Summaries of Russian documentation of mass mortality events in saiga during the 1970s and 1980s

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1. Introduction

This review is a summary of Russian sources on suspected outbreaks of pasteurellosis in the saiga antelope prior to 2015. This literature consists of translations of the relevant sections of the annual saiga expedition and aerial count reports; reports of government commissions investigating the larger mortality events; and published articles. Footnotes by the reviewer provide additional or contextual information to support interpretation of the summarised articles.

In many of the reviewed reports, authors do not distinguish between haemorrhagic septicaemia (the proximate cause of the 2015 die-off) and pneumonic pasteurellosis, which has a different pathology and aetiology. In such cases the term pasteurellosis is used to mean either of these two syndromes. Some of the veterinary terms could not be translated with full confidence and in such cases the Russian terms are also provided. In addition to the events described here, a mass mortality also occurred in 2012, documentation of which is already available in English (Zuther, 2012). The locations described in this document are available in GIS format at https://catalogue.ceh.ac.uk/documents/8ad12782-e939-4834-830a-c89e503a298b.

2. 1949 Mortality event in captive saiga

(i) Published paper on 1949 die-off (Shamatava et al., 1951)

The authors could find no recorded cases of pasteurellosis in saiga in the literature, so this may be the first documented case.

The outbreak: In spring 1949, a number of saiga died over a short period of time at Tbilisi zoo. The saiga were transported to the zoo from Azerbaijan on the 30th January 1949. Five animals died; dates of deaths are listed in Table 1.

Table 1. Dates of death of individual saiga in 1949 die-off

Animal Number	First symptoms	Death
1	18 Feb	– died in a few hours
2	31 March	– died in a few hours
3	3 April	6 April
4	5 April	7 April
5	5 May	21 April

The final (5th) saiga was treated with anti-pasteurella serum. It fell ill on the 5th May, and was treated on the 7th. It showed some improvement and was given a second dose on the 8th. Its temperature fell back to normal, general health improved, but on the 16th its condition deteriorated rapidly. A third dose was administered, but it died on the 21st.

The authors note that the appearance of the disease was preceded by a sharp drop in temperature – and that the saiga were kept outdoors and thus exposed to the weather.

Necropsy: There were large numbers of spotted haemorrhages on serous and mucous membranes of internal organs. Haemorrhages were particularly serious on membranes of the digestive tract, respiratory organs and on endocardium and pleura. Pulmonary tissue was congested and oedematous, dark red, and a foamy liquid was present in bronchial and bronchiole spaces. Under the pleura and the mucous membranes of bronchi or bronchiole were many spotted haemorrhages. The spleen was slightly swollen and loose, edges rounded, and dark in colour. Under the capsule were numerous small haemorrhages.

In addition to the above described haemorrhages, puffiness and hyperaemia of the mucous membranes of the intestines and abomasum, and in particular the pylorus were also observed. Lymphatic nodes were enlarged; on incision they were found to be humid, of an uneven red-pink colour, and spotted with small haemorrhages. Mesenteric lymph nodes sometimes exhibited necrosis, with collapse of the tissue into a fine grained mass.

Tissue degeneration was discovered in the liver, kidneys and heart - particularly in the case of the liver which was enlarged with rounded edged and highly congested. Liver tissues was dark red, lightly broken up, and upon on incision had a grainy aspect. In central nervous system was observed hyperaemia in neural material and on membranes.

In the more chronic instance of the disease (saiga number 5), in addition to the above- mentioned changes, fibrous pleuropneumonia was observed in the lungs - several parts of both lungs were inflamed. The tissues in these inflamed parts were unevenly thickened. 'Hepatisation' of lung tissue was observed in places. On incision, colour was uneven, grey-red, bright red and grey-yellow in colour. Histological examination of inflamed lung tissues revealed infiltration of serous, sero-fibrous, and fibrous-cellular exudate in alveolar cavities and interstitial tissues, and areas of necrotised tissue. Inflammation was also seen on the pleura - thickening of pleural layer and accumulation in pleural cavity of a serous-fibrous exudate. Other than this, in this chronic case, changes in other organs were the same as that of the acute cases, but less marked.

Microbiology: Pathogens were isolated from the fifth animal, which as noted above took 16 days to die and had been treated with serum. The authors note that a similar organism was isolated from two other dead saiga by another worker (N. C. Lekveishvili). Infection of mice using emulsified organ tissue from the saiga had no effect. But a pure culture of *Pasteurella* bacteria isolated the lymph nodes was lethal to laboratory animals.

Morphology: smears from bouillon culture (both from primary and passenger cultures) and from blood and organs, revealed microbes shaped like short rods, with an ovoid form, distributed singly or in pairs. They exhibit a strong bipolar intense colouring. These microbes were stained using Giemsa dye – and belong to the group of Gram-negative bacteria. The size was 0.8-0.9 microns in length by 0.3 microns across. They were motionless and did not form spores.

