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Supplementary Materials for

Saigas on the brink: Multidisciplinary analysis of the factors influencing mass mortality events

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This PDF file includes:

increased virulence and invasion of a commensal parasite such as *P. multocida*, subsequent septicemia in infected hosts, and/or enhanced transmission.

- table S5. Comparison of means for cases and controls using the full data set (all MME years); real dates of onset or 9 May; and climate metrics aggregated over the 10 days to onset.
- table S6. Long-term climate anomaly data at Kostanai sites (ERA data using actual die-off site, NCEP data using pixel, covering entire Torgai area).

Supplementary Text

Disease in saigas and links to life history

Saigas stand out for the scale and frequency of die-offs reported, including in 1951, 1974, 1981, 1984, 1988, 2010, 2011, 2012, when thousands to hundreds of thousands of animals died. But details are often sparse and the term MME cannot be applied in all cases and co-factors such as weather, which is highly variable in this region, are rarely mentioned except where heavy snow fall or dzhut has occurred. Dzhut is where ice forms over the snow restricting access to vegetation causing starvation and reported historically as the major cause MMEs in saigas. It is only since 1974 that MMEs, as defined by Fey and others, were reported to be due to pasteurellosis of one type or another. Of these, the five listed in table S2 led to the death of over 10,000 animals. On one occasion, in 1988, this was also associated with a dzhut.

An important question is whether earlier saiga mortalities involved pasteurellosis, but historical records provide no evidence for this. It seems unlikely that such events would have escaped the attention of Soviet scientists, given that similar mortalities in Mongolian gazelles were described in 1871. In the case of saiga MMEs, diagnosis of pasteurellosis was generally made on the basis of bacteriological isolates from probes from dead animals, with a lack of descriptive pathology, either gross or histological, to match to the isolated pathogen and little attention to other factors that might have been important in these outbreaks. Most probably this was a result of the remoteness of these central Asian populations from human centers and late interventions, a lack of fresh material, and no ecological and epidemiological data collected due to a narrow diagnostic approach.

Saigas are seasonal long distance migrants, with the extent of migration ranging up to 1500 km each way in certain populations such as that of Betpak-Dala in Central Kazakhstan . Their migratory nature means they are able to benefit from the periodic peak in food availability and quality and thereby reach population sizes which are orders of magnitude greater than those of resident ungulate species. On the other hand, both food and climatic conditions encountered within a certain seasonal range may have consequences that carry over to their next seasonal range and affect crucial life cycle processes such as birth, lactation, and survival and future reproduction. Such carry-over effects may render animals vulnerable to extreme and unusual weather events, as well as disease, during stressful periods.

With similar body size and diet, saigas are sympatric to domestic sheep and goats and may share pastures even if they rarely come into direct contact. This means saigas are also vulnerable to disease transmission from livestock through pasture and water sources. Other potentially ecologically significant diseases that have affected saigas in the past include Foot and Mouth Disease, Peste des Petits Ruminants; and potentially also anthrax and *Erysipelothrix*.

Description of the outbreak

The team which observed the outbreak was part of an international collaborative effort running from 2012 to date under the authority of the government of Kazakhstan, undertaking annual monitoring of saiga population health at calving. This represented a unique opportunity as rarely have such events, which occur periodically but rarely at this scale, been scientifically studied in depth. Research of this nature is highly challenging, in such vast and

remote terrain and with such an elusive species. In 2014 the team had witnessed a successful calving season, an apparently healthy, growing population, since its decline to low thousands from poaching at the end of the 20th Century.

The females of the over 240,000 strong Betpak-Dala population aggregated for calving between 48 and 51 degrees northern latitude and 61 and 69 degrees eastern longitude in around 15 distinct clusters in early May 2015 with time of first calving varying by some days depending on the location (Fig. 1). The Government authorities carry out aerial census prior to this period to ascertain the numbers, which provides some indications on developing concentrations, and supports protection and monitoring of the calving process later on.

A collaborative research team, including scientific staff from the Research Institute of Biological Safety Problems (RIBSP) of the Ministry of Education and Science in Gvardeyskiy, wildlife rangers of the Committee of Forestry and Wildlife of Ministry of Agriculture, biologists and veterinarians from the Association for the Conservation of Biodiversity of Kazakhstan (ACBK) and Royal Veterinary College undertook annual biological and veterinary monitoring of saiga in Kazakhstan from 2012 onwards. The first information about the saiga deaths in 2015 came from the Torgai area (Zholoba area near former Kaynar village of Amangeldy district of Kostanai oblast) on May 10, 2015, where about 62,000 saiga were concentrated for calving. The team located the saiga herd and took observations. At the calving area, saiga carcasses were found which had been dead for 1-2 days, suggesting mortality had commenced on or around May 9th, 2015. On May 11th 100 carcasses were recorded; on May 12th up to 400; on May 13th more than 1000; between 13th and 16th another 20,000 were estimated dead; and by the 19th there were no surviving saigas in this location. The morbidity and mortality was estimated at 100% although it is not impossible that a few animals moved out of the immediate area. A second research team was then formed with other scientists joining to observe another calving aggregation of 8,000 saiga in Ortakara area near Lake Tengiz, Akmola oblast (Fig. 1). The first carcasses were observed by rangers on 20th May and had been dead for 1-2 days. Many hundreds of sick and dead animals were observed on 23rd May when the scientific team arrived (fig. S2). The last cases of death in the area were observed on May 25, 2015. No survivors were observed and similar morbidities and mortalities estimated compared to the Torgai site. The areas of the die-offs as part of the wider MME were approximately 26 km^2 in Tengiz and 81 km^2 in Torgai (Fig. 1).

Gross pathology

A total of 33 dead saigas (9 calves, of which 4 were male and 5 female, and 24 adult females), from the two studied sites, underwent thorough post mortem examination by AK, SW (Torgai), RK and MO (Tengiz). Samples were taken from dead and moribund animals according to an agreed method. The gross pathology was mostly consistent in both groups of animals. External parasites were not observed, except a single engorged tick on one carcass. The winter coat was mostly shed with a few tufts remaining on some animals. Animals were generally in very good body condition, with 76% having abundant mesenteric/omental fat (fig. S2d) which in small ruminants correlates significantly with live weight and body condition . In the ten adult animals necropsied immediately after death, there were generalized hemorrhages, ecchymoses and petechiae, in the subcutis (fig S2e), on multiple serosal surfaces, extensively within the sub-endocardium of the heart (fig. S2f), and within lungs, lymph nodes (S2g) and skeletal musculature. All organs were diffusely congested, and there was blood stained fluid in serous cavities (fig. S2e). Multifocally, the pharyngeal and

oesophageal mucosae were replaced by diptheritic membranes in some cases. The trachea and bronchi were filled with frothy fluid, and the lungs were markedly congested and edematous (fig S2h). Livers were enlarged and friable and gall bladders were prominent. The rumen was full and there was an accumulation of fluid in the intestines. Many cases showed catarrh, congestion and reddening of the mucosae, with multifocal subserosal hemorrhages and congested vasculature (fig S2i). Diffusely, lymph nodes were edematous and hemorrhagic, and this finding was less marked in the thoracic and peripheral lymph nodes when compared to the mesenteric and omental lymph nodes. The spleen was of normal size and shape with occasional serosal hemorrhages.

In calves, hemorrhages were restricted to the heart and lungs, and additional findings included wetting of the fur around the muzzle from excess salivation, and fluid gastrointestinal contents. Eight of the calves had clotted milk in the stomach indicating they had recently suckled, suggesting that starvation was not the cause of death in these cases.

Histopathology

Microscopic descriptions for 18 of 33 necropsies are as follows:

Block Number	**Morphological diagnosis**
F3457/1, Adult	Spleen: multifocal, marginal zone neutrophils with intravascular gram negative bacterial emboli Liver: random, focal necrosis with intralesional bacteria and neutrophilic infiltrate.
F3457/2, Adult	Spleen: multifocal, intravascular, gram negative bacterial emboli Liver: multifocal, random, single cell necrosis Lung: diffuse congestion and multifocal atelectasis Kidney, heart: multifocal congestion
F3457/3, Adult	Spleen: multifocal, marginal zone neutrophils with intravascular gram negative bacterial emboli Liver: multifocal, random, single cell necrosis
F3457/4, adult	Spleen: marginal zone neutrophils Liver: multifocal, random necrosis with intralesional gram negative bacteria
F3457/5, adult	Spleen: marginal zone neutrophils with intravascular gram-

	negative bacterial emboli Liver: random, single cell necrosis. Lung: perivascular edema and hemorrhage and multifocal atelectasis
F3457/6, adult	Spleen: multifocal necrosis with intralesional and intravascular gram negative bacterial emboli Liver: multifocal, random necrosis with intralesional gram negative bacteria Lung: perivascular hemorrhage and multifocal atelectasis
F3457/7, adult	Spleen: multifocal necrosis with intralesional gram negative bacteria Liver: multifocal, random necrosis with intralesional bacteria Lung: bacterial emboli in alveolar capillaries
F3457/8,adult	Spleen: multifocal marginal zone neutrophils with intravascular gram negative bacteria emboli Liver: multifocal, random, single cell necrosis
F3457/9,adult	Spleen: multifocal marginal zone neutrophils
F3457/10,adult	Spleen: multifocal marginal zone neutrophils
F3457/11, adult	Spleen: multifocal necrosis Liver: multifocal, random necrosis and sinusoidal neutrophilia. Lymph node: subcapsular, cortical and medullary hemorrhage with multifocal necrosis Lung: emphysema

F3457/12, adult	Spleen: diffuse follicular hyperplasia Lymph node: necrotising lymphadenitis and steatitis with intralesional gram negative bacteria Lung: multifocal intra-alveolar hemorrhage
F3457/13, juv	Liver: multifocal, random necrosis
F3457/14,juv	Lung: intrabronchiolar hemorrhage and multifocal atelectasis
F3457/15,juv	Liver: multifocal, random, necrosis and sinusoidal neutrophilia
F3457/16,juv	Liver: multifocal, random necrosis
F3457/17,juv	Spleen: marginal zone neutrophils and multifocal necrosis Liver: multifocal, random necrosis, with intralesional gram negative bacteria and sinusoidal neutrophilia Lymph node: follicular hyperplasia Lung: diffuse congestion
F3457/18,juv	Spleen: diffuse congestion, follicular atrophy Liver: multifocal, random, necrosis with sinusoidal neutrophilia Lymph node: lymphoid depletion and sinus histiocytosis Lung: diffuse congestion and multifocal atelectasis

Bacteriology

At the RIBSP, PCR was negative for *Coxiella burnetti* (causative agent of Q Fever), *Mycoplasma ovipneumoniae*, *Erysipelothrix rhusiopathiae* and *Listeria*. In the samples of pathological material from 30 out of 32 saigas, DNA of *Pasteurella multocida* type B was detected; and in samples from 10 out of 24 saigas, alpha-toxin of *C. perfringens* was detected.

