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1 Seroprevalence of infectious diseases in saiga antelope (*Saiga tatarica tatarica*) in Kazakhstan
2 2012-2014

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16

17 ABSTRACT

18 286 serum samples were collected from three sub-populations of saiga in Kazakhstan
19 (Betpakdala, Ustyurt and Volga-Ural) between 2012 and 2014, and were tested for the presence
20 of antibodies to *Brucella* spp., bluetongue virus, peste des petits ruminants (PPR) virus, Akabane
21 virus, Schmallenberg virus, *Chlamydomphila*, *Toxoplasma*, *Mycobacterium avium* subspecies
22 *paratuberculosis* and *Coxiella burnetii* (Q Fever). Seropositives to *Coxiella burnetii* of saiga
23 were detected and the adjusted seroprevalence of Q Fever antibodies was 0.07 (95% confidence
24 interval (CI): 0.03-0.10). Seropositives to Akabane virus were detected in all three populations
25 and the adjusted seroprevalence values for this virus were very high (all were >0.13). Lower
26 adjusted seroprevalence values were estimated for PPR Virus and *Mycobacterium avium* subsp.
27 *paratuberculosis* (0.005 and 0.006). No seropositives for bluetongue, *Toxoplasma*, *Brucella* or
28 Schmallenberg were detected.

29

30 **Keywords**

31 Saiga, antelope, surveillance, seroprevalence, wildlife.

32

33 **Introduction**

34 The role of wildlife in the maintenance and spread of infectious diseases in Kazakhstan is not
35 known. The interface between domestic and wild animals is a potential concern in the control of
36 infectious diseases and of increasing research interest (Weitholter et al., 2015) but the
37 significance of wildlife reservoirs in relation to livestock or human diseases is largely unknown
38 or putative in the majority of cases (Miller et al., 2013; Kock, 2014). The risk of direct
39 transmission of infection between free-ranging populations of wildlife and domestic animals is
40 low and more likely to occur through indirect routes such as insect vectors, fomites, watering and
41 supplementary feeding points or contamination of shared pasture or where populations are
42 restricted through fencing (Kock, 2014). It has been shown that some wild animals for example,

43 in the United States (Miller et al., 2013), are a potential constraint to control of infectious
44 diseases in livestock but the links are putative. More usually the main driver of ongoing
45 transmission is the domestic animal and when infection is controlled in these populations
46 prevalence in wildlife declines (Serrano et al., 2012).

47 Most of the global population of saiga are found in the Republic of Kazakhstan, where it is one
48 of the most numerous species of wild cloven-hooved animals. The saiga migrates large distances
49 twice-annually. After a dramatic collapse from over a million animals in 1993, to an estimated
50 178,000 in 2000 based on aerial and vehicle surveys (Milner Gulland et al., 2001) and an
51 estimated 20,000 in 2003 (Anon, 2015), the saiga population had significantly increased to an
52 estimated 216,500 animals by aerial survey in 2014 (unpublished data, ACBK 2014). In spring
53 2015 over 150,000 saiga in the Betpakdala population in the Republic of Kazakhstan died over
54 the course of a few weeks, and the mortality event is still being investigated (Anon 2015). As the
55 saiga population increases, the overall area of the habitat occupied by saiga can be expected to
56 increase, as well as the density of saiga. It is likely that this will result in increased proximity
57 between saiga and domestic livestock, with a resultant increased risk of indirect disease
58 transmission both from saiga to domestic livestock and vice versa. Saiga is a migratory species
59 and the population range is many thousands of kilometers a year including in the western
60 subpopulation, transboundary movements.

61 Review of literature suggests that saiga may be susceptible to livestock diseases. For example
62 foot-and-mouth disease (FMD) has been reported to cause epidemic disease in saiga 1955, 1956,
63 1958, 1967, 1969 and 1974 (Kindyakov et al., 1970; Fadeev & Sludskii, 1982) but mortalities
64 were only significant in 1967. Pasteurellosis has also been reported in 1974, 1981, 1984, 1988,
65 2010 and 2011 as a cause of mass mortality but pathological evidence to justify this diagnosis is
66 scanty (Statsenko 1980; Fadeev & Sludskii, 1982; Aikimbaev et al., 1985). These outbreaks
67 involved many tens of thousands of animals. In many cases opportunistic bacteria might be a
68 proximate cause of death but not the underlying cause. Isolation of *Pasteurella* spp. from healthy