The bouillon culture of these organisms (PH 7.4) became muddied or opaque after 18-24 hours. At the bottom of the tube a white precipitate collects. Upon shaking, the precipitate rose in braids/strings. On Agar plates, after 24-30 hours appeared a culture of dew-drop shaped cells, in translucent columns. The columns merged and formed a thin layer, which later thickened to form a plaque.

Liquid from this isolated culture did not coagulate. The isolated strain had weakly enzymatic properties (expresses mildly fermentative properties). It did not break down maltose, galactose, glycerine or sucrose but broke down glucose and lactose with resulting production of acid.

The strain is pathogenic to white mice and rabbits, which died 24 hours after infection from the bouillon culture. The same strain was isolated from these, and used to infect more mice, which died in 20 hours, and so on for four passages. In the last round, mice died within 12 hours and rabbits within 18 hours.

The strain was identified as a type of *Pasteurella* bacteria – but the species was not defined. The authors noted that the isolated strain was not highly virulent because the saiga from which it was isolated died from a non-acute form of pasteurellosis (i.e. the fifth animal, to which serum had been administered).

3. 1974 Mortality Event - Betpak-dala population

(i) Published paper on 1974 die-off (Statsenko, 1980)

Location: The die-off occurred in Kurgal'dzhinskii nature reserve, which is located close to lake Tengiz. In the reserve are a number of islands located where the Nura and Kulanutpes rivers meet. Around 1000 saiga live on these islands, but in 1974, perhaps due to the drought in the southern regions of the republic, they appeared in unusually large numbers on the fields of the sovkhoz adjacent to the reserve. These animals, which started to damage the crops, were driven away with aeroplanes and vehicles. Searching for cover, they ran for some distance. A massive treatment of crops on this sovkhoz with the herbicide 2.4D '*Butioviye Efira*' (literally crushed ether) was conducted form the 10th June onwards.

Timing: From 13-14th June the nature reserve services recorded around 10,000 saiga on the islands. Two days later (15-16 June) a massive die-off began on the territory of the nature reserve. Within 48 hours around 1000 animals died, of which 500 were observed on the land and the same number in the water (from a plane). Most of the dead were adult males and well-fed females. Those left alive – with the exception of 300-400 head, then abandoned the islands.

Autopsy and lab analysis: Research was carried out on one of the islands on which 500 saiga had died (some carcasses were already rotten by the time of the study). On the island were 300-600 live saiga including sick individuals left behind from the main herd which were easy to catch. One was caught for necropsy, along with two fresh carcasses from the die-off.

All three necropsied animals were females and had been [otherwise] healthy and in an average nutritional state. Fat depositions were observed under the skin. All three exhibited similar symptoms. The two which had already died of disease displayed rigor mortis.[2] In the subcutaneous tissue of the sub-mandibular area, neck and inter-maxillary space were found a large number of dotted and striped haemorrhages, which were also sometimes observed in the skeletal muscles. Blood was dark red, uncoagulated, with loose/soft dark red clots. From the nasal cavity was a small amount of pink foamy liquid. Mucous membranes of the oral cavity, nose and conjunctiva, were cyanotic. Vessels were highly 'injected' (blood filled). The lymph nodes (submandibular, pre-scapular, subscapular and deep cervical) were swollen and spongy. The surface of the lymph nodes was dark red, with smooth external edges; tissue around the nodes was oedematous.

In the sick animal the surface of incisions were a grey-pink colour and covered with small spotted haemorrhages. Large haemorrhages were found in the cortical layer. Incisions resulted in the seepage of an abundant watery liquid. The mucous membranes of the pharynx, oesophagus, larynx, and trachea were pink with a blueish hue. At the bifurcation, the mucous membranes were dark red, and on the surface of the trachea and bronchi were full of a pink foamy liquid.

The surface of the lungs was pink, with a blueish hue. On the pleura of all three animals were a large number of pathogenic haemorrhages. The consistency of the lungs was consolidated. From the surface of the incision

[2] There are inconsistencies here regarding timing. If the animals died in two days, it seems odd that some display rigor mortis whilst others are rotten – the die-off may have continued for a while longer following the initial rapid mortality in the first two days.

came a foamy bloody liquid. Spotty and stripy haemorrhages in abundance were observed on the costal pleura, especially in intercostal blood vessels.

The heart was in systole – in the heart cavities had accumulated a small amount of yellow liquid. The epicardium was covered with spotty and stripy haemorrhages, especially on the pericardial and epicardial fat (the latter showed strongly in all animals). The spleen was slightly enlarged, of a doughy consistence, and the capsule was tight, shiny.

The liver was also slightly enlarged, red-brown, and full of blood. Consistency was soft/slack. The liver was marked by a number of small, poorly differentiated dark red areas. The kidneys were full of blood, especially in the medulla [or possibly the cortex] and intermediate layers [в мозговом и промежуточном слоях]. The consistence of the kidneys was slack and small lighter brown patches were observed on the surface.

The rumen was full of fodder. Fodder in the [probably] omasum or abomasum [в поджелудках] was very moist, the large intestine (the mucous membranes of which were normal) was full of half liquid faecal matter. In the rectum faeces were well formed.

Vessels in the brain membranes were highly 'injected', with some small haemorrhages; brain matter was of a springy-dense consistency. The choroid plexus was oedematous and dark red.

Diagnosis: Oblast veterinary workers also found analogous pathological changes during autopsies carried out earlier on. The overall pathology is a haemorrhagic syndrome – haemorrhagic lymphadenitis, splenitis, pulmonary oedema, general phlebostasis [венозный застой], degeneration of the liver [дистрофия печен] and kidneys.

Laboratory research in Kurgal'dzhinskii and Tselinogad oblasts found a *Pasteurella* spp. organism, highly pathogenic to white mice. Analogous micro-organisms were found under the microscope in the material collected by the investigative commission, but no details are given.

In view of the recent spraying with herbicide, the contents of the rumen and material from the liver & kidneys were examined. The presence of compounds from the herbicides was not confirmed.

The authors conclude that Pasteurellosis[3] was the cause, and that it was triggered by acute stress experienced by the saiga when they were chased for many kilometres from the agricultural fields, although they still do not completely exclude a possible role for the herbicide.

[3] The authors do not specify which form.

4. 1981 Mortality Event- Betpak-dala population

(i) Aerial report 1981 (Institute of Zoology and Department of Reserves and Hunting, 1981)

Date and location of deaths: Deaths were recorded from 24th to 28th May just north of the river Uly-zhylanshyk. Deaths occurred after calving, which had occurred south of the river. Females with calves then moved north to summer areas, which is where the die-off occurred. The females and calves crossed the river Uly-zhylanshyk close to village of Aigyrkum and died on the territory between the rivers Turgai and Uly-zhylanshyk.[4]

Carcass counts and mortality estimation: Carcasses were counted on 10th June, by which time the sovkhoz had organised some removal of carcasses, so although 44,000 carcasses were counted from the plane, total deaths were estimated at 60,000-70,000.

Carcass Density: The die-off area covered around 1300km^2, a relatively small territory. The area of high density of saiga carcasses was small and density abruptly dropped off outside this area. The density of carcasses ranged from:

- 80 per km^2 (over area of 220km^2 (or 15x15km) total of 18,000 dead)
- 30 per km^2 (over area of 400km^2; total of 12,000 dead)
- 11 per km^2 (over area of 600 km^2; total of 7000 dead).

On 11th June a plane also flew just south of the die-off area - no saiga were seen, so authors concluded that migration of remaining saiga was over and no saiga were likely to arrive in die off area from the south. Locations surveyed at that time were:

- West: lake Saga
- South: lake Donsar
- East: Metstansia Brali
- North: Sovhoz Rodnik (village Aigyrkum)

Weather: Winter was not severe; there was no *dzhut* or mass mortality at that time. But spring was cold and vegetation green-up late. Precipitation was high across the whole of Kazakhstan, from the 9-14 April. This fell as snow in much of Aktiubinsk and Dzhezkazgan oblasts, and cold temperatures also recorded in the south at this time. Wet conditions continued right up to the end of April. This led to high water levels in rivers and other water bodies and flooding of major rivers such as Chu, Sarysu and Emba. This led in turn to a change in migration routes. During the annual count (14-15 April) saiga were not seen in their usual territories for this period (Kalmakkyrgan, Baikonur, Miur), being located in the pri-Aral Karakum areas instead. The saiga were not seen in their usual haunts along the River Turgai, passing closer to Dzhezkazgan town instead. Overall the high humidity, rainfall and cold affected all three populations, but did not lead directly to any mass mortality at the time (April). However, it led to late migration – many saiga stayed in wintering grounds for longer and had to change migration routes due to flooding.

Conclusions and diagnosis: In the conclusions pastuerellosis is given as the cause but no detail is given on lab analysis or necropsy.

[4] The report includes a map of the overall area – which has been digitised. This map suggests that some animals died at the actual calving site also, which is corroborated by local observers. The GIS file reflects these observations.

(ii) Expedition report 1981 (Institute of Zoology and Department of Reserves and Hunting, 1982)

Migration and weather: The report includes little material on the actual die off, but a lot on movements generally. It again mentions the late migration of Betpak-dala population due to cold weather – snow melted only slowly at the end of the winter. At the end of May, saiga populations were noted on the 'virgin' sovkhoz of Karaganda Oblast; at the borders of Tselinograd, Dzhezkazgan and Turgai oblasts; between the Turgai and Uly-zhylanshyk rivers and in the Mugodzharski Mountains.

