Foot-and-mouth disease in Wakhan District, Afghanistan

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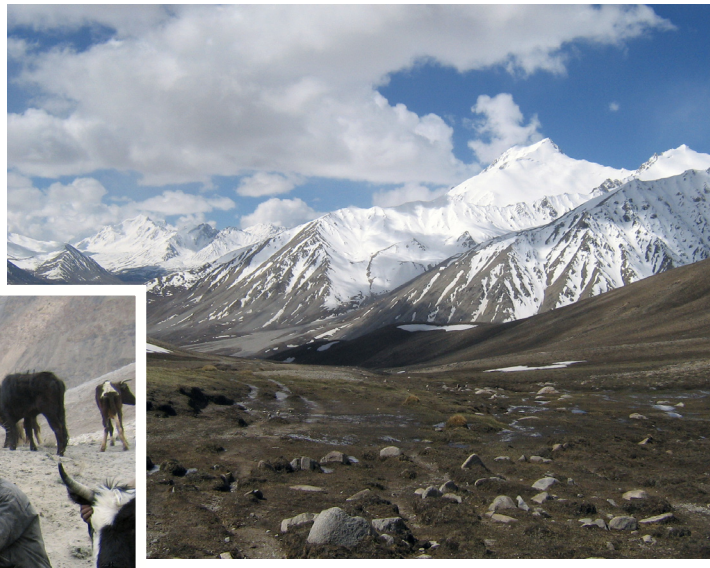

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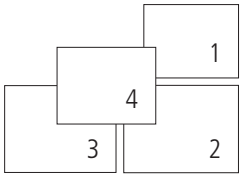

Cover photos:

1. Livestock pastures in Big Pamir at the end of aestivation, Wakhan District, September 2009.
2. Dr Ali Madad about to take a blood sample from a restrained cow before inoculating a foot-and-mouth disease vaccine, as part of a vaccine test trial, Wakhan District, May 2009.
3. Close-up of a necrotic lesion of the foot in a sub-adult yak affected by foot-and-mouth disease, Big Pamir, September 2008.
4. Drs Ali Madad and Hafizullah vaccinating a cow against foot-and-mouth disease during the first mass-vaccination campaign ever carried out in this region of Afghanistan, Wakhan District, Badakhshan Province, May 2009.

All photographs in the report: Afghanistan Ecosystem Health Project Team, WCS

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General introduction

Foot-and-mouth disease (FMD) is an extremely contagious viral (family Picornaviridae) disease of cloven-hoofed domestic and wild animals. It is endemic in most of Asia (including the Middle East), Africa, and South America. There are seven immunologically distinct serotypes and over 60 subtypes of the FMD virus (FMDV). The disease is endemic in Afghanistan where it occurs as regular epizootics. It has a direct effect on food security as it drastically reduces milk production in cows, reduces their fertility rate and incapacitates breeding bulls and oxen. A new serotype (Asia 1) was identified in Afghanistan in March 2001 (S. Yingst / CVL–Kabul pers. comm.), bringing the total of known serotypes to three for the country (A, O and Asia 1). The virus is very stable at low temperatures and can survive in frozen tissues. It may persist for days to weeks in organic matter under moist and cool temperatures. It is however inactivated on dry surfaces and by UV radiation (sunlight). Transmission primarily occurs by respiratory aerosols and direct or indirect contact with infected animals. Sheep and goats are occasionally considered maintenance hosts, and sometimes present very mild signs. Cattle are generally the first species to manifest signs of FMD and are therefore considered 'indicators' of this disease. Recovered or vaccinated cattle exposed to diseased animals can be healthy carriers for 6 to 24 months; sheep can be carriers for 4 to 6 months.

Because of their remoteness the Pamirs in the Wakhan District of the province of Badakhshan, has rarely been surveyed for infectious diseases in animals. Four days drive from Kabul, often on a rough track just to access the outreaches of this infamous mountain range has rendered health investigations in this region arduous and logistically expensive to carry out. In 2008, we have shown in a punctual serological survey that 51.3% and 75% of the sheep and domestic yak (*Bos grunniens*), respectively, had antibodies against FMD (Ostrowski et al., 2009). In addition two yak tested with virus neutralization test (VNT) had positive antibody titers against Asia 1 Shamir serotype (and not against serotypes A and O) indicating that at least this FMDV type actively circulated in Wakhan/Pamirs in 2008. Yet apart of this fragmentary information nothing is known about the epidemiological status of the disease in this remote stretch of land bordered in the south by Pakistan, in the east by China and in the north by Tajikistan. Whether the disease is endemic in this region as in most localities in Afghanistan, whether it expresses in seasonal epizootics, in interannual cycles, and whether large scale vaccination campaigns are achievable in this logistically challenging area is unknown.

The purposes of the present work were: 1/ to undertake a questionnaire survey among Wakhi herders in order to better understand the descriptive epidemiology of the disease in domestic ungulates in the Pamir ecosystem, 2/ to repeat a serological survey of livestock in 2009 and compare the results to those measured in 2008, to better approximate the population exposure level and detect the presence of other serotypes of the FMDV, and 3/ to test the serological response of cattle injected with the vaccine distributed by the Dutch Committee for Afghanistan (DCA), and implement a mass-vaccination campaign on cattle and yak. The lessons harvested during this pilot project will hopefully be applicable to other livestock communities across the fragile altitude ecosystems of Central Asia.

Background

This document is written in the continuity of our 2006, 2007 and 2008 missions in this region of Afghanistan (Ostrowski, 2006; Ostrowski et al., 2007; Ostrowski et al., 2009). To summarize our earlier findings in Wakhan and Pamirs of Afghanistan, