saiga correlated with autolysis and freezing artifact, and that seen in the ibex was not available for histologic examination. Evidence of aspiration was also identified in lung of 2/4 saiga with no evidence of PPRV infection, and was considered a common sequella of illness or debilitation, not directly linked to PPRV status. An additional gross finding was enlarged or red discolored lymph nodes in body cavities in four PPRV-positive cases (3 saiga, 1 goitered gazelle). This correlated histologically with sinus histiocytosis and/or congestion in three cases and was not examined in one saiga. However, post-mortem carcass condition and emaciation may have complicated assessment of lymph node size and color. The finding was observed in both PPRV-positive and negative animals and was not consistent across those infected.

Histologically, the oral/pharyngeal mucosa in 4/7 examined saiga and 1/1 goitered gazelle was disrupted by mild to marked multifocal erosion to ulceration with epithelial degeneration and necrosis, variable inflammation (necrotizing stomatitis), rare syncytial cells in all cases, and intranuclear viral inclusion bodies in one saiga. The degree of inflammation correlated with surface bacterial colonization. The liver demonstrated acute, multifocal, random hepatocellular degeneration and necrosis with varying absent to mild acute inflammation (necrotizing hepatitis) (4/4 saiga and 2/2 goitered gazelle), as well as intranuclear and/or intracytoplasmic viral inclusion bodies and syncytia in one saiga and both goitered gazelle. In one of the saiga and both goitered gazelle, there was additionally mild to moderate necrosis of cholangiolar epithelium, with intranuclear and intracytoplasmic viral inclusions in the saiga and one goitered gazelle. Mild cholestasis, predominantly intracanalicular, was noted in association with the liver lesions in two affected saiga. Additional viral histologic lesions included mild to moderate bronchiolar epithelial necrosis multifocally in the lung (1/4 saiga and 1/2 goitered gazelle) and mild multifocal necrosis of crypt epithelium in the intestine (1/4 saiga and 0/2
goitered gazelle), with few syncytia and inclusion bodies (intracytoplasmic) at both sites. Post-mortem artifacts hindered intestinal evaluation some cases. Concurrent diseases in PPR-infected animals included one saiga with stomatitis typical of parapoxviral infection (contagious ecthyma) and one goitered gazelle with bacterial sepsis as characterized by mild foci of inflammation with intralesional bacteria in the heart and liver and intravascular bacteria in the kidney. Atrophy of adipose tissue was histologically confirmed in all cases in which adipose tissue was examined (3/3 PPRV-positive and 3/3 PPRV-negative animals). Lymphoid depletion in lymph nodes and/or spleen was identified histologically in 7/8 and 3/4 PPRV-positive and – negative samples, respectively. Among the animals without evidence of PPRV (4 saiga, 1 ibex), two saiga had histologic evidence of other infections (disseminated mycosis and small intestinal endoparasitism, respectively). The detailed observations for each animal are available online https://doi.org/10.6084/m9.figshare.7502258.v1

1.3 Laboratory diagnostic procedures

Samples sent to the State Central Veterinary Laboratory (SCVL) for PPRV confirmation were processed usinga gel-based reverse transcription polymerase chain reaction (RT-PCR), carried out according to Couacy-Hymann et al. (2002) (1) using Qiagen One-step RT-PCR kit (QIAGEN). Additional diagnostic procedures carried out on cases submitted to the State Central Veterinary Laboratory included antibody detection using ID Screen® PPR Competition (IDvet, Montpellier, France), antigen detection using ID Screen® PPR Antigen Capture (IDvet, Montpellier, France), antigen detection using Peste-Test® (The Pirbright Institute, UK).

Detailed results of these tests are available in the data file https://doi.org/10.6084/m9.figshare.7502264.v1

The combined results of histology examination and these additional diagnostic procedures provide a strong support for PPRV being the causative agent of the observed mortality.