In June they continued on to the north, not to their usual summer areas, but much further south – to the rivers Kulanutepe, Kon, Uly-zhylanshyk, Ul'koyal, and the Mugodzharski Mountains.[5] Males migrated directly to summer grounds, whilst females stayed longer around the calving areas.[6] The authors note that snow melted late at calving areas, but there is no mention of bad weather during the calving period itself.[7]

Mortality: Most deaths concerned females and calves.[8]

Locations of die-off: Some locational information is given for the die-off areas, which were centered on Krupskoi and Amangeldy sovkhoz (this concurs with the map from the aerial report). Dead saiga were also observed by expedition members on the territory of sovkhoz Schliemann and Jubilee of Turgai Oblast. At these locations, six dead saiga were observed along the road, over a distance of 30km. Two more were found on the territory of sovkhoz Koslova in Kustanai Oblast.[9]

5. 1984 Mortality Event – Ural population

(i) Commission Report 1984 (Aikimbaev et al., 1984)[10]

Mortality: The authors state that around 10% of the Ural herd died and that the period of mortality was relatively long: stretching from the end of February to 16th March. The animals did not die at once, or over a short period, the event was said to affect mostly weaker animals at the edges of the herd. Between 16th and 20th March the event subsided and only a small number of individual animals were found to be sick. The authors link the end of the event to warming temperatures.[11] The area with corpses was surveyed from the air between the 12th and 14th April.[12] Corpses were found mostly in an area of 50 x 85km around Kyzyl Kapkan, with fewer distributed around Aibas and Urdin anti-plague units.

Live saiga observations: Whilst flying over the area, herds of living saiga were noticed, mostly around Kyzyl Kapkan and 50-60km east of there. Each of these herds was between 1.5 and 3 thousand animals.[13]

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[5] Some of these areas are effectively the same as the spring ones.

[6] During the die-off few males were present - it affected females and calves moving north following calving.

[7] The report mentions warm weather during calving on p9, but it is not clear whether this concerns the Ustiurt population only, or all populations.

[8] It is not clear whether these are from the same syndrome or whether calf deaths were due to starvation following death of the mother.

[9] These areas are much further north than the die-off area recorded by the aerial survey and these deaths are likely to represent background mortality.

[10] The date of this report is the 20th April 1984 – so before the later April flights and census established the full extent of the damage – which was found to have reduced the total Ural herd by much more than the 10% given here.

[11] The aerial report mentions deaths continuing into April.

[12] The aerial report states that the April counts were from 26-29th of that month.

[13] This suggests that the animals still living in mid-April were found in roughly the same places as those that died at the end of March.

Diagnosis and sampling: Ground expeditions were undertaken from 10-20 April.[14] Autopsy and analysis from the April trip is discussed in this report, but information is identical to that in Aikimbaev et al. (1985), summarised below and so not repeated here.

Conclusions: The proximate causal pathogen was identified as *Pasteurella haemolytica*.[15] Virulence was considered to have been initiated by a very fast increase in saiga numbers, their high density, and cold weather. Given the unusual form of *pasteurella* in saiga from Urdin area, (showing a weaker, more chronic form of pasteurellosis) the question is raised of role of individual animals in carrying disease between epidemics. Here, although some animals died, mortality rates were much lower than in other areas (see also Aikimbaev et al. (1985) below).

(ii) Aerial report 1984 (Institute of Zoology and Department of Reserves and Hunting, 1984)

Timing: Die-off occurred from the end of February to middle of March, but authors note deaths running into April also and individual cases in May.[16]

Counts in early March: Carcasses were first observed during the March saiga census.[17] On these flights a total distance of 2370km was flown, and 1276 carcasses were observed.[18] Overall the density of dead saiga ranged from 0.7 to 2.0 per km^2.

Routes for the March flights and numbers of saiga recorded:

- 25 Feb: Guriev – Ganiushkino – Guriev: 111 carcasses
- 01 March: Guriev – Azgir – Guriev: 185 carcasses
- 03 March Guriev – Kalmykovo – Guriev: 183 carcasses
- 06 March Guriev – Terenty – Guriev: 797 carcasses
- 02 March – Guriev – Karabay- Taisagai –Gurivev – no dead saiga seen, 20,000 seen alive on that trip
- 06 March – Guriev – Bikey – Nosxx Sam – R Emba, Guriev – again no dead ones seen, not clear whether live ones observed.

Most carcasses were observed in the northern part of Dengiz district (present Kurmangazy district) the south-east part of Kovobogatin district of Guriev (present Atyrau) oblast. Following migration of this group to Ural (West Kazakhstan) oblast, deaths were also registered with analogous symptoms in Taipan and Dzhangalin districts of that oblast.

Population affected: The report states that a commission visited the field from 7 to 10 March – they estimated density of corpses from '03'[19] to 2 per km^2. From a sample of 86 dead animals they calculated that 33% of the dead were adults, and 68% young individuals. So over half of the dead were those born just the year before. A more detailed breakdown is also given:

- Adult males: 15%
- Young females (coming up to 1 year old): 44%

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[14] And from 7-10 March according to aerial report.