69 saiga and other wildlife species has been reported and without pathological diagnosis isolation of
70 these pathogens alone cannot be considered a diagnosis (Besser et al., 2013). Other pathogens
71 such as *Listeria*, *Brucella*, *Coccidia*, *Toxoplasma*, *Clostridia*, *E. coli* and *Streptococcus* have
72 been detected, sometimes over half a century ago and only on a few occasions. Furthermore their
73 role in disease of saiga is unproven and the epidemiological significance is unknown (Galuzo et
74 al., 1963; Tilga, 1964; Aykimbaev et al., 1985; Ivanov et al., 1998).

75 Given the paucity of knowledge on diseases of saiga, it is important not to speculate on historical
76 isolation of pathogens as evidence of disease, without reliable epidemiological and pathological
77 evidence to support the diagnosis. Work undertaken in Kazakhstan since 2012 is the first attempt
78 to examine saiga mortalities systematically using agreed protocols and more detailed eco-
79 epidemiological investigations.

80 The paper describes findings of research to determine the current seroprevalence of bluetongue,
81 PPR, Schmallenberg, Q fever, *Toxoplasma*, *Chlamydomphila*, Akabane, *Mycobacterium avium*
82 subsp. *paratuberculosis* and *Brucella* in different subpopulations of saiga in Kazakhstan between
83 2012 and 2014.

84

85 **Materials and methods**

86 These studies were conducted as part of a national epizootic monitoring program on saiga
87 infectious diseases in Kazakhstan and research towards the development of disease prevention
88 methodology initiated after recommendations of an independent review commissioned by Fauna
89 and Flora International (FFI) under the Convention of Migratory Species of the United Nations
90 Two hundred and eighty-six blood samples were collected from free-ranging captured saiga, 47
91 of them in 2012, 109 in 2013, and 130 in 2014 (Fig 1). Serum was extracted in the field and
92 frozen at -196°C (in liquid nitrogen).

93 Sera were taken from adult saiga during the rut period in autumn, when the saiga gather together
94 in three distinct mass herds (Betpakdala, Ustyurt and Volga-Ural). The locations of the herds

95 were identified using satellite collars that had been previously fitted to saiga from all three
96 populations. All mass herds were sampled. Individual saiga were selected on the basis that they
97 could be caught, because saiga are extremely fearful of humans and catching specific individuals
98 is very difficult. The saiga were captured by trained staff. Saiga were individually herded into a
99 raised net using motorbikes, and then physically restrained for sampling. The average time from
100 the point of first chasing the animal to capture was 3-4 minutes and from physical restraint to
101 releasing them was 3-4 minutes (total time 6-8 minutes). If it was not possible to catch the
102 animal during 4 minutes, chasing was stopped. Manipulations included radio collaring,
103 biological measurements and sampling including for this study. Whole blood was taken from the
104 jugular vein using the vacutainer method for serum collection (Becton Dickonson – USA) and a
105 1¼ inch, 21 gauge needle. Serum was transported at -196⁰C to the laboratory within 30 days and
106 placed in a -20⁰C freezer.

107 For the detection of antibodies to bluetongue, PPR virus, *Toxoplasma*, *Coxiella burnetti*,
108 *Mycobacterium avium* subsp. *paratuberculosis*, *Chlamydomphila*, Akabane virus and
109 Schmallenberg virus, we used commercial ELISA kits (ID-Vet, France).

110 We used a competitive ELISA (SVANOVIR) for detection of anti-*Brucella* antibodies
111 The apparent seroprevalence values were estimated as the number of seropositives divided by the
112 number tested. In order to adjust for the tests used, sensitivity and specificity values were
113 obtained from the manufacturers; adjusted seroprevalence values and 95% confidence intervals
114 were estimated according to the method described by Rogan and Gladen (1978), using an online
115 tool (AusVet, 2015a). If a range of values for sensitivity and specificity were given by the
116 manufacturer, the lower values were used. The 95% confidence intervals incorporate uncertainty
117 in the true sensitivity and specificity of the test, taking into account the sample sizes that the
118 sensitivity and specificity were estimated from.

