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

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ABSTRACT

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

Keywords

Saiga, antelope, surveillance, seroprevalence, wildlife.

Introduction

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

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

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

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

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

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

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

Materials and methods

These studies were conducted as part of a national epizootic monitoring program on saiga infectious diseases in Kazakhstan and research towards the development of disease prevention methodology initiated after recommendations of an independent review commissioned by Fauna and Flora International (FFI) under the Convention of Migratory Species of the United Nations

Two hundred and eighty-six blood samples were collected from free-ranging captured saiga, 47 of them in 2012, 109 in 2013, and 130 in 2014 (Fig 1). Serum was extracted in the field and frozen at -196^{O}C (in liquid nitrogen).

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

were identified using satellite collars that had been previously fitted to saiga from all three populations. All mass herds were sampled. Individual saiga were selected on the basis that they could be caught, because saiga are extremely fearful of humans and catching specific individuals is very difficult. The saiga were captured by trained staff. Saiga were individually herded into a raised net using motorbikes, and then physically restrained for sampling. The average time from the point of first chasing the animal to capture was 3-4 minutes and from physical restraint to releasing them was 3-4 minutes (total time 6-8 minutes). If it was not possible to catch the animal during 4 minutes, chasing was stopped. Manipulations included radio collaring, biological measurements and sampling including for this study. Whole blood was taken from the jugular vein using the vacutainer method for serum collection (Becton Dickonson – USA) and a 1¼ inch, 21 gauge needle. Serum was transported at -196^{0}C to the laboratory within 30 days and placed in a -20^{0}C freezer.

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

We used a competitive ELISA (SVANOVIR) for detection of anti-*Brucella* antibodies

The apparent seroprevalence values were estimated as the number of seropositives divided by the number tested. In order to adjust for the tests used, sensitivity and specificity values were obtained from the manufacturers; adjusted seroprevalence values and 95% confidence intervals were estimated according to the method described by Rogan and Gladen (1978), using an online tool (AusVet, 2015a). If a range of values for sensitivity and specificity were given by the manufacturer, the lower values were used. The 95% confidence intervals incorporate uncertainty in the true sensitivity and specificity of the test, taking into account the sample sizes that the sensitivity and specificity were estimated from.

A risk factor analysis was conducted in R version 3.2.0 (R Core Team, 2015) to assess the evidence for a statistical association between age, gender, location or year of study and individual saiga being seropositive to each pathogen.

Age was categorized as 6 months or younger or older than 6 months; year of study was categorized as 2012, 2013 or 2014; and location was categorized as "Betpak dala", "Ural" or "Ustiurt" – the three distinct aggregations of saiga in Kazakhstan.

The Fisher's exact test was used to assess any statistical significance of associations between serological status for each pathogen and each risk factor. Risk factors with a univariable p value of <0.2 were included in a multivariable logistic regression model, and subsequently dropped from the model if they had a p value of >0.1 and they did not affect the odds ratios of the other variables by more than 5%. Interaction terms were added to the model, and their significance tested using the likelihood ratio test.

Results

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

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

Pathogen	Apparent seroprevalence (number sampled)	Sensitivity (number tested)	Specificity (number tested)	Adjusted seroprevalence (95% confidence interval)
Coxiella burnetti	0.06 (256)	0.93 (14)	1.0 (250)	0.07 (0.03 - 0.10)
Mycobacterium avium subsp. paratuberculosis	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)

Tables 2 and 3 show the final results of the multivariable logistic regression models for the two pathogens for which there were more than one seropositive: *Coxiella burnetti* and Akabane virus.

Saiga were more likely to be seropositive to *Coxiella burnetti* if they were over 6 months ($p = 0.007$) and female ($p = 0.04$). There was no association between *Coxiella burnetti* serological status and location ($p = 0.46$). There was an apparent increased risk of positive *Coxiella burnetti* status in 2014 compared with 2013, but this association became statistically insignificant after controlling for the confounding effects of age and gender ($p= 0.34$).

Saiga had sixty times the odds of being seropositive to Akabane virus in 2012 compared to 2013 ($p <0,001$). There was no association between Akabane serostatus and age ($p = 0.86$). There was an apparent increased risk of positive Akabane status in the Ustiurt herd, and in females, but these associations became statistically insignificant after controlling for the confounding effect of year of sampling ($p = 0.998$ and $p = 0.27$, respectively).

Table 2. Logistic regression analysis of risk factors for positive Coxiella burnetti serostatus in 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?

Table 3. Logistic regression analysis of risk factors for positive Akabane virus serostatus in 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?

Discussion

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

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

Akabane disease is widespread throughout the world (Kono et al., 2008), but there is no data on the prevalence of the disease in domestic and wild animals in the countries of Central Asia, including Kazakhstan. Our results have shown that animals in all three populations were 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.

Lundervold reported the possibility of bluetongue and PPR virus infection in saiga in Kazakhstan (Lundervold et al., 2004). Our study detected no seropositives to bluetongue, PPR, Schmallenberg or *Chlamydophila* but it is noteworthy that PPR has recently been confirmed in small ruminants in southern Kazakhstan for the first time in 2014 (Kock et al., 2015).

The analysis is based on sensitivity and specificity values obtained from the manufacturers of the tests, however none of the tests have been specifically validated in saiga. Conservative estimates were therefore made, where possible, however it is possible that test accuracy could have been under- or over-estimated.

An important assumption of the analysis is that the saiga were randomly sampled. In practice, this is not possible, and so the results should be interpreted cautiously. Under or over-sampling of a certain age-group or gender could have biased the results.

Conclusion

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

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Conflict of interest

None.

Figure captions

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