[15] Suggesting that this was pneumonic pasteurellosis and not haemorrhagic septicaemia.

[16] It is not clear whether syndrome is the same.

[17] The die-off had not finished by then, so these must be seen as early numbers.

[18] These numbers were extrapolated up to 13,300, for a total area covered of 15,600km^2, but the methodology used is unclear.

[19] Should be 30?

- Adult females: 17%
- Young males: 23%
- Total young animals: 68%

Weather: The winter was not severe, with snow cover no higher than 10-15cm and pasture in good condition (p29); the authors rule out the effect of the rut on males and conclude that the die-off is not related to this.

Symptoms and necropsy (March): [20]Authors note that most of the dead saiga were young born in 1983 and that their nutritional state was satisfactory. The antelope staggered around and did not react to sounds made by humans, or show signs of fear as they would normally do. During external inspection of the bodies, a blood foamy effusion from the nose was observed. Autopsies were conducted on 21 Saiga. Veterinary personnel noted an accumulation of bloody liquid in the trachea, in the kidneys they noted congestion and presence of perirenal fat. The intestines and rumen were full of fodder. The chest cavity exhibited presence of a bloody liquid and also signs of a fibrous exudative inflammation of the lungs and pleura. Some signs of haemorrhage were noted on the lungs. The lungs were oedematous, and a bloody seepage was observed on incision. The heart muscles were 'loose' or flabby and haemorrhages are visible on the atria of some of the animals. The liver was blood-filled, with no particular characteristic changes. Lesions of intestines, stomach or spleen were not noted. The samples from the 21 saiga were taken to the oblast veterinary laboratory. In Dengiz district lab a *Pasteurella* spp. of bacteria was identified (Эисп. No. 118 6 March 1984). The Ural oblast veterinary laboratory found *Pasteurella* bacteria[21] pathogenic to white mice, guinea pigs and rabbits. Material from 12 saiga was sent to other labs for research into possible viruses. In Guriev [currently Atyrau] oblast veterinary laboratory diagnostic research continued. Chemical analysis was undertaken of the rumen contents of 4 saiga, presence of alkaloids was not noted, search for chloro-phosphoorganic pesticides or 'mineral poisons' continued at the oblast laboratory.

Aerial counts April: Aerial counts were made from the 26th April to 28th of April, from one side of the plane only. The plane flew over an area of 464km^2 and overall 1815 dead saiga were counted – with a carcass density ranging from 1.8 to 27.9 per km^2. Apart from carcasses recorded above, there may have been others but it was not possible to fly over the entire territory between the Volga and Ural rivers.

Flight Routes:

- 26 April - Uralsk-Furmanovo – Suiunduk – New Ushtagan – New Kazaanka – Dzhangala
- 27 April – Dzhangala –Novaya Kazaknka – Fakeev – R. Bermrlai - Dzhangala
- 28 April – Dzhangala – Akhmetshkola – Tas – Makhambet – Inderbor - Dzhangala,
- 29 April – Dzhangala – Novaya Kazanka - Aralsor - Furmanova – Uralsk

Of these dates –the highest densities of dead saiga were seen on 28th April [*Reviewer's note: but dates missing in some cases*]. A number of locations are listed at which particularly high densities of dead saiga were observed. These included ***Atyrau oblast****:* Novotroitskoe, Mahambet, Azgir, Novie Ushtagan, sovkhoz Siunduk, Ur. Terekty. ***Ural Oblast***: Malaya Bogda, Kalmykovo village, Taipak, lake Edilsor, Novaya Kazanka, Aralsor, around Khanov. One hunter also made his own personal observations from the plane:

- 26th 160 live 628 dead;
- 27th 1567 live 658 dead;
- 28th 3044 live 1234 dead.

[20] This report was produced in March 1984, before the April analyses described in the Aikimbaev et al. paper below. It thus concerns samples taken in March only.

[21] And other cocci – poorly legible

- In total over 3 flying days, 4741 live saiga and 2420 dead were recorded.

Mortality estimate: The Ural count was repeated in May, during the calving – on the 24th, 25th and 27th in order to investigate the total impact of the die-off. The total recorded number of live saiga at that time was 40,000 adults (or 50,000 according to the model used for correcting count data).[22]

(iii) Published paper on 1984 die-off (Aikimbaev et al., 1985)

Sampling: The die-off occurred in the first two weeks of March – it was mostly over by the 16th. The first set of samples was collected at the beginning of March, during the event. Samples were taken from a number of dead animals, and direct cultures made from parts of lungs, liver, spleen and blood, from which *Pasteurella haemolytica* was isolated at the Central Asian anti-plague institute.