Gram-negative cocci-ovoid bacteria consistent with *Pasteurella* were cultured from biological material and from milk of the sick and dead saigas. The isolated bacterial cultures of *Pasteurella* produced indole, did not grow in bile and MacConkey's medium, did not produce the urease enzyme, methyl red and Voges-Proskauer tests were negative, and they did not liquefy gelatin, nor coagulate milk. TLVro- and micromorphological characteristics of the bacterial isolates, cultural signs of growth in liquid and solid media, biochemical, saccharolytic and tinctorial features confirmed the bacteria as *Pasteurella multocida*. PCR with specific primers confirmed *Pasteurella multocida* type B.

At the Republican State enterprise "National Veterinary Reference Centre" of the Ministry of Agriculture, samples from 5 animals from Kostanai, Aktobe and Akmola oblasts were taken and isolates of *Pasteurella* were confirmed; in addition, anthrax, paratuberculosis, campylobacteriosis, listeriosis and brucellosis tests were negative.

At the Federal Governmental Budgetary Institution "Federal Centre for Animal Health" in Russia, samples from 4 animals were confirmed positive for *P. multocida* serotype B. At the regional reference laboratory at Kostanay samples tested positive for *Pasteurella*.

At the Pirbright Institute *Pasteurella multocida* sequences were isolated from all the 12 samples along with other bacterial commensals and pathogens including rickettsia.

At the Friedrich Loeffler Institute, a random subset of 4 FTA card dried blood samples out of 11 submitted were analyzed by NGS and RNA sequence reads. Approximately 80% of the reads had high similarity to *Pasteurella multocida* sequences.

Bacterial visualization in tissue sections

Formalin fixed tissue samples from three individuals were tested using Fluorescence In Situ Hybridization (FISH). Both *P. multocida* and *Clostridium* were found present in all samples (fig. S3). *Pasteurella multocida* was found mostly in the liver, consistently but generally at lower abundance in intestine and lung, and occasionally in myocardium, kidney and spleen. Results are consistent with systemic invasion of *P. multocida*, with subsequent hematogenous spread, with other opportunistic bacteria such as *C. perfringens* invading only later and post-mortem.

Mineral analysis

Mineral deficiencies can cause pathological symptoms in wildlife . The individual and mean values for liver tissue minerals from three adult saiga sampled at necropsy based on ppm wet weight are shown in table S3. The mean values when compared to domestic ruminants, antelope, deer, and wild mountain caprines were all above the range of deficiency and likely to be adequate for normal metabolism. Marginal copper was found in two livers and deficient zinc in two livers, based on sheep values. This general level of variation and range has been reported previously for saigas which died apparently from natural mortality but the true normal range for all minerals is unknown in this species. The Cu:Mo ratios, which are sometimes associated with deficiencies, were in the normal range for ruminants. From these results, further study of the copper and zinc levels in saigas is a priority to establish the true population range and its variation with season, age, sex and condition.

Virology

At the RIBSP, PCR results for FMD, PPR, sheep pox, akabane virus, Aujesky's disease, viral diarrhea, Visna-Maedi and MCF were negative in all 32 cases, except for one case with a positive result for sheep pox virus. 96 samples inoculated on LKC and Vero cells did not show CPEs within three blind passages. The control flasks did not show any CPEs and the monolayers remained intact during the observation period. At electron microscopic examination in samples from saiga virus particles were not found.

33 samples of pathological material from 10 saigas taken in Akmola, Kostanai and Aktobe oblasts were sent by the National Veterinary Reference Centre (NVRC) to the Institute of Microbiology and Virology of the Ministry of Education and Science for virology, with the only positive result a virus-specific RNA belonging to the Flaviviridae. In the Reference laboratory in Russia, 4 samples from saiga from the Kostanai, Akmole and Aktobe oblasts tested for FMD, bluetongue, sheep pox and PPR were negative. At the FLI in Germany none of the obtained sequence reads from the FTA analysis showed significant similarity to known viral sequences in the INSDC databases. At the Pirbright Institute in the UK all samples of FTA card dried blood were negative for the tested viruses.

Hypotheses for testing

A set of hypotheses for co-factors, together with the rationale from the literature and evidence for and against the hypothesis is shown in table S4. The evidence is most consistent with a climatic trigger for pathogen invasion (hypothesis 1), perhaps potentiated by nutritional or physiological stress during calving and lactation (hypothesis 3). Evidence for a primary nutritional driver (hypothesis 3), microbial co-infection or toxicosis (hypotheses 2 and 4), or enhanced transmission of *P. multocida* between saigas (hypothesis 5) is weak. All hypotheses, however, require further investigation in order conclusively to confirm or deny them.

Detailed modelling results

NDVI patterns were analyzed using two separate datasets, covering the years 1998-2016 and 1982-1997 (table S4). NDVI was unusually low in the 3rd week of April 2015 compared to controls in that sub-dataset, due to a late spring. The 2015 MODIS spring snow anomaly was also negative and large for the same reason. However, the NDVI anomaly was not large over all case sites and had disappeared by the 4th week of April; the index was *higher* at 1988 die-off sites than controls (although the GIMMs data covering this earlier part of the dataset are less reliable than the PROBA-V or SPOT-VEGETATION dataset).

For these reasons, and because these variables were not available for the entire dataset, they were not selected for multivariate analysis. But the possible impact of long winters was represented in the ERA-generated snow cover days variable (number of days with snow cover from 1st January), which covered the whole time series. Other variables not selected for further analysis included metrics of maximum or mean temperature and metrics of temperature variability, which if anything appeared *lower* at cases than controls, although it was expected that higher temperature variability may make animals more susceptible to infection.

Overall, in univariate analysis, metrics of minimum temperature, relative humidity and dew point temperature showed the strongest positive relationships with the outcome variable.

Snow cover days, total precipitation, days with precipitation, soil moisture and wind gust variables looked like possible candidates when plotted, although they did not emerge as significant in Bayesian tests including the effect of year (fig. S4; table S5). These eight variables (which with the exception of wind gust were positively associated with die-offs) were included in a principal components analysis (PCA). Results indicate that the first dimension, capturing 46% of the variation, encapsulates predominantly humidity and precipitation variables (fig. S5). The second dimension, with 20%, includes principally dew point temperature, humidity and minimum temperature up to die-off.

Of the Bayesian multivariate models, the strongest emerged for the 10 day aggregates using estimated real onset dates where available and 9th May for other cases, including both models using natural climate variables and those using PCA dimensions. A model using standard 9th May onset dates for all sites was also constructed using natural climate variables, but the PCA dimension models using this dataset were not significant.

Most of the variables made no contribution to the model, having small coefficient estimates and confidence intervals crossing zero. This confirmed the findings of univariate analysis. The model was quickly reduced to the variables temperature, dew point temperature and relative humidity (WAIC 10.35), to which temperature made the smallest contribution. Its removal resulted in a model with a higher WAIC (12.7), probably as the two remaining variables are so closely related, whilst models with temperature and dew point temperature alone had insignificant coefficients. The combination of humidity and temperature in the model resulted in the lowest WAIC of any model (9.56), full convergence and largest coefficient estimates. The inclusion of latitude does not improve this model, as this variable is probably accounted for by the climate variables themselves.

This final model using 'real' climate variables contained only temperature and humidity metrics aggregated over the 10 days to onset (with intercept of -27.31, SE=11.33; β scaled mean of daily maximum humidity = 5.21, SE=2.3; β scaled average daily minimum temperature = 3.42; SE=1.5). The estimate for the grouping effects (31 levels) was 13.06 (SE=11.33). These coefficients, which are in logits, are extremely large and demonstrate threshold effects, which are particularly strong in the case of humidity (Fig. 4a, main text). The effect of minimum temperature is less clear, with 2015 appearing warm, 1988 average and 1981 cold (Fig. 4b, main text). But relative humidity is strongly related to temperature, as a warmer atmosphere can hold more moisture, thus the extremely high relative humidity at low temperatures observed in 1981 corresponds to a far lower *absolute* atmospheric moisture content than that of 2015, which exhibited both high humidity and high temperatures. Despite this relationship, interaction effects between humidity and temperature were not significant. It is possible that the extent to which the atmosphere is saturated with moisture is as important as absolute moisture content, although for the moment a biological basis on which to link this to pathogenesis is unknown.

The model using PCA dimensions one and two (β dimension 1 = 3.59, SE=1.66; β dimension 2 =4.61, SE=1.56) predominantly represents combinations of humidity, precipitation, dewpoint temperature and minimum temperature. Wind gust and snow cover period contributed little. Results of the anomaly analysis are discussed in the main body and summarized in table S6.

