Detection of antibodies to peste-des-petits-ruminants virus in the semi-domesticated yak

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Abstract
Peste des petits ruminants (PPR) is a highly contagious and acute viral disease of small ruminants. Occasionally, PPR can affect wildlife with devastating results, such as the recent outbreaks in Mongolian saiga. The yak (Bos grunniens and Bos mutus) is a large ruminant found throughout the Himalayan region of south Central Asia, the Tibetan Plateau and as far north as Mongolia and Russia. In Pakistan, yaks are confined to the high plateau of the Northern Areas, from Gilgit to the valley of Ladakh. In Tajikistan, yaks are present in districts in the north, east and southeast of the country. Commonly, yak intermingle with domestic animals during winter when mountainous summer pastures are covered with snow, and with wildlife during summer pastures. PPR is considered endemic in sheep and goats in Pakistan and Tajikistan. In this study, we investigated the potential presence of antibodies to peste-des-petits-ruminants virus (PPRV) in yak populations using ELISA. A total of 250 (Pakistan) and 85 (Tajikistan) serum samples of healthy yaks were collected. None of the Tajik yaks were seropositive (95% confidence interval (CI) 0.0–4.2%), while 23 of 250 (9.2%; 95% CI 5.9–13.5%) yaks sampled in Pakistan were found positive. Whether PPRV is continuously circulating among yaks or seroconversion reflects spill-over from outbreaks of PPR in domestic animals remains unknown. Due to the herding practices, yak might transmit PPR from domestic to wild ruminants. Differences in contacts between yaks and domestic animals in Pakistan and Tajikistan, or the low sample size, could explain that no Tajik yaks were seropositive.

Keywords Morbillivirus · Epidemiology · Serology · Sheep · Goats · Wildlife

The yak (Bos grunniens and Bos mutus) is a long-haired bovine found throughout the Himalayan region of south Central Asia, the Tibetan Plateau and as far north as Mongolia and Russia. Yak can be classified as wild, semi-domestic and domestic. In Pakistan, yaks are confined to the high plateau of the Northern Areas, from Gilgit to the valley of Ladakh, with an estimated population of 14,900 (Khan 1996). In Tajikistan, yaks are kept in three areas: in the northern Sharishston district (n = 200; occasionally these animals are using a shared pasture with Ayni district), in the eastern Murghob district of the

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Pamir province \((n = 17,000)\), and in the southeastern Shurobod district \((n = 100)\). Yaks are important assets for the livelihood of people in these areas. They are kept for meat as well as for milk and wool production. Yaks are mainly kept as semi-domesticated, where they come near human populations in winter to get shelter and feed and share grazing and watering areas with domestic animals (Hashmi et al. 2017). During the summer, yaks are migrating to summer pastures in mountainous areas on high altitude, where intermingling with wildlife occur.

Peste des petits ruminants (PPR) is a highly contagious and acute viral disease of mainly small ruminants, such as goats and sheep, that also can affect wild animals (Aziz-Ul-Rahman et al. 2018; Munir 2014). The disease is caused by peste-des-petits-ruminants virus (PPRV), classified as a Small ruminant morbillivirus (Amarasinghe et al. 2018). Recently, PPR has caused devastating outbreaks among the endangered Mongolian saiga \((Saiga tatarica mongolica)\) and is considered a threat to wildlife conservation (Aguilar et al. 2018). Presence of PPR has been recognised in Pakistan since 1991 (Abubakar and Munir 2014), and the first outbreak of PPR in Tajikistan was reported in 2004 (Kwiatek et al. 2007). The disease is now considered endemic in the sheep and goat populations of these countries (Abubakar and Munir 2014; Shonazarov 2012). Bovines, such as cattle and buffaloes, can be infected by PPRV, resulting in subclinical infection (cattle and African buffaloes; Syncerus caffer; Albina et al. 2013) or in some cases clinical signs of PPR (water buffaloes; Bubalus bubalis) (Govindarajan et al. 1997). Previous studies from China have indicated yak to seroconvert to PPRV. During a PPR outbreak among goats in Tibet, nine yaks without clinical signs were sampled (Zhao et al. 2009). One of the yaks had seroconverted, most likely as a result of spill-over from the outbreak in goats. A seroprevalence study of yak in various regions of China indicated an overall seroprevalence of 11.2%, with a higher seroprevalence in Tibet (bordering to Pakistan) and Sichuan (Li et al. 2018). In this study, the aim was to investigate the presence of antibodies to PPRV in yaks in Pakistan and Tajikistan, where PPR is endemic in domestic animals.

A total of 250 serum samples of apparently healthy yaks were collected from four different localities of Gilgit-Baltistan (GB), Pakistan in October–November 2014 (early winter) (Fig. 1; Table 1). In Tajikistan, 85 apparently healthy yaks over 2 years of age (13 females, 67 males and in 5 animals, sex was not recorded) were sampled during June–August 2016 in the districts of Sharihston \((n = 22)\), Ayni (bordering to Sharihston, and yaks originating from Sharihston were using a shared pasture; \(n = 13\)) and Murghob \((n = 50)\) (Fig. 1). In all sampling areas, yak intermingled with domestic animals (including sheep and goats) and wildlife, depending on season. Serum samples were heat inactivated before being analysed in duplicate according to the manufacturer’s instructions using a commercial competitive ELISA (ID-screen PPR competition ELISA, IDVet, Grabels, France) detecting antibodies specific to the nucleoprotein of PPRV (Libeau et al. 1995). Statistical analysis was performed using a Chi-square test with a \(p\) value of 0.05 considered significant, and 95% confidence intervals were calculated.