A second set of samples was collected on 12-15 April, so by this time the carcasses were over one month old. Dead animals displayed signs of bleeding from nose, and on necropsy, hyperaemia, and consolidation of the lungs. Hyperaemia and some enlarging of liver and spleen were also observed. The chest cavity was full of a bloody substance.

The April sampling was conducted as follows:

- Kyzyl Kapkan (12-14 km SE of Kyzyl Kapkan) – 2 saiga corpses, one sick animal, 5 healthy saiga & 1 dead Korsac (*Vulpes corsa).*
- Aibas (2km west of Aibas): dead dog which (according to locals) had been eating saiga and dead no longer than 7 days.
- Urdin (Shaman - 60m South east of Urdin) dying saiga – with gangrenous abscess and purulent discharge from anus.

From all these animals smears were taken from internal organs and blood, cultured on Hottinger Agar and used to infect guinea pigs (intraperitoneally) and mice (subcutaneously) using emulsion from lungs, liver, spleen and blood. In total, 44 mice and 27 guinea pigs were infected. Of these, 6 guinea pigs and 5 mice died. Of the passenger animals died: 4 guinea pigs infected with material from the sick saiga found in the vicinity of Kyzyl Kapkan; the dog; and a saiga which had been lying in a polluted lake for two about weeks.

The bioassayed animals, including passenger animals, died within 2-3 days following infection. The pathology included hyperaemia, and abrupt densified and swollen tissue around the site of initial infection, haemorrhages of subcutaneous blood vessels, enlargement and hyperaemia of liver and spleen.

From the corpses of the dead animals collected in the field, were obtained number of unspecific cultures of bacteria, explained by the age of the samples. However, from the smear of the liver and spleen of the sick saiga found close to Kyzyl Kapan, the dog and the saiga from the dirty water, and from the dead white mice and guinea pigs were visible small 'heaped' 'encapsulated' gram negative coccobacillus, whose morphology is characteristic of *Pasteurella spp*.. Upon direct culture of material from the abscess and the purulent fluids from the dying saiga from Urdin area, was extracted gram negative and other micro-organisms, whose morphology was also reminiscent of *Pasteurella* spp. The surviving lab animals were killed 6-7 days after infection and from these no pathological infections could be found.

[22] It is the lower figure of 40,000 which is quoted in Table 25 (p225) of Sokolov and Zhirnov (1998). Given that in 1983 the equivalent number was 150,000, this would give a total mortality of 110,000. Overall in 1984 there were three aerial counts: In March (the standard annual survey, during which mortality was already observed); in April during which carcasses counted (but not exhaustive enough to estimate total mortality); and in May (calving) to produce a new, post-die-off estimate of total numbers.

Cultures of liver, spleen and lung, from those guinea pigs and mice infected with the fluid from the abscess and anus of the dying saiga close to Urdin, following 24 hours after infection, displayed a single type of culture, made up of transparent round columns, also reminiscent of *Pasteurella* growth. From the white mice smears from the liver yielded gram negative cocobacillus identified as *P. Haemolytica*.

In addition, 12 sera were studied from healthy saiga from the territory of Kyzyl Kapkan anti-plague unit and the above mentioned two sick saiga[23] having antibodies to *P. Haemolytica*, with negative results.

Conclusions: The authors' studies in Ural oblast at the end of February and beginning of March 1984, revealed culture of a bacteria identified as *P. Haemolytica*. Samples collected in April were shown to be lethal to lab animals. Authors attribute the cause to high saiga numbers and high densities during the period of cold weather. The unusual form of *Pasteurella* in the saiga from Urdin area, where there was no mass mortality (thus suggesting weak, chronic Pasteurellosis) raises the question of role of individual animals in carrying disease between epidemics. Here, although some animals died, there was not such a mass die off as in other areas.

(iv) Published paper on 1984 die-off (Martinevskii et al., 1985)

In addition to the above study, Martinevskii et al. (1985) investigated some of the characteristics of two of the *P. haemolytica* strains involved in the 1984 outbreak – Nos. 7 and 70. These had the following characteristics:

- The organism has an optimal growth temperature of 37°C on Hottinger Agar, Martin and blood Agar, forming round or more rarely oval colonies.
- The culture causes lysis of blood cells on Hottinger agar with 5% blood of guinea pigs.
- Does not form hydrogen sulphide or indole.[24]
- Gives positive reaction to denitrification.
- Negative reaction to nitrification and to Voges-Proskauer test.
- Exhibits presence of oxidase.
- Does not contain fibrinolysin, coagulase, phenylalanine deaminase, lysine-, arginine, ornithine decarboxylase, does not liquefy gelatine. Does not ferment arabinose, mannose, lactose, trehalose melibiose, adonitol, dulcitol, salicin, or 'exudin'.[25]
- Ferments (with production of acid but without gas): glucose, xylose, galactose, sucrose, maltose, mannose and mannitol.
- Of the three types in the literature (A, T and Z) the strain isolated from saiga differs in terms of its reaction with mannose, xylose and in lack of agglutination on exposure to therapeutic serum and serum from guinea pigs immunised using the reference strain *P. haemolytica* 1059.[26]

[23] Unclear here which ones.