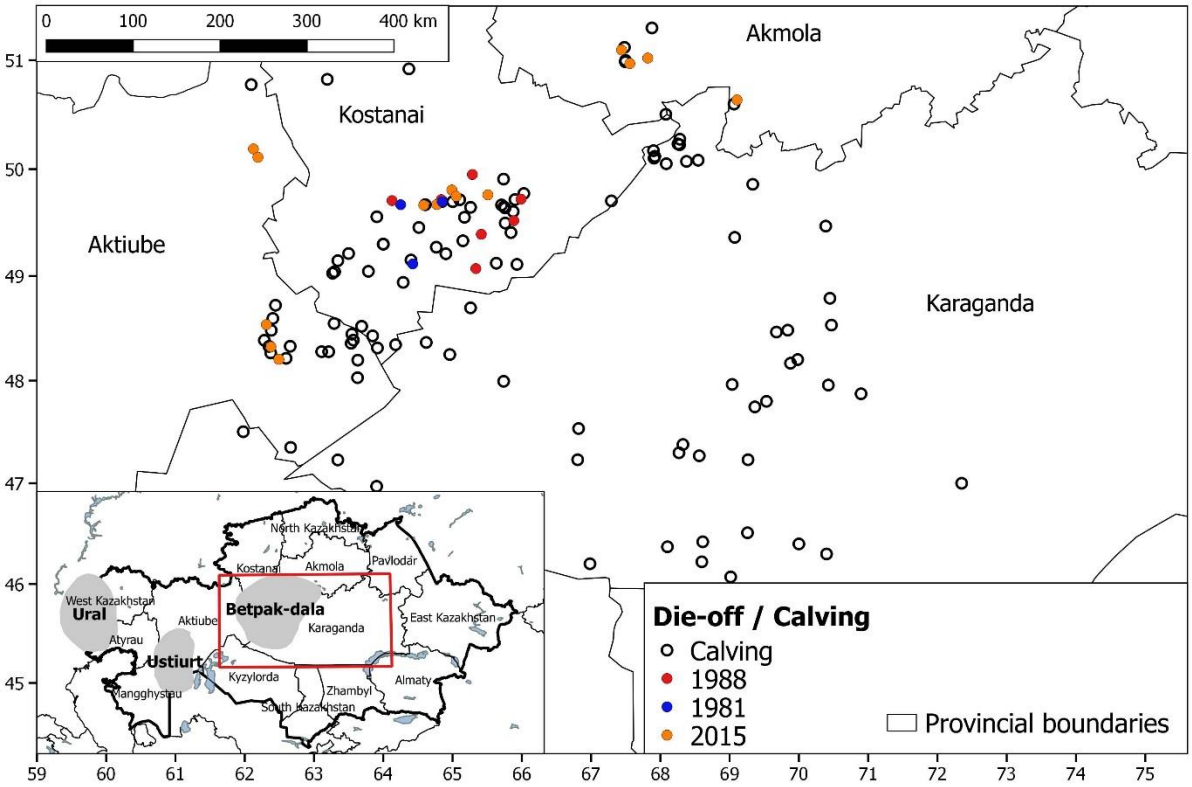


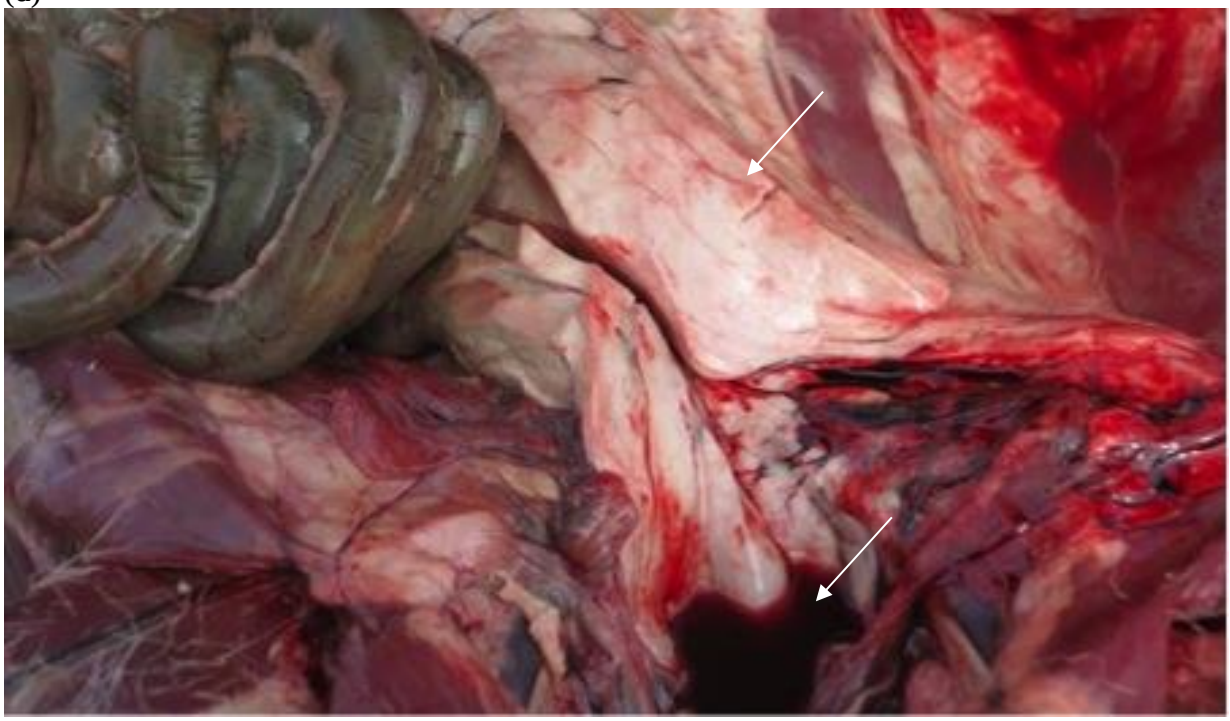
fig. S1. Locations of the three *P. multocida*–associated disease events (1981, 1988, and 2015) in the Betpak-dala population and control sites at which calving proceeded without a die-off since 1979, used in the climate analysis (*x* and *y* units are longitude and latitude).

(a)

(b)

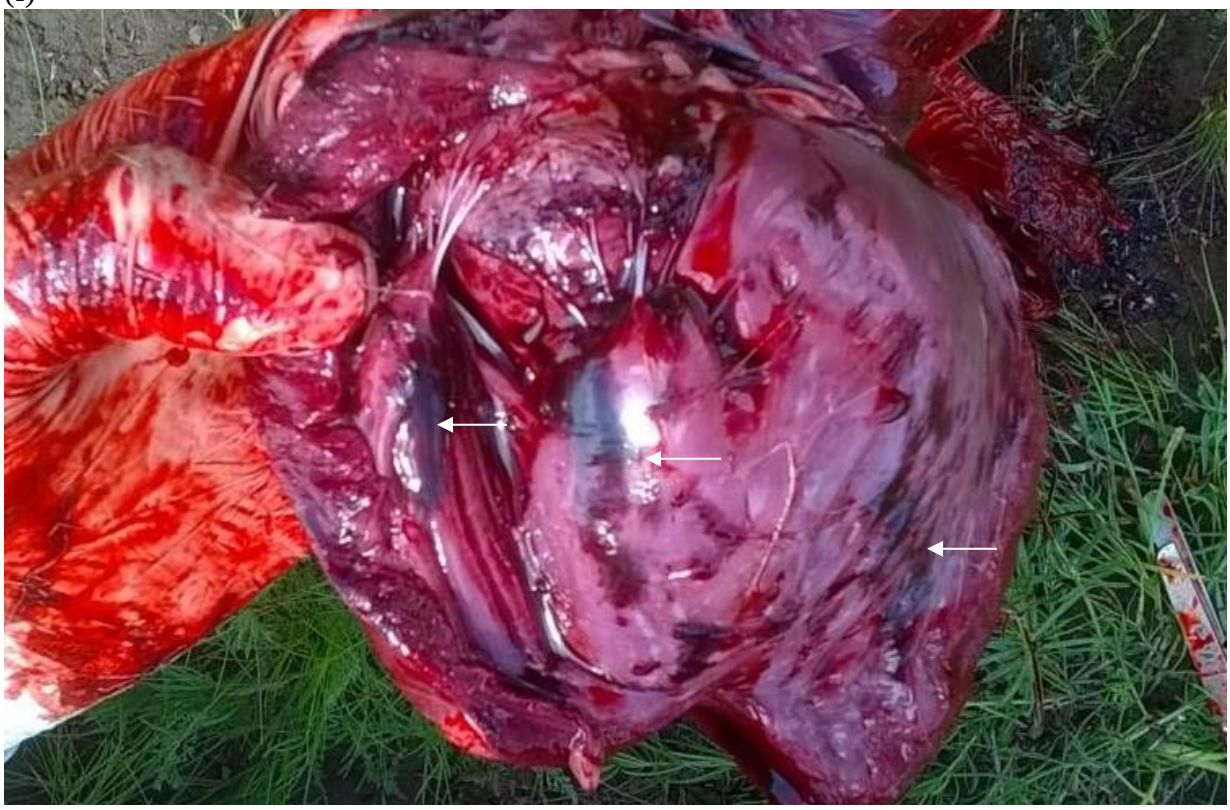
(c)

(d)

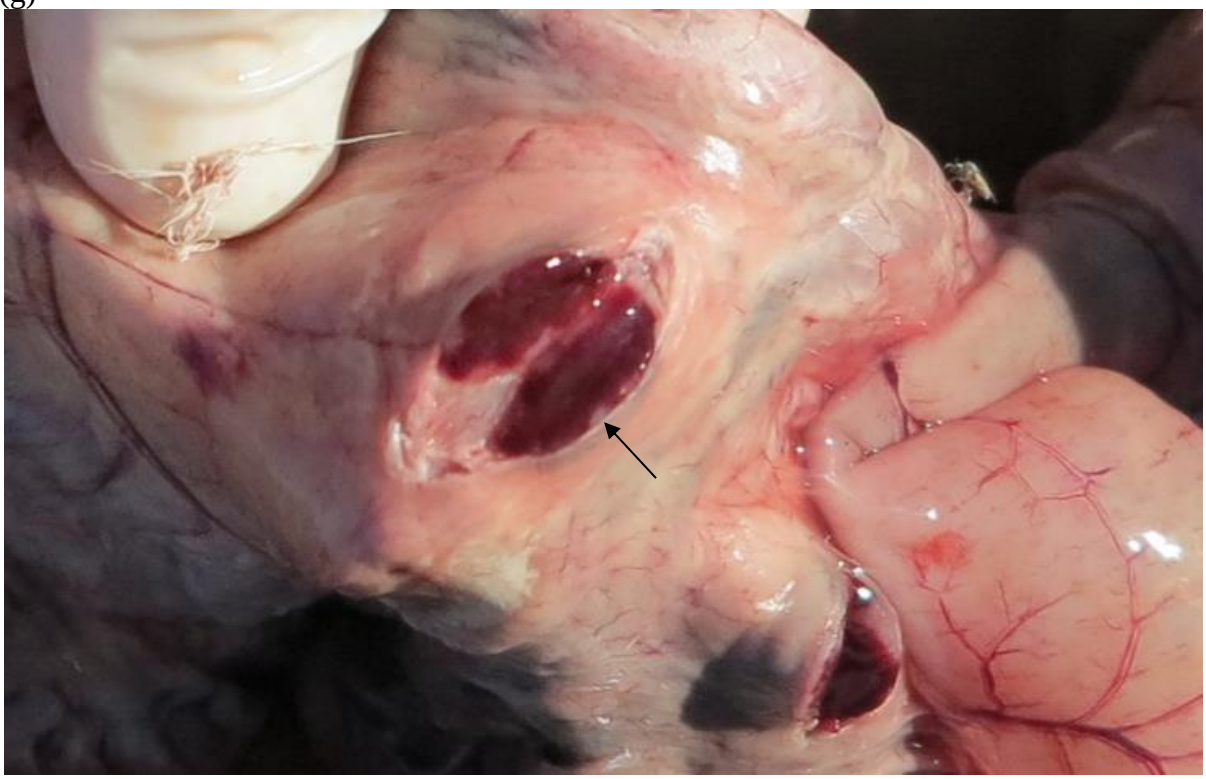


(e)

(f)

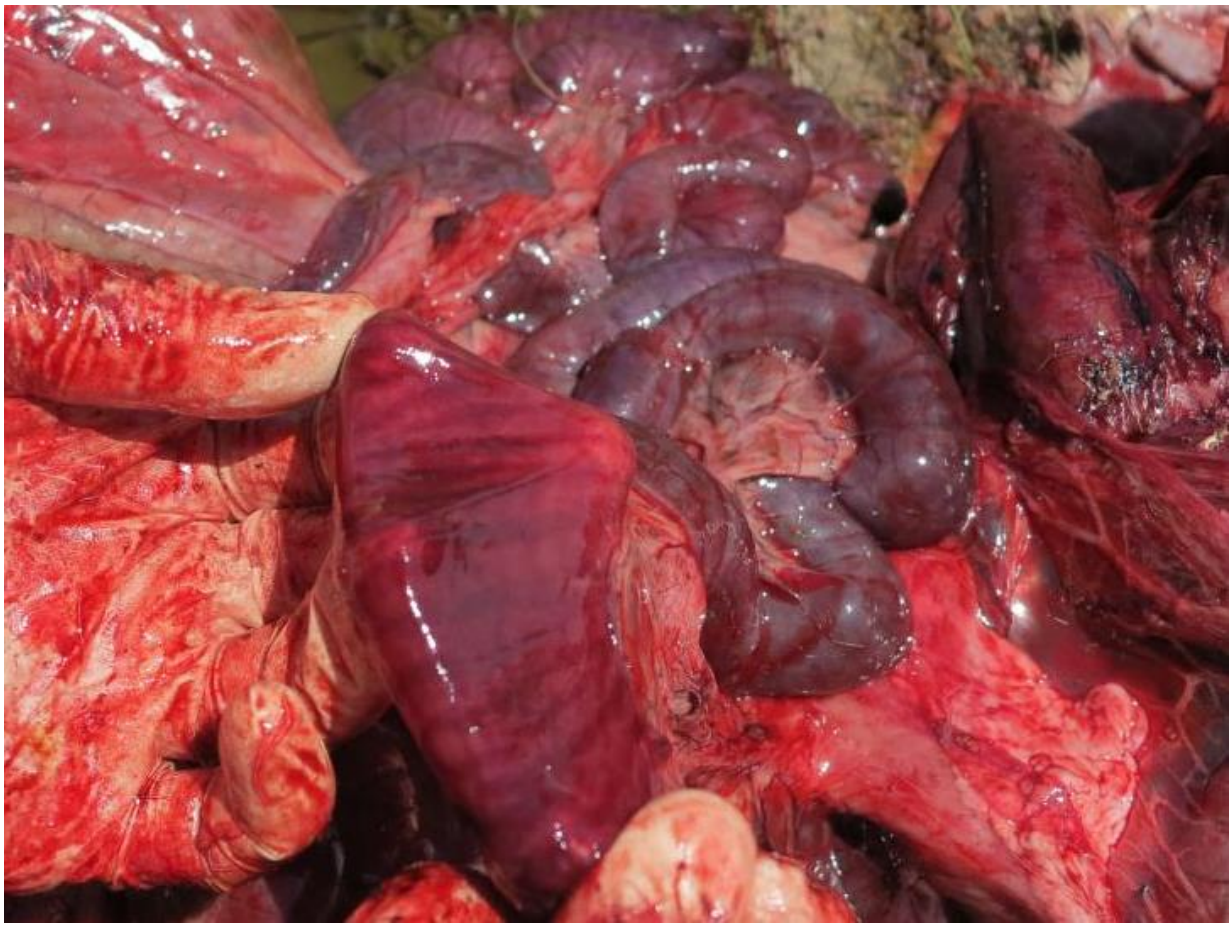


(g)



(h)

(i)



(j)

fig. S2. Photographs depicting the die-off of saiga and their clinical signs and pathology. (**A**) Saiga die-off in Torgai region, central Kazakhstan, in May 2015 with lone calf, which

died some hours later. Showing the even spacing of carcasses suggestive of animals falling where they were grazing. (**B**) Dead female saiga showing terminal diarrhea. (**C**) Saiga calf suckling from a dead mother. It is proposed the calves were infected through this route in the absence of commensal bacteria in their upper respiratory tract or gut in the first couple of days of life. They died hours after their mothers in observed cases. (**D**) Mesenteric/omental fat showing that the necropsied animal was in good condition and blood stained fluid in the serous cavity (white arrows). (**E**) Subcutis, ecchymoses and petechiae, characteristic of the septicemia caused by *Pasteurella multocida*. (**F**) Heart, sub-endocardial hemorrhages. (**G**) Mesenteric lymph node, congested and edematous (**H**) Lung, congestion and edema (**I**) catarrh, congestion and intense reddening of the mucosae of the intestine (**J**) Photograph of the 1988 die-off, for comparison with (A), showing identical distribution and posture of carcasses. Photo taken by the Institute of Zoology, Kazakhstan.

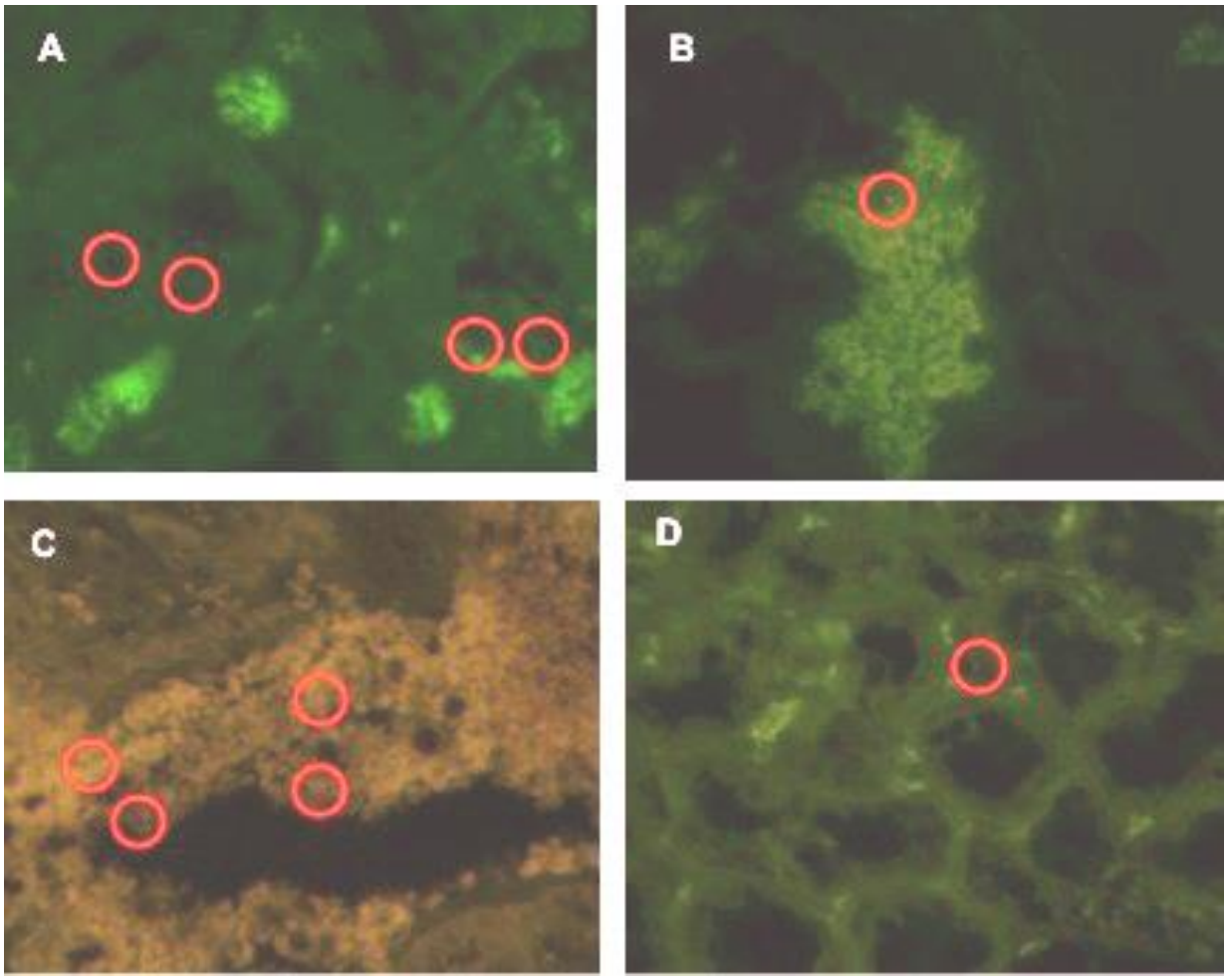


fig. S3. Fluorescence in situ hydridization (FISH) photomicrographs showing position of fluorescing *P. multocida* bacteria. *Pasteurella multocida* (red, circled) within (**A**) intestinal mucosa, (**B**) intestinal blood vessel, (**C**) liver and (**D**) lung.

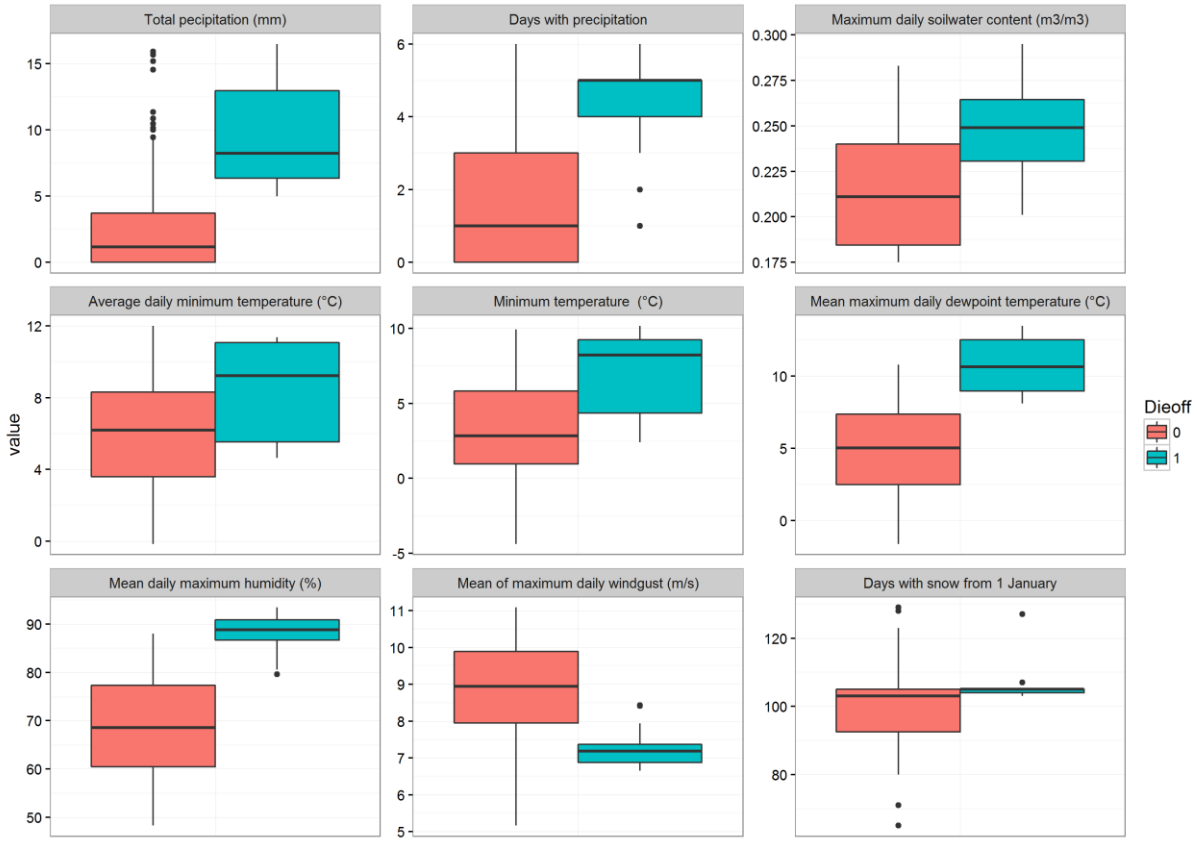


fig. S4. Box plots for cases (die-off sites) and controls (no die-off) using the full data set, with real dates of onset if available or 9 May if not, showing the relationship between cases/controls and selected climate metrics aggregated over 10-day periods before onset.

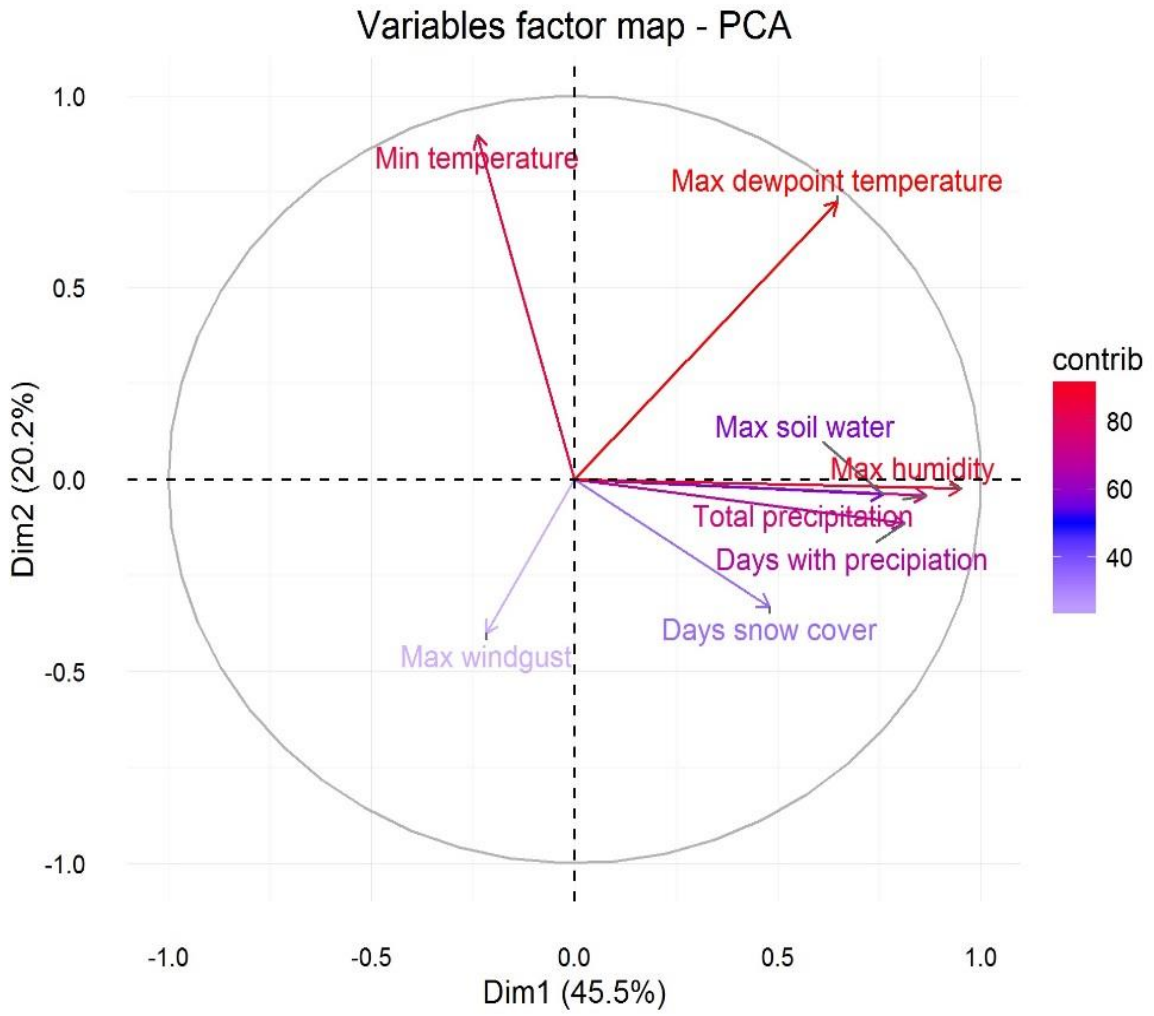


fig. S5. Contributions of variables to components 1 and 2 of the PCA using the full data set, with real dates of onset if available or 9 May if not. The loads of the variables into the first two PCA components are also shown here:

Variable	Aggregate type	Component 1	Component 2
Precipitation	Total (mm)	-0.4533036	-0.03250537
Precipitation	Number of days	-0.4256831	-0.08986582
Soil water	Maximum of daily means (m^3/ m^3)	-0.3976953	-0.03039895
Temperature	Mean of daily minimum (°C)	0.1259684	0.7052
Dew point temperature	Mean of daily maximum (°C)	-0.3384141	0.5694721
Relative humidity	Mean of daily maximum (%)	-0.4983222	-0.0186722
Wind gust	Mean of daily maximum (m/s)	0.1144289	-0.3160734
Snow cover days	Number of days from 1 Jan	-0.2516685	-0.2609341

table S1. PCR primers used for microorganism detection.