119 A risk factor analysis was conducted in R version 3.2.0 (R Core Team, 2015) to assess the
120 evidence for a statistical association between age, gender, location or year of study and
121 individual saiga being seropositive to each pathogen.
122 Age was categorized as 6 months or younger or older than 6 months; year of study was
123 categorized as 2012, 2013 or 2014; and location was categorized as “Betpak dala”, “Ural” or
124 “Ustiurt” – the three distinct aggregations of saiga in Kazakhstan.
125 The Fisher’s exact test was used to assess any statistical significance of associations between
126 serological status for each pathogen and each risk factor. Risk factors with a univariable p value
127 of <0.2 were included in a multivariable logistic regression model, and subsequently dropped
128 from the model if they had a p value of >0.1 and they did not affect the odds ratios of the other
129 variables by more than 5%. Interaction terms were added to the model, and their significance
130 tested using the likelihood ratio test.

131

132 **Results**

133 Table 1 shows the apparent and adjusted seroprevalence values for *Coxiella burnetti*,
134 *Mycobacterium avium* subsp. *paratuberculosis*, Akabane virus and PPR virus. There were no
135 seropositives to the following pathogens: *Brucella* (0/288), Bluetongue virus (0/268),
136 *Toxoplasma* (0/346), Schmallerberg virus (0/346) or *Chlamydomphila* (0/346).

137

138 Table 1. Apparent and adjusted seroprevalence of several pathogens in saiga antelope.
 139 Also shown are the sensitivity and specificity values, and the number of samples on which these
 140 estimates were based, obtained from the manufacturers of the ELISAs used. The adjusted
 141 seroprevalence and 95% confidence interval takes into account uncertainty in the true sensitivity
 142 and specificity values.

Pathogen	Apparent seroprevalence (number sampled)	Sensitivity (number tested)	Specificity (number tested)	Adjusted seroprevalence (95% confidence interval)
<i>Coxiella burnetti</i>	0.06 (256)	0.93 (14)	1.0 (250)	0.07 (0.03 - 0.10)
<i>Mycobacterium avium subsp. paratuberculosis</i>	0.003 (346)	0.57 (102)	1.0 (800)	0.005 (0 - 0.02)
Akabane virus	0.10 (346)	0.78 (50)	1.0 (324)	0.13 (0.09 - 0.18)
PPR Virus	0.006 (346)	1.0 (28)	1.0 (391)	0.006 (0 - 0.014)

143
 144 Tables 2 and 3 show the final results of the multivariable logistic regression models for the two
 145 pathogens for which there were more than one seropositive: *Coxiella burnetti* and Akabane
 146 virus.
 147 Saiga were more likely to be seropositive to *Coxiella burnetti* if they were over 6 months ($p =$
 148 0.007) and female ($p = 0.04$). There was no association between *Coxiella burnetti* serological
 149 status and location ($p = 0.46$). There was an apparent increased risk of positive *Coxiella burnetti*
 150 status in 2014 compared with 2013, but this association became statistically insignificant after
 151 controlling for the confounding effects of age and gender ($p = 0.34$).
 152 Saiga had sixty times the odds of being seropositive to Akabane virus in 2012 compared to 2013
 153 ($p < 0.001$). There was no association between Akabane serostatus and age ($p = 0.86$). There was
 154 an apparent increased risk of positive Akabane status in the Ustiurt herd, and in females, but
 155 these associations became statistically insignificant after controlling for the confounding effect
 156 of year of sampling ($p = 0.998$ and $p = 0.27$, respectively).
 157

158 Table 2. Logistic regression analysis of risk factors for positive *Coxiella burnetti* serostatus in
 159 saiga.

Risk factor	Subgroup	Apparent seroprevalence (number sampled)	Odds ratio (95% confidence interval)	p value
Age	<= 6 months	0.008 (119)	baseline	-
	> 6 months	0.12 (137)	16.4 (2.1 – 127.1)	0.007
Gender	Male	0.04 (136)	baseline	-
	Female	0.09 (120)	3.2 (1.1 – 9.8)	0.04?

160

161 Table 3. Logistic regression analysis of risk factors for positive Akabane virus serostatus in
 162 saiga.

Risk factor	Subgroup	Apparent seroprevalence (number sampled)	Odds ratio (95% confidence interval)	p value
Year	2012	0.81 (36)	60.4 (19.6 – 186)	<0,001
	2013	0.06 (109)	baseline	-
	2014	0.0 (130)	0	0.99?