[24] The indole test differentiates *P. multocida* and the various biotypes types formerly known as *P. haemolytica*.

[25] No translation for latter.

[26] *B. trehalosi* (former T strain of *P. haemolytica*) is distinguished from the former A strain (now known as *Mannheimia haemolytica* by fermenting trehalose but not L-arabinose and usually not galactose or D-xylose. This organism is thus not *B. trehalosi*. The Z strain referred to above was not found in literature.

The strain is virulent to guinea pigs and white mice, which die within between 2 and 4 days following infection, exhibiting pathology characteristic of haemorrhagic septicaemia.[27] Cultures were often contaminated by another Gram-negative bacteria which produce gas on breakdown of glucose and other carbohydrates, but this organism is not an antagonist of the *Pasteurella* strain isolated here. *Pasteurella* could be isolated from such mixed culture following injection into laboratory animals and subsequent culture of smears from lungs, liver spleen or blood. The bacteria isolated from saiga are highly sensitive to antibiotics such as streptomycin, penicillin, polymyxin, erythromycin, rifampicin, tetracycline and oleomycin.

The pathology observed upon necropsy of seven saiga is described and were similar between these animals. The lungs were swollen with blood vessels of all dimensions full of blood and extensive focal haemorrhages. In one saiga was observed purulent inflammation in the bronchi and oedematous liquid in the alveoli. In the spleens of 5 individuals were noted circulatory disorders and areas of sub-capsular necrosis (forming a red pulp). In the liver was observed also various stages of necrosis. The kidneys were also affected by circulatory disorders, diffuse necrotic exudative glomerulonephritis and necrotic 'neurosis'. The adrenal glands of two saiga were blood-filled and haemorrhages were observed in the мозговий слой [medulla?]. The authors speculate that cold weather may have brought on the disease.

6. 1988 Mortality Event – Betpak-dala population

(i) Commission Report 1988 (Turgai Regional Executive Committee, 1988)

Timing: Die-off occurred from 14th May to 22th May 1988 in Amangeldy and Dzhangeldin raions of Turgai oblast. The first incidence of death was noted on the 12th May at two sovkhoz (Rodnik and Dzhangildin), and was observed at others on the 14th. Mass deaths occurred from 16th to 22nd May over a larger area.

Mortality: By 25th May 255,000 carcasses of adults were counted. Of these, 8,000 were males and the rest females. In percentages the death toll constituted 3% males and 97% females, of which 30% died directly after calving and the rest were pregnant). Most of the saiga were undernourished. This initial count was undertaken by a team of veterinary specialists who visited the site. At the die off sites, each time they observed small numbers of live adult saiga (in poor condition) and calves without mothers. This suggests calves did not die from the initial syndrome.

On the 26th May, an aerial count was conducted to obtain the final numbers.[28] The estimate was revised to 434,000 adults, which were reported by sovkhoz (in thousands) as follows:

- Krupskoi 151
- Imanova 90
- Dzhangildina – 45
- Rodnik – 9
- Sarytrgaiskii – 7
- Karaturgaiskii – 5
- Enbek – 110
- Kyzyl Askerckii (Dzhangil'dinsk raion) – 18

[27] Although this syndrome is caused by *P. multocida* and the organism in question here is said to be *P. haemolytica*.

[28] But many carcasses had been buried by then.

This number was problematic as the aerial count conducted earlier in the year estimated total adult numbers to be 360,000. Given the 400,000+ reported to have died plus the ~200,000 still observed to be living further east, this caused some to revise this total pre-die-off Betpak-dala population up to 600,000.[29]

Weather: The 1987/8 winter was a very hard one for the Betpak-dala population – in eastern Moiynkum and the Chu mountains snow depth reached 40-60cm. At end of February temperatures were -35C. Around 20,000 saiga died (see more on *dzhut* in the saiga expedition report of that year). Most of the animals were exhausted. In addition, the river Chu was very high that year and acted as a barrier, delaying the migration of the saiga. The high waters in the Turgai basin concentrated saiga over a smaller territory than usual. In April and the first half of May, the weather was cold and wet, again affecting the general condition of the saiga. According to the meteorological station in Arkayka, during the first ten days of May the temperature was very low, dropping in the space of 24 hours. Rainfall was 10-23 mm compared to an average of 4-5mm.

Livestock: During the die-off period, on the sovkhoz concerned were grazing 5,000 cattle, 2,000 horses and 120,000 sheep. But no infection was noted in these animals.