Species/ disease	Primer/ toxins	Primer sequences	Amplicon size bp	Reference
Pasteurella multocida	KMT1T7	ATC CGC TAT TTA CCC AGT GG	456	78
	KMT1SP6	GCT GTA AAC GAA CTC GC AC		
Mannheimia haemolytica	Mhgcp	TGGGCAATACGAAC TACTCGGG	227	79
	MhgcpR	CTTTAATCGTATTC GCAG		
Bibersteinia trehalosi	BtsodAF	GCC TGC GGA CAA ACG TGT TG	144	79
	BtsodAR	TTTCAACAGAACCA AAATCAC GAA TG		
Mycoplasma ovipneumoniae	LMFI	TGA ACG GAA TAT GTT AGC TT	361	80
	LMRI	GAC TTC ATC CTG CAC TCT GT		
Clostridium toxins	CPA (alpha toxin)	GTTGATAGCGCAGG ACATGTTAAG CATGTAGTCATCTG TTCCAGCATC	402	81
	CPB (beta toxin) β	ACTATACAGACAGA TCATTCAACC TTAGGAGCAGTTAG AACTACAGAC	236	
	CPE (epsilon toxin) ε	ACTGCAACTACTAC TCATACTGTG CTGGTGCCTTAATA GAAAGACTCC	541	
	CPI (iota toxin) ι	GCGATGAAAAGCCT ACACCACTAC GGTATATCCTCCAC GCATATAGTC	317	
Q fever	Trans1	TAT GTA TCC ACC GTA GCC AGT C	687	82
	Trans2	CCC AAC AAC ACC TCC TTA TTC		
	Trans3	GTA ACG ATG CGC AGG CGA T		
	Trans4	CCA CCG CTT CGC TCG CTA		
Visna Maedi	Fex	TGA CAC AGA AAA TGT AAC CGC AAG	291	83
	Rex	CCA CGT TGG GCG CCA GCT GCG AGA		
	Rin	TTG CAC GGA ATT		

		AGT AAC G		
	Fin	AAG TCA TGT AC AGC TGA TGC TT		
Akabane virus	AKA F1	TAACTACGCATTGC AATGGC	709	84
	AKA R1	TAAGCTTAGATCTG GATACC		
	AKA F2	GAAGGCCAAGATG GTCTTAC	230	
	AKA R2	GGCATCACAATTGT TCAGC		
Malignant Catarrhal Fever	B9.15	AAGCTTCAGCTTAC TCCCTTTACTCT	460	85
	Bax 7525	AAGATAAGCACCA GTTATGCATCTGAT AAA		
	Bax 556	AGTCTGGGGTATAT GAATCCAGATGGCT CTC	238	
	Bax755	TTCTGGGGTAGTGG CGAGCGAAGGCTTC		
Theileria annulata	F	ATGCTGCAAATGAG GAT	768	86
	R	GGACTGATGAGAA GACGATGAG		
Babesia sp.	BJ1	GTCTTGTAATTGGA ATGATGG	425	87
	BN2	TAGTTTATGGTTAG GACTACG		
Anaplasma marginale	AM-F	TTGGCAAGGCAGCA GCTT	95	88
	AM-R	TTCCGCGAGCATGT GCAT		
Erysipelothrix rhusiopathiae	MO101	AGATGCCATAGAA ACTGGTA	407	89
	M0102	CTGTATCCGCCATA ACTA		

table S2. A summary of the largest MMEs in saiga attributed to Pasteurellaceae-related syndromes. Sources are internal field reports except where numbered.

Syndrome/ Organism isolated	Year	Population	Deaths	Mortality as % of regional population[1]	Source
Hemorrhagic septicemia/ *P. multocida* [*C. perfringens*]	9-29 May 2015	Betpak-dala	~210,000	~88%	(IUCN/SSC Antelope Specialist Group & Saiga Conservation Alliance, 2015)
'Pasteurellosis' - probably hemorrhagic septicemia/ *P. multocida*	12-22 May 1988	Betpak-dala	270,000	73%	(Institute of Zoology and Betpak-dala State Hunting Organisation, 1988, 1989, Torgai Regional Executive Committee, 1988)
'Pasteurellosis'; possibly pneumonic form/ *P. haemolytica*	March-April 1984	Ural	110,000	73%	(Institute of Zoology and Department of Reserves and Hunting, 1984, 1985)
'Pasteurellosis' official cause ; no detail on syndrome or organism	24-28 May 1981	Betpak-dala	~70,000	~15%[2]	(Institute of Zoology and Department of Reserves and Hunting, 1981, 1982)
Symptoms resemble bloat or fog-fever/ *P. multocida*/ [*C. perfringens*]	18-29 May 2010[3]	Ural	12,000	75%	(R. Kock, "Final Report of Mission to Kazakhstan September 13th to October 1st 2011 and related analysis". Royal Veterinary College, United Kingdom (2011). R. Kock, et al. A retrospective assessment of saiga antelope *Saiga tatarica* die-off in Western Kazakhstan 2010-2011. *Saiga News* 1-4 (2014).)

[1] i.e. of the affected population (Betpak-dala, Ural or Ustiurt)

[2] Although it is arguable as to whether the mortality observed in this event qualifies as 'substantial' (using Fey et al.'s definition of an MME, (2)), we include it here as a significant event in terms of numbers dying, and because of its relevance to the overall analysis of Pasteurella-assigned mortality events in saigas.

[3] In 2011 an additional 400 animals calving at the same location died, whilst those calving elsewhere were unaffected. There are no official data on calving in other places, however.

table S3. Results of an ICP-MS analysis of livers (w/w) of three dead saigas from May 2015 (Tengiz group). Dark green = within normal limits. light green = marginal, yellow = deficit, as assessed using ovine parameters (73).

	Content										
Sample ID	g/kg		mg/kg		mg/kg		mg/kg		mg/kg		Ratio
	Iron	SE	Cobalt	SE	Copper	SE	Zinc	SE	Molybdenum	SE	Cu:Mo
1	0.125	0.038	0.045	0.001	11.7	2	46.4	8.3	1.71	0.51	6.4
2	0.137	0.02	0.033	0.006	6.35	0.7	29.4	2.5	0.634	0.07	45
3	0.164	0.038	0.025	0.015	24.2	2	24.2	0.9	0.463	0.051	51
Mean	0.142	0.02	0.034	0.007	14.1	9.16	33.3	11.6	0.935	0.68	

table S4. Assuming a diagnosis of HS as a primary cause of death, this table is a list of possible stressors, which may cause suppressed immunity in the host or increased virulence and invasion of a commensal parasite such as *P. multocida*, subsequent septicemia in infected hosts, and/or enhanced transmission. Thus, the proximate cause of HS is assumed throughout – which accounts for the peracute syndrome. The question is whether each trigger could interact with *P. multocida* in some way and whether each can explain the observed particularities of the outbreak. The hypotheses listed here were initially set up from the literature reviewed and from preliminary field observations. Subsequently, additional laboratory, statistical and field studies were conducted to test them further and evaluate their likelihood. Hypotheses focus on factors triggering pathogen invasion in the short term; longer term influence on *P. multocida* carriage and host susceptibility might also be important in the disease dynamics. Given that causation might be multifactorial, competing hypotheses are not mutually exclusive at this stage of the investigation. The first column lists hypothetical causes of HS (1, 2 etc.) and then potential mechanisms (a, b, c etc.) linking these to pathogen invasion.

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
1. Climatic factors trigger *P. multocida* growth and virulence in adult saigas with latent infection				
1a. Some unspecified climatic factor triggers either loss of host immunity or increased pathogen virulence. *P. multocida* commensal in the respiratory or alimentary tracts, then proliferates, invades and causes HS	Climatic driver would be consistent with multiple near-synchronous events across a large landscape. Climatic factors have been associated with pasteurellosis in other wild ungulate die-offs (15-17, 90, 91).	Saigas experience highly variable climate, within and between years: what was special about MME years and die-off sites? Assumes widespread carriage of *P. multocida* by saigas at high prevalence, which still needs to be tested. Mortality of calves after dams not consistent with synchronous	Statistical analysis supports links with specific climatic factors (see 1b-d below). Only 1/35 saiga calves tested positive for *P. multocida* at age 2 days in 2016: suggests *P. multocida* infection of calves does not normally occur in utero. Hence delayed mortality of calves could be secondary to acute bacterial invasion of females, as a result of	High