1.4 Phylogenetic Analysis

On two of the saiga samples, sequencing of the C-terminus portion of the N-gene were performed by The Pirbright Institute, UK, following shipment of samples on dry ice. Tissue samples were homogenized and total RNA extracted following previously described methods (2). Viral RNA was reverse transcribed and amplified as previously described (3) using the
superscript III One-Step RT-PCR kit (Invitrogen, Carlsbad, USA). PCR amplicons were purified using the GE Healthcare Illustra GFXPCR purification kit (GE Healthcare, Pittsburgh, USA) according to the manufacturer’s instructions and sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, USA) on an ABI 3730 machine. Sequences were assembled and analyzed using SeqMan pro (DNASTar Lasergene 13.0).

The partial N-gene sequences of PPRV (n = 56) available in GenBank for South, Central, and East Asia through September 2018 were retrieved and used for constructing a neighborhood-joining phylogenetic tree, including the two partial N-gene sequences obtained from Mongolian saiga in this study. Alignments of the N-gene sequences were made using the Clustal W program and used for construction of distance matrices using the Kimura 2-parameter nucleotide substitution model (4) as implemented in the programme MEGA 6.0 (5). A maximum-likelihood phylogenetic tree was generated using MEGA 6.0, and the robustness of tree topology was assessed using 1,000 bootstrap replicates.

2. Government carcass disposal

Following the beginning of the outbreak in saiga, the Inter-Ministerial Working Group on Saiga Mass Mortality was created and included the Ministry of Nature Environment and Tourism (MNET), the Ministry of Food, Agriculture and Light Industry (MoFALI), the National Emergency Management Agency (NEMA), the General Agency of Specialized Inspection (GASI), SCVL, and representatives of academic institutions, non-governmental conservation organizations, and other international organizations. This working group initiated the implementation of an emergency response together with provincial-level counterparts of these agencies in the Khovd and Gobi-Altai provinces, and proceeded to restrict livestock movements, and collect and destroy saiga carcasses, between January 8 and February 28, 2017. This effort was supported by local veterinarians, citizens, non-governmental organization staff, provincial government officials, and other international experts.
3. Saiga Population surveys

3.1 Data preparation

Despite efforts to standardize data collection, deviations from the protocol were reported by field biologists, or identified following initial data inspection. These broadly fall under three categories: 1) observations made outside of transects, 2) observations made after spending an excessive amount of time in one location along the transect, and 3) duplicate observations of the same individual or group. To address these issues in the data we excluded observations that: 1) did not align on transects, effectively resulting in an initial truncation of observations made over 2 km away from any transect line, 2) were made after 10 minutes from the same location along the transect, and 3) were less than 500 m distance from a group of the same size (+/-4 individuals) based on exact group location (calculated using the bearing and distance from the point of observation; R code available upon request for procedure 2 and 3).

The May 2017 survey was particularly problematic, with significant missing data due to a gap in scientific supervision of field staff. These missing data seemed to cluster on particular transects, and when this was the case, the entire transect was excluded from the data (thereby reducing the overall survey effort for this time point).

3.2 Data analysis

Distance sampling data were analyzed using the software Distance v7.2 (6). Data were right truncated to improve the fit of the detection function model. Different key functions and adjustment term combinations were considered to model the detection function (half-normal with cosine or hermite polynomial adjustment terms or either hazard rate or uniform key functions with cosine or simple polynomial adjustment terms). Various statistics (Kolmogorov-Smirnov, Cramer-von Mises, and to a lesser extent chi-squared, as it does not prioritize the fit of the detection function close to the transect line, which is the most important for unbiased estimation of detectability) and graphical diagnostics (QQ-plot and the plot of the detection function versus the actual data) were used to measure goodness-of-fit of the model. Akaike’s Information Criteria (AIC) (Akaike, 1973) was used in the selection of the detection function for those models with adequate fit at distances near zero (7). Models were fitted separately for each survey and were stratified by the three regions within the home range (Durgun Steppe, Khuisiin Gobi and Sharga Gobi) when sample size was sufficient. In addition, to avoid positively biased
estimates of group size (that can arise if smaller groups further from the transect line tend to be missed), we fitted a regression of the natural logarithm of group size against the probability of detection at distance x from the transect line within each survey region. If the regression was statistically significant at the 15% level, then the expected group size based on the regression was used, otherwise average group size was used to estimate population density and abundance. Results included in this manuscript were obtained using the data cleaning process described above. For the purpose of a sensitivity analysis, results obtained with raw data are presented below in the Appendix Table.