163

164 Discussion

165 Currently the role of saiga in the transmission of infectious diseases of ruminants in Kazakhstan
 166 is unknown. The present studies were conducted to explore the seroprevalence of pathogens in
 167 different populations of saiga in Kazakhstan.

168 There is no data about the prevalence of Q fever among wild and domestic animals in the
 169 territory of Kazakhstan in the available literature. Our studies detected seropositives to *Coxiella*
 170 *burnetti* in the Betpakdala population in 2014. Previously cases of Q fever in deer and mouflon
 171 (Ruiz-Fons et al., 2008; López-Olvera et al., 2009) have been described, but so far it is not clear
 172 whether wildlife is a reservoir for disease in livestock.

173 Akabane disease is widespread throughout the world (Kono et al., 2008), but there is no data on
 174 the prevalence of the disease in domestic and wild animals in the countries of Central Asia,
 175 including Kazakhstan. Our results have shown that animals in all three populations were
 176 seropositive to Akabane virus by this test. A high percentage of seropositive animals in all

177 studied regions indicate widespread infection in the studied regions most probably via the
178 *Culicoides* midge which are also abundant in the habitat. The high seroprevalence in this species
179 might indicate that it is a natural reservoir of infection for Akabane virus in this region.

180 *Mycobacterium avium* subsp. *paratuberculosis* has been isolated in free-ranging, captive and
181 semi-captive wildlife in Europe but rarely is disease reported outside of farmed wildlife
182 (Glawischnig et al., 2006). In Kazakhstan paratuberculosis in domestic and wild animals has not
183 been reported. In the present study one seropositive animal to paratuberculosis was detected in
184 the Volga-Ural population but the significance of this finding is uncertain. In the study we used
185 ID Screen® Paratuberculosis Indirect, which is designed to detect antibodies in sheep, goats and
186 cattle. It is possible that the one positive result represents a false positive due to a non-specific
187 reaction. The monitoring of paratuberculosis in domestic and wild animals and standardization of
188 tests for wildlife, including the saiga, needs more research.

189 Previously it was shown that saiga showed positive antibodies to *Toxoplasma* but the
190 significance of this finding is unknown (Galuzo et al., 1963). In our study using an ID Screen®
191 Toxoplasmosis Indirect Multi-species kit, antibodies to *Toxoplasma* were not found.

192 Brucellosis in wild and captive saiga has been previously reported (Ivanov et al., 1998) however
193 this was during a period when the saiga and livestock populations in Kazakhstan were much
194 higher. The relative importance of wildlife in the transmission of *Brucella* spp. varies according
195 to the context (Ferroglio et al., 2007; Lopez-Olvera et al., 2009;). In Kazakhstan, brucellosis is
196 common amongst domestic livestock, and it is thought that saiga are currently unlikely to play a
197 role in its transmission, due to their relatively low population and lack of close contact with
198 livestock at calving times (when *Brucella* would be transmitted via placenta and placental fluids)
199 (Beauvais et al., 2014). Our study found no saiga with antibodies to brucellosis, supporting the
200 assertion that they are not involved in the ongoing transmission of the disease amongst domestic
201 livestock.

202 Lundervold reported the possibility of bluetongue and PPR virus infection in saiga in Kazakhstan
203 (Lundervold et al., 2004). Our study detected no seropositives to bluetongue, PPR,
204 Schmallerberg or *Chlamydomphila* but it is noteworthy that PPR has recently been confirmed in
205 small ruminants in southern Kazakhstan for the first time in 2014 (Kock et al., 2015).
206 The analysis is based on sensitivity and specificity values obtained from the manufacturers of the
207 tests, however none of the tests have been specifically validated in saiga. Conservative estimates
208 were therefore made, where possible, however it is possible that test accuracy could have been
209 under- or over-estimated.
210 An important assumption of the analysis is that the saiga were randomly sampled. In practice,
211 this is not possible, and so the results should be interpreted cautiously. Under or over-sampling
212 of a certain age-group or gender could have biased the results.

213

214 **Conclusion**

215 These data, for the first time, indicate probable infection of saiga population with Akabane virus
216 of which it may be a natural host and, *Coxiella burnettii*, the cause of Q fever a disease of
217 ruminants and a zoonosis. Negative results from tests detecting antibodies to a range of other
218 infectious diseases suggest that these infections and/or diseases are not likely prevalent in the
219 recently sampled populations of saiga in Kazakhstan but absence cannot be confirmed for certain
220 from this relatively small sample size.