None of the sampled Tajik yaks were found seropositive (95% CI 0.0–4.2%), whereas 23 out of 250 serum samples from yaks in Pakistan (9.2%; 95% CI 5.9–13.5%; Table 1) were found positive for PPRV antibodies. At the time of sampling, no active clinical outbreak was reported in this area. A higher proportion of animals older than 2 years of age was seropositive compared with animals \(\leq\) 2 years (11.9% vs. 6.1%; \(p = 0.116;\) Chi-square test), although this was not considered statistically significant. Presence of seropositive
animals in all four localities in GB indicates that PPR is widespread in the area. A higher proportion of females was positive for PPR in comparison with males (11.0% vs. 6.3%; \( p = 0.217 \); Chi-square test), although this was not statistically significant.

Whether PPRV is continuously circulating among yaks or seroconversion reflects spill-over from outbreaks of PPR in domestic animals remains unknown. Overall seroprevalence in goats sampled in 2005–2006 in GB was estimated at 24.0%, with a lower seroprevalence in Ghizer district (14.5%) (Abubakar et al. 2017). During the period 2005–2007, three PPR outbreaks in GB were reported (Zahur et al. 2014). At the time of sampling, no active clinical outbreak in GB was reported, but a few months later, an outbreak was reported, indicating the ongoing circulation of PPRV in this area. Especially during winter, yaks intermingle with domestic animals and could then be exposed to PPRV excreted by infected sheep and goats. Differences in contact patterns between yaks and domestic animals in Pakistan and Tajikistan, or the low sample size, could explain that Tajik analysed yaks were not seropositive. A recently reported study on yaks in neighbouring Tibet showed that males were more likely to be seropositive than females (approx. 25% in males vs. 10% in females), with an increasing proportion of seropositive animals due to age (Li et al. 2018), reflecting the higher chance of being exposed to PPRV with rising age. In our study, there was similarly a tendency for animals over 2 years of age to more likely be seropositive, but in contrast female yaks had a higher proportion of seropositivity than male yaks (11% in female yaks vs. 6.3% in male yaks), although this was not statistically significant. Overall, 15.5% (95% CI 11.7–19.9%) of Tibetan yaks were seropositive for PPR (Li et al. 2018), while in GB, Pakistan, the seroprevalence was significantly lower (9.2%; 95% CI 5.9–13.5%; \( p < 0.05 \); Chi-square test); however, the same ELISA was not used in the two studies, which could influence these results. Tibet was the first region in China to encounter a PPR outbreak in 2007 with a seroprevalence of 34.1% in goats (Liu et al. 2018). Due to a successful stamping out, no PPR outbreaks were reported in Tibet after 2010. The study on yak in Tibet does unfortunately not state which year the sampling occurred (Li et al. 2018). Thus, it is unclear whether the Tibetan yaks seroconverted during the period of active PPR outbreaks, as a spill-over effect, or if it reflects a potential silent virus circulation within the yak or other animal populations. It is also not known whether yak can develop clinical signs of PPR. Other bovines are commonly subclinically infected (Albina et al. 2013), and yaks investigated during a PPR outbreak did not develop clinical signs (Zhao et al. 2009). Variation in susceptibility to PPRV infection between species is probably due to host receptor affinity (Kumar et al. 2014), as suggested by the fact that Vero cells expressing the goat signalling lymphocyte activation molecule (SLAM) receptor are more prone to PPRV infection than cells expressing the bovine SLAM receptor (Adombi et al. 2011). Circulation of PPRV in domestic animals intermingling with wildlife has been shown to be a threat to endangered wildlife species (Aziz-ul-Rahman et al. 2018), such as the Mongolian saiga (Aguilar et al. 2018). Semi-domestic yaks are intermingling with both domestic and wild animals, depending on season, and might therefore be a potential source for such spill-over effects from domestic animals to wildlife. Although other bovine species are considered dead-end-hosts for PPRV, investigations are needed to elucidate if yak could play a role in disease transmission to domestic and wild animals.

Table 1: Seropositivity to PPRV for yaks sampled in Pakistan

| Variable          | Analysed (positive) | % positive (95% CI) |
|-------------------|---------------------|---------------------|
| Total             | 250 (23)            | 9.2 (5.9–13.5)      |
| Female            | 155 (17)            | 11.0 (6.0–15.9)     |
| Male              | 95 (6)              | 6.3 (1.4–11.2)      |
| ≤2 years          | 115 (7)             | 6.1 (2.5–12.1)      |
| >2 years          | 135 (16)            | 11.9 (6.9–18.5)     |
| Phundar area 1    | 58 (5)              | 8.6 (2.9–19.0)      |
| Phundar area 2    | 64 (8)              | 12.5 (5.6–23.2)     |
| Ghizer area 1     | 67 (7)              | 10.4 (4.3–20.3)     |
| Ghizer area 2     | 61 (3)              | 4.9 (1.0–13.7)      |

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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