3.3 Population survey results and discussion

Data was right truncated at 1 km, except for May 2017 where 5% of the data were truncated (~700 m). For the first two surveys, detection functions were fitted to stratified data. Almost all the final detection function models were half-normal with cosine adjustment terms. The exception was a uniform with cosine adjustment terms for Sharga for March 2017 and a half-normal with no adjustment terms for April 2018. With the exception of the April 2018 survey, there were indications of size bias in average group size estimation for all strata and for each of the other surveys. Thus, expected group size was used to estimate individual density and abundance for all surveys, except for April 2018. For the purpose of a sensitivity analysis, results obtained with raw data are presented below in the Appendix Table.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time (days) since first PPR confirmation</th>
<th>Total effort (km)</th>
<th>Individual (group) counts</th>
<th>Density of Individuals [95% CI]</th>
<th>Abundance [95% CI]</th>
<th>Expected cluster size [95% CI]</th>
<th>Average Detection probability Live saiga [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 2017</td>
<td>30</td>
<td>1,505</td>
<td>2,366 (373)</td>
<td>1.33 [1.01, 1.75]</td>
<td>28,890</td>
<td>D: 4.0 [3.3, 4.7] K: 3.5 [3.0, 4.2] S: 6.3 [4.8, 8.2]</td>
<td>D: 0.35 [0.29, 0.42] K: 0.35 [0.30, 0.40] S: 0.42 [0.35, 0.49]</td>
</tr>
<tr>
<td>March 2017</td>
<td>90</td>
<td>1,505</td>
<td>2,310 (174)</td>
<td>0.88 [0.54, 1.45]</td>
<td>19,188</td>
<td>D: 10.9 [7.9, 14.9] K: 11.7 [8.7, 15.8] S: 5.5 [4.3, 6.9]</td>
<td>D: 0.42 [0.35, 0.50] K: 0.73 [0.61, 0.88] S: 0.58 [0.50, 0.68]</td>
</tr>
<tr>
<td>May 2017</td>
<td>150</td>
<td>1,263</td>
<td>749 (159)</td>
<td>0.40 [0.28, 0.58]</td>
<td>8,772</td>
<td>D: 2.6 [2.1, 3.3] K: 2.6 [2.1, 3.2] S: 2.9 [1.6, 3.3]</td>
<td>0.51 [0.46, 0.57]</td>
</tr>
<tr>
<td>April 2018</td>
<td>480</td>
<td>1,505</td>
<td>369 (46)</td>
<td>0.24 [0.14, 0.42]</td>
<td>5,142</td>
<td>D: 11.7 [7.5, 18.1] K: 10.9 [6.1, 19.6] S: 6.3 [4.4, 8.9]</td>
<td>0.51 [0.40, 0.65]</td>
</tr>
</tbody>
</table>

b. D: Durgun Steppe, K: Khuisiin Gobi, S: Sharga Gobi

As expected, estimates were higher using the raw datasets, particularly for the January 2017 survey, however the total population decline was consistently estimated at 82%.
Despite all efforts deployed in the field and the best intentions of field personnel, only a portion of the home range of a species can be searched, and only a fraction of animals will be detected in this searched area due to the inevitable issue of imperfect detection (7–11). In the distance sampling analysis, the estimated detection probabilities for live animals ranged from 0.35 to 0.69. Assuming detection probabilities are similar for carcasses (although likely lower), this would suggest that the 5,425 animals collected and destroyed only represented about half of the carcasses present in the searched area, which in turn represents just a fraction of the actual outbreak area. This explains the gap observed between carcass count and total mortality estimated from population monitoring, which highlights the importance of using robust population estimation methods in the assessment of infectious disease impact (12).

4. References