221

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225

226

227

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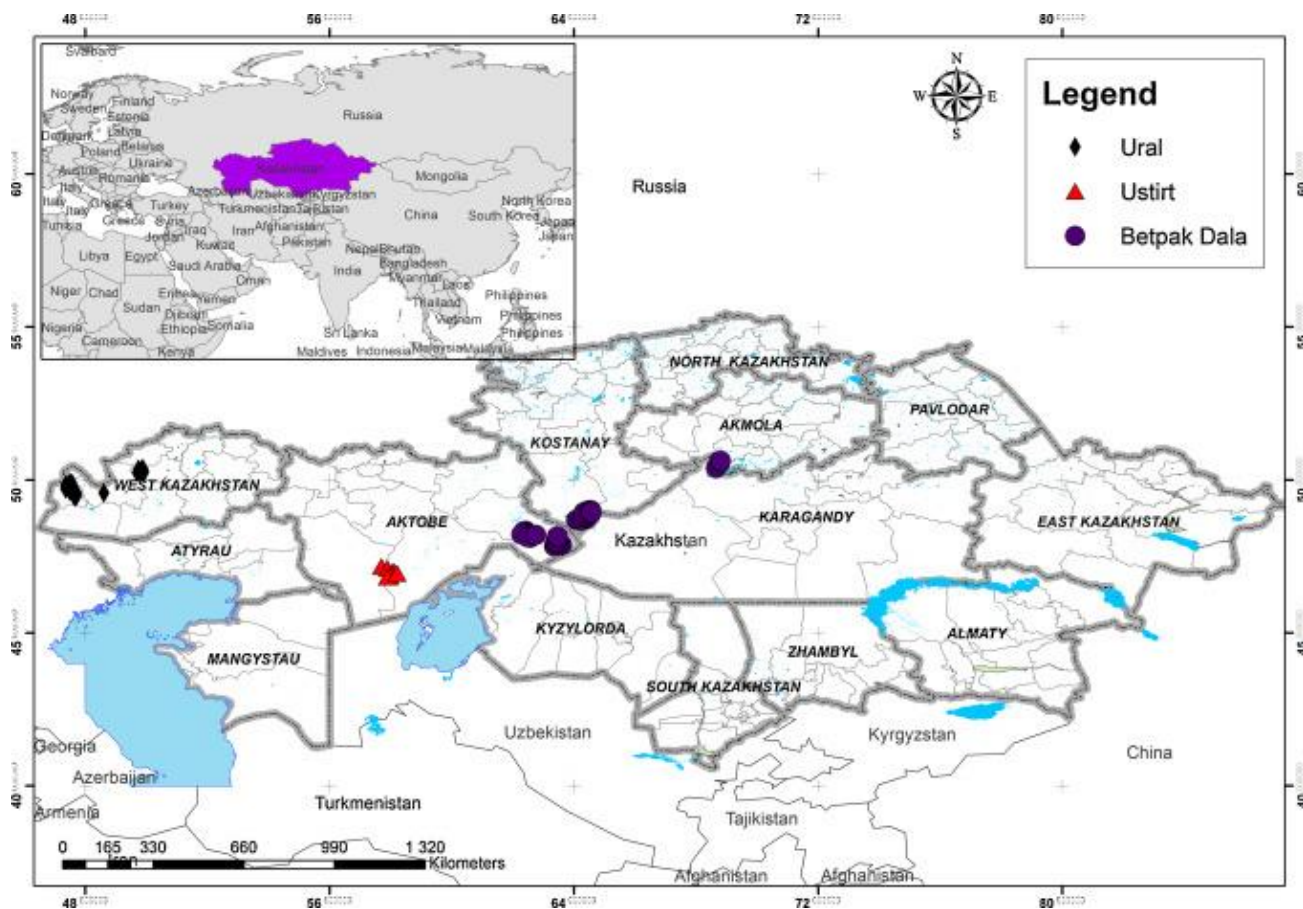
300 **Conflict of interest**

301 None.

302

303 **Figure captions**

304 Figure 1. Map of Kazakhstan showing the locations where samples were collected.



305