Symptoms and necropsy:

- Most saiga were in poor condition following harsh winter and *dzhut* conditions
- Corpses exhibited inter-maxillary and sub-mandibula oedema
- Bloody froth from nose and from the anus was observed. Around the carcasses was observed a liquid stercoraceous mass mixed with blood.
- During necropsy, haemorrhagic lesions were noted in the internal organs, lymph nodes and mucous membranes.
- Sick animals exhibited strong depression, breathlessness, shaky walking and muscular twitching.
- Sick animals would fall over repeatedly, getting up with difficulty, some were observed in a comatose state.

Details of lab analysis: initial samples collected on 16th May and analysed locally (in Amangeldy and Turgai oblast veterinary labs) already suggested *Pasteurella*. Further samples were taken between 20th and 25th of May and initially taken to oblast veterinary and epidemiological stations. For analysis they used different culture media & laboratory animals to check biochemical composition of cultures. Research eliminated anthrax, cattle plague, sheep pox, leptospirosis, listeria, and other diseases. Samples were taken to labs in Kazan, Russia and to KazNIVI, Volgograd NII MZ SSSR and Kazakh Republic Veterinary Laboratory. Clinical analyses suggest *Pasteurella* – the pathogen was said to exhibit biochemical characteristics of *Pasteurella multocida*, not different from those strains present in the environment.

At the same time as the veterinary samples were collected, analyses were conducted on plants, soils and water, looking for nitrates, nitrites, pesticides, and other toxic substances. The plant samples consisted mainly of *Stipa* spp., *Poa bulbosa, Anabasis* and *Atriplex* spp. They found alpha isomer GKHTSG and some poisonous species including *klopovnika, gorchaka, rogogl, avnika, bryntsa liuikovykh* (Rus.). On many plants was observed the development of various types of fungi, the toxic properties of which were said to merit further study. On the territories of Turgai oblast, numerous herbicides are used including *granozan, dendrobatsellin, dilen, oktopan, butalon, avddeks, triallat* and others. These were applied from the air and on the ground from 6 May to 20 June. Radioactivity levels in soils, vegetation and livestock products were equivalent to normal

[29] The aerial count report authors believe the 434,000 estimate to be erroneous - see below.

background levels. Having said this, the level in saiga livers was 2497 bk/kg, compared to an expected norm of 600 bk/kg.

Causes: Official diagnosis is pasteurellosis caused by *Pasteurella multocida.*[30] The report notes that this bacteria is found in healthy animals and becomes highly virulent under conditions of stress. The *P. Multocida* strain involved is the same as the one naturally present in the environment. The report concludes that cold weather lowered resistance to the disease.

(ii) Aerial Report 1988 (Institute of Zoology and Betpak-dala State Hunting Organisation, 1988)

Mortality: The official number of dead (as from map) of 430,000 is overstated. The authors believe this is the case because the sovkhoz workers were paid to bury carcasses per head. The aerial team could not definitely confirm this figure because at the moment of the count the burial of carcasses was well underway and some centres of infection (die-off sites) could already not be observed. So the aerial team suggest a number of 270,000 deaths in total. The team, both with vehicle and plane, also observed in detail the eastern wing of the Betpak-dala population - east of Dzhezkazgan town in May-June. Here, they observed 180-190,000 adults, so there were not no massive die offs there and calving proceeded normally.

(iii) Expedition report (Institute of Zoology and Betpak-dala State Hunting Organisation, 1989)

The expedition report mostly repeats the information in the Commission report above. They suggest that pasteurellosis could have been triggered by cold weather – during first 10 days of May temperatures were between 1 and 5 ºC. They do not exclude possible toxins in the environment, mentioning high radioactivity in liver.

They list the die-off locations as above, noting that their size ranged from 1-2 km^2 up to 100-130 km^2. The number of carcasses in some areas reached 40-50 per ha. Most of the dead were females, concentrated in calving areas. Adult males made up 3-8% of total adults dead. They conclude that the total number of deaths was probably around 270,000.

7. 2013 Mortality Event - Betpak-dala population

Lastly, we include here details of a small mortality event which occurred in 2013. Source: Kazakh News article, ASTANA, September 10 (Novosti Kazakhstana, 2013).

On 7th September, the Ministry of Environmental Protection (MEP) reported Saiga carcasses found on the southern, western and northern shores of Lake Tengiz in Karaganda and Akmola regions. As a result of land-based and aerial surveys, the total number of dead animals was found to be 493 individuals. It is not clear when the animals died, but presumably death occurred sometime in the first week of September.

The head of the Committee for Forestry and Hunting of the MEP, Bakytbek Duisekeev, suggested that results of the examination of biological and pathological data shows that the cause of death of animals was pasteurellosis. But the carcasses were too decomposed for firm diagnosis (Orynbayaev, pers. comm.).

[30] The Kazakh lab (Aikimbaev and Martinevskii) reported *P. haemolytica* but the Russian lab results identified *P. multocida*. They thus erased *haemolytica* out throughout the report, replacing it with *P. multocida* (Grachev pers. comm.).

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