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
		bacterial invasion due to common experience of climatic factor by latent bacteria.	subsequent transmission to calves in milk (as observed in the field).	
1b. Rising ambient temperatures and humidity trigger pathogen growth and virulence. Post-transcriptional modification of virulence-associated proteins in altered oronasal-pharyngeal environment (92). Conditions optimal for growth of commensal P.multocida population.	Some literature on HS records warm wet conditions at time of outbreaks. Humidity and temperature changes affect large areas, so can account for sudden onset in multiple locations. Index case times vary, which may correlate with weather fronts moving across the landscape.		Case-control study suggests higher minimum temperatures and atmospheric moisture at case sites in comparison to controls. Long term temperature anomalies: minimum temperatures at or above 95th percentile 3-5 days before die-off in 1988 and 2015 (Torgai meteo station).	High
1c. A sudden drop in temperature (perhaps combined with precipitation and wind) just before die-off causes a loss of host immunity Energy metabolism shifts from immune system to temperature regulation. Oropharyngeal environment changes, affecting the	Timing of die-offs likely to coincide with molt (loss of winter coat). Some literature on HS records cold, wet and windy conditions at time of outbreaks. Cold is known to compromise some immune cell functions.	Males as well as females were affected (metabolic shift away from immune system more likely in lactating females).	Statistical comparisons suggest that temperatures not significantly lower at die-off sites than at control sites except in 1981. Temperature increases followed by sharp drops recorded at die-off sites, but are also common at controls. Long term temperature anomalies do not suggest	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
mucosal barrier or its microbiome, and hence resistance to opportunistic invasion of commensals.			anomalously low temperatures before die-offs in 2015 or 1988. Invading bacteria appear to be specifically *P. multocida*, as opposed to an expected mixed invasion following general lapse of defenses.	
1d. A sudden drop in temperature just before die-off causes an increase in pathogen virulence	Some literature on HS records cold, wet and windy conditions at time of outbreaks (90). Evidence in literature for cold shock triggering virulence expression in respiratory commensal bacteria.		See 1c above.	Low
2. Co-infection with secondary pathogen enables invasion of *P. multocida*				
2a. Co-infection with another organism which acts with *P. multocida* to cause mortality event. Co-infection causes reduction in immune-	Some diseases involving *P. multocida* infections are multi-factorial and sometimes associated with other bacteria or viruses. Morbilliviruses are often	In order to account for sudden onset at multiple locations, putative secondary pathogen must have been carried in recently infected/recovered or healthy animals and no	Virology negative including for vesicular disease and other viruses; no pathogens identified consistently by Next Generation Sequencing other than *P. multocida.* Known viruses eliminated by PCR	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
competence, or raised body temperatures via fever, triggering change in RNA structures of pathogen. Predisposing infections could have been acquired at other locations earlier in the migration.	associated with immunosuppressive episodes such as drought, malnutrition and raised blood parasite loads as co-infections. High parasite loads sometimes associated with pasteurellosis in the literature.	evidence for this clinically. In most cases of HS in wildlife *P. multocida* was the only organism isolated. Multi-pathogen etiology is more characteristic of pneumonic pasteurellosis. Evidence of bacterial septicemia as primary cause of death is strong.	(FMD; bluetongue; peste des petits ruminants; capripox; epizootic hemorrhagic disease). Anthrax, paratuberculosis, brucellosis, listeriosis, campylobacteriosis all negative. Histopathology shows hematogenous spread of a Gram-negative bacteria; no signs attributable to other pathogens, other than in 3 individuals with inclusion bodies in respiratory epithelial cells suggestive of a possible paramyxovirus but there were no associated histopathological changes that would be expected from this infection. Evidence so far only for presence of *Clostridia perfringens*, *Theileria annulata and PPRV* (see specific hypotheses 2b-d below).	
2b. Another pathogen, such as PPRV, invades	PPR incursion into small livestock in Kazakhstan in 2014 close to southern migration	No evidence of unusual mortality prior to the event; no unusual mortality in livestock	Possible paramyxovirus inclusions found in three individuals from Tengiz cluster	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
prior to mortality event. Thereby lowering immunity or predisposing saiga to infection by *P. multocida* in other ways.	routes of saiga antelope.	in the region; animals in good condition.	but these need to be confirmed with EM or immunohistochemistry.	
2c. Theileriosis interacts with *P. multocida*, possibly as a result of environmental stressors. Lowered host immune-competence leads to invasion by *P. multocida*.	*Theileria annulata* isolated from some of the dead animals examined (c. 40%). Protozoan infection (*Theileria annulata*) can cause immunosuppression, most likely when immune-naïve hosts move into an area with reservoir of infection in ticks, which could have come from livestock, leading to sudden and synchronous exposure.	No consistent pathology associated with infection with this parasite was found. Onset in livestock can be acute and severe but wild antelope are generally highly tolerant of *Theileria* infection. Few livestock in the area in 2015 or 2013, so source of infection unclear.	*Theileria annulata* isolated in c.40% of cases using PCR, so cannot account for high mortality rate; ticks only recorded in ~3%. No histopathological sign of an increase in the number of *Theileria* in diseased tissues. Few ticks found during 2016 sampling on saigas or in environment; few ticks and no reports of tick-borne diseases being a problem in livestock near die-off areas generally or in 2015 or 2016, including in animals brought into the area for the first time.	Low
2d. Clostridial disease interacts with *P. multocida*, possibly as a result of environmental	Can account for peracute syndrome.	Non-preferential impact on animals of different ages unlikely. No recorded cases of HS	Clostridial alpha toxin identified from PCR tests on blood samples (10/24) - so only from 43% of carcasses.	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
stressors. Opportunist response to *Pasteurella* septicaemia overwhelms immune system.		related to coinfection between *Clostridia* and *P. multocida*. Lack of evidence of unusual diet at die-off sites that could have affected gut environment and hence clostridial overgrowth. No consistent symptoms or pathology attributable to clostridial disease.	*C. perfringens* isolated from carcasses at Aktobe branch of RSE (Veterinary State Laboratory). The presence of some *Clostridia* is not inconsistent with the primary disease HS; terminal invasion will occur but no evidence of primary factor. FISH identified no general invasion of bacteria; rather, it suggested a *P. multocida* specific event.	
3. Poor general nutrition increases susceptibility of saigas to bacterial invasion				
3a. Changes in nutritional status (possibly combined with high parasite burden) make saiga susceptible to pathogen. Changes in nutritional status cause nutrient partitioning away from	Consistent with broad landscape effect. Mortality occurred during lactation, a time of nutritional vulnerability. Evidence from literature for links between lactation,	Deaths of males harder to explain. Unlikely to result in massive mortality; more survivors would be expected. The course of the MME would be expected to be prolonged.	Comparison of saiga energy requirements and forage plant quality suggest energy deficits in April & May in lactating females; protein deficits in April likely, but in May unlikely, even in poor years and under parasite burdens.	Low as main driver, but nutritional stress during calving and lactation could potentiate

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
immune system e.g.: *Phenology:* mismatch of forage quantity/quality peak with calving time or location; *Species*: change in pasture composition. These changes could occur in calving areas or at other times of the year and in other parts of the range.	nutritional stress (especially protein) and immunosuppression (93). Evidence from data and literature for recent changes in plant phenological patterns in northern Kazakhstan. .	Animals in 2015 were in moderate to good condition, though this does not rule out acutely limited labile protein; rumen function appeared good with highly nutritious pasture content and healthy ruminal fluids, and normal papillation. Vegetation composition highly variable between die-off sites.		climatic factors under hypothesis 1
3b. Migration results in highly variable nutrition between years, influenced by climate variation and plant dynamics. Circumstances in 2015 resulted in acute undernutrition and immunosuppression, enabling pathogen invasion.	Energetics of the saiga are linked to migration patterns and season, immunity and physiological resilience. Opportunities or challenges to optimal nutrition, energy conservation, e.g. severe winters can lead to high mortalities. Poor body condition was associated with MME in 1988. Might be subtle effects immediately prior to calving if low NDVI not affecting fat reserves or overall body condition but leading to low	Unlikely that the population would be affected by these factors in a uniform way given age and sex variation, and differing migration distances and experiences of different sub-groups of saiga over the landscape.	In 2015, deaths occurred across large latitudinal range, exhibiting large differences between days from snowmelt to onset of death. NDVI & snow anomaly data suggest late snow cover and green-up in 2015, but conditions normal by May; literature suggests severe winter & cold wet spring in 1988; in 1981 wet cold April. So no consistency between MME years. Comparison of difference in	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
	glycogen reserves, which are in demand in last trimester, and partitioning of energy away from immune responses.		timing of calving and days from key phenological points such as peak NDVI or green-up) between case and control sites pending.	
3c. Nutrition affects gut microbiome and pathogen invasion. Plant phenology and nutritional content could affect gut microbiome and weaken innate defenses against invasion.			Preliminary finding from FISH that *P. multocida* was found in gut submucosa more than in the lung tissues. No evidence for unusual plant composition or phenology at die-off sites.	Low
4. Micro-nutrient deficiency, toxicosis or environmental pathogen cause mortality directly or by predisposing to bacterial invasion.				
4a. Geochemical anomalies lead to altered concentrations of Cu antagonists (Mo, S, B or Fe) in forage plants to compromise immunity and/or trigger virulence in	Accounts for association with rapid development of vegetation and concurrency of events - young plants contain high concentrations of elements. If plant development affected	Although inter-annually repeated occurrence of die-off of variable severity at certain sites suggests a possible role for local hydrological, soil & geological conditions, in 2015 sites saiga were affected across	Speculative – as no information on plant / soil mineral content currently available. Difficult to identify given likely dynamic and complex biogeochemistry of	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
P. multocida. Long to short term extensive hydrological anomalies such as rise of ground water flooding in combination with temperature anomalies increase concentration of Cu antagonists in soil and then vegetation, which is hungrily ingested by already Cu-compromised post-calving saiga. These ingested antagonistic elements (i) impair Cu status and lower host immunity (ii) affect iron metabolism and trigger virulence of *P. multocida*. Postulated syndromes include copper deficiency due to excess of Cu antagonists (Mo, S, B, Fe), possibly in association with other elemental deficiencies (Zn, Co).	by stressors (weather, soil chemistry) elements can rapidly reach extremely high concentrations; MME years tended to be wet or characterized by flooding and rise of ground waters). Elements of concern are found in arid environments in ground waters and can be accumulated at geochemical barriers. Effect of hypocupraemia is most prominent in females, which predominate in the die-off sites and suffer heaviest deficit of Cu, but not exclusively limited to gravidity or lactation. Multiple plant species can accumulate Mo, S, B and iron, some are better accumulators than the others. Links between copper deficiency and (i) pasteurellosis outbreaks in wild and domestic ungulates; (ii) immune response to *Pasteurella* organisms. Implicated antagonists are	their entire range. No variation in disease or mortality within aggregations, in spite of micro-relief. Clinical syndrome, gross and histopathology not consistent with reported toxicoses and deficiencies in other ruminants. The likely individual variation in mineral deficiency should produce variable clinical outcomes and not a die-off or MME. Immune-suppression mechanism alone seems unlikely to account for the timing of the deaths and its variation across the landscape and high morbidity and peracute deaths.	phenomenon. Marginal tissue copper detected in 2/3 tested saiga from die-off, but no convincing evidence as yet of critical hepatic copper level in saiga Case-control analysis of cumulative precipitation & soil moisture in period up to calving suggest that both were unusually high at 1981 site and at most 2015 sites, but were less so in 1988 and at the Irghiz cluster of 2015 sites. No evidence of issues in livestock; more work needed on whether husbandry practices reduce susceptibility to mineral deficiencies.	

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
	widely present in the environment and become more biologically available under damp, alkaline and reducing conditions typical for arid areas. Some evidence for association of (i) blood iron availability with *P. multocida* virulence; (ii) good evidence for decline of innate immunity in hyporupraemic ruminants. (iii) some evidence for hypocupraemia related alteration of iron metabolism of importance for (i).			
4b. Toxic plant species at calving sites cause death of animals. Climatic conditions lead to emergence of previously unusual toxic species. Algal toxicosis from water sources reduces immunity.	Would explain peracute syndrome.	Clinical syndrome, gross and histopathology, not consistent with plant toxicosis. Botanical data suggest that vegetation composition was very different between 2015 die-off sites. Only one genus, *Artemisia*, was present at all sites. Prevalence of toxic species similar to other years. Saigas tend to feed on a wide	No reports of high morbidity or mortality in livestock grazing steppe pastures near die-off sites in 2015, either to veterinary authorities or from farmer interviews in 2016; isolated instances of mortality related to known toxic plants but those plants not reported to be more abundant than usual in spring 2015. No reports of unusual water color or	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
		range of plant species over a wide area so eating concentrations of toxic plants is unlikely.	appearance of vegetation.	
4c. Altered growth or phenology of non-toxic plants species causes disease. Unusual growth conditions generate toxic or physiologically harmful metabolites in plants normally eaten by saigas.	Would explain peracute syndrome (94). Exposure to lush plant growth after scarcity, e.g. following rain or under-nutrition, can cause fatal bloat or emphysema fog fever in farmed ruminants. Rainfall was high in 2015. Bloat-related disease was associated with MME in saiga in western Kazakhstan in >70% in 2010 and a smaller die-off of ~ 10% in 2011 in the exact same location at calving.	Clinical syndrome, gross and histopathology, not consistent with bloat, fog fever or other metabolic toxicosis.	Vegetation not reported to be any more lush than usual in spring 2015 (95), and bloat or fog fever syndromes were not noted in farmer interviews in 2016.	Low
4d. Perturbation of microbiome leading to overgrowth of *Pasteurella*. Gut conditions affected by dietary change, e.g. increased concentrations of minerals or phytochemicals	Phytochemicals such as sesquiterpene lactones are present in *Artemisia* (96) and known to affect bacteria (97). Concentrations are seasonally variable so climatic factors resulting in change in plant physiology could explain	Plant species in die-off areas have been present in the region for some time, under a range of climatic conditions: *Artemisia* species are ubiquitous. There is no known mechanism connecting phytochemicals to *P. multocida* invasion or	*Artemisia* was the only genus of plant that was eaten by saiga at all six die-off sites that were examined by a botanist. In every case, *A. nitrosa*, *A. pauciflora* or both were present. This genus is common in the saiga habitat and in past	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
(98)	landscape-level effect and relatively recent occurrence of MME events.	massive disruption of microbiome. Saiga - and animals in general – are known to avoid high concentrations of phytochemicals because of their taste or other adaptive reasons.	surveys of saiga diet, and no shifts in vegetation in the die-off areas have been noted by scientists or by farmers interviewed in 2016. Phytochemical concentrations were not analyzed.	
4e. Exposure to toxins. Migrating saigas encounter environmental or other toxins, predisposing to pathogen invasion.	Could account for peracute syndrome. Some toxins known to cause immunosuppression.	Near-simultaneous exposure of separate groups across such a large area unlikely.	No toxins found by state laboratory investigations of die-off sites. No unusual illness or mortality observed in livestock or people.	Low
5. Unusual conditions promote transmission of *P. multocida*				
5a. Unusually wet conditions or chemical components in the environment favor survival and spread of *Pasteurella multocida* in the environment	Survival of *Pasteurella* in the environment increases in wet conditions.	Even in wet conditions *Pasteurella* do not survive for long periods in the environment. Almost simultaneous events at geographically distinct sites not suggestive of horizontal transmission.	No environmental isolates of *Pasteurella* from water bodies during 2016 field mission.	Low

Hypothesis and potential mechanisms	Factors in support of hypothesis : Initial field observations & literature	Factors making hypothesis unlikely: Initial field observations & literature	Follow-up laboratory, statistical and field evidence for or against hypothesis	Likelihood
5b. Animal fecal shedding into a flooded environment exposes population to novel virulent strain	Would explain disease in a population lacking resistance to a new strain.	Almost simultaneous events at geographically distinct sites not suggestive of horizontal transmission.	No reports of unusual disease in livestock in 2015, or excessive local flooding, from farmer interviews in 2016.	Low
5c. Mosquitos spread virulent strain quickly across calving aggregation	Large numbers of insect vectors present at the sites.	Almost simultaneous events at geographically distinct sites not suggestive of vector-borne infection cycle.	Insects, especially mosquitoes, reported to be even more abundant in 2016; no reports of suspicious human or livestock disease in either year.	Low

table S5. Comparison of means for cases and controls using the full data set (all MME years); real dates of onset or 9 May; and climate metrics aggregated over the 10 days to onset.

Predictor	Aggregate type	Sample size[4]	Mean Controls (SE)	Mean cases (SE)	Bayesian model		
					Coefficient of standardized variable	Standard error	Confidence intervals
Precipitation (ERA)	Number of days	131	3.5 (0.22)	6.4 (0.50)	0.04	1.56	-3.09; 3.06
Precipitation (ERA)	Total (mm)	131	6.17 (0.61)	16.0 (1.92)	-1.77	1.48	-4.93; 0.82
Precipitation (GPCC)	Total (mm)	125	2.6 (0.40)	8.2 (2.70)	-2.73	1.48	-6.08;-0.29
Dew point temperature	Mean of daily maximum (°C)	131	4.9 (0.22)	8.9 (0.32)	5.57	2.06	1.7; 9.79
Relative humidity	Mean of daily maximum (%)	131	71.2 (0.86)	84.6 (0.83)	4.64	2.03	0.79; 8.81
Snow cover days	Number of days from 1 Jan	131	99.7 (1.04)	104.6 (0.24)	0.21	2.4	-4.6; 4.98
Soil water	Maximum of daily mean (m^3/ m^3)	131	0.23 (0.003)	0.25 (0.006)	-0.72	1.52	-3.94; 2.11
Temperature	Average of daily mean (°C)	131	13.5 (0.24)	14.3 (0.62)	3.52	1.74	0.41; 7.28
Temperature	Average of daily maximum (°C)	131	19.9 (0.27)	20.3 (0.74)	3.73	1.78	0.61; 7.50
Temperature	Average of daily minimum (°C)	131	5.9 (0.23)	7.4 (0.54)	3.83	1.66	0.98; 7.41
Temperature	Maximum (°C)	131	26.1 (0.27)	25.7 (0.70)	0.02	1.77	-3.43; 3.59
Temperature	Minimum (°C)	131	1.3 (0.27)	3.2 (0.40)	3.21	1.42	0.86; 6.36
Temperature difference	Maximum daily variation (°C)	131	18.1 (0.15)	17.5 (0.35)	0.95	1.88	-2.53; 5.07
Temperature difference	Total variation over 10 days	131	24.8 (0.28)	22.4 (0.58)	-3.58*	1.5	-6.91; -1
Temperature difference	Difference in daily minimum	131	9.7 (0.25)	7.8 (0.47)	-2.82	1.28	-57.1; 0.77
Windgust	Mean of daily maximum (m/s)	131	8.8 (0.09)	7.9 (0.12)	-3.67*	1.76	-7.39; -0.53
NDVI anomaly (sd x100) 1998-2016	1st week May	96	-12.9	-27.56	These metrics cover only subsets of the dataset; models do not converge		
	4th week April	96	-20.70	-48.57			
	3rd week April	96	-20.51	-122.11			

[4] The sample size for die-off/control comparisons at real onset/ 9 May is 131, as 2015 die-off sites without dates are removed. The GPCC dataset covers the years 1988-2016.

(2015 die-off sites vs controls)					
Snow anomaly (1- probability of snow x 10000). 2000-2016 (2015 die-off sites vs controls)	2nd week April	95	-425	-3795	
	1st week April	95	-1469	-6983	
	4th week March	95	-2085	-6458	
NDVI anomaly (sd x100) 1982-1997 (1988 die-off sites vs controls	1st week May	91	-63.5	-108.47	
	4th week April	91	-13.2	59.64	
	3rd week April	91	0.033	84.04	

*Mild converge problems

table S6. Long-term climate anomaly data at Kostanai sites (ERA data using actual die-off site, NCEP data using pixel, covering entire Torgai area). Mean = long-term mean (1980-2016). Precipitable water is an indicator of atmospheric humidity. It does not indicate how much it will rain but constitutes an instantaneous value of the amount of moisture in the air above a location. Period 1 = 1st-15th May, Period 2 = 16th-30th May.

		ERA dataset: 1979-2016								NCEP: 1948-2016		
Data type	Year	Total precipitation		Mean of max daily humidity		Mean of max daily dewpoint temperature		Mean of min daily temperature		Average precipitable water	Average relative humidity	Average temp
	Period	1	2	1	2	1	2	1	2	May	May	May
Real values	1981	26.07	41.76	87.31	85.3	8.62	7.9	7.46	5.05	18.63	74.33	13.63
	1988	23.36	14.72	83.38	72.27	7.53	6.16	6.44	7.02	16.02	70.86	13.8
	2015	14.58	23.83	84.55	89.01	9.59	12.09	8.5	9.57	20.32	80.41	16.02
	Mean	**11.04**	**11.28**	**75.8**	**70.19**	**6.21**	**7.92**	**6.24**	**9.6**	**17.18**	**64.32**	**15.61**
Cumulative probability of value (using empirical cumulative distribution function)	1981	0.05	0	0.03	0.05	0.16	0.45	0.21	0.97	0.23	0.14	0.81
	1988	0.08	0.29	0.13	0.45	0.26	0.76	0.45	0.87	0.71	0.25	0.78
	2015	0.29	0.11	0.11	0	0	0	0.08	0.5	0	0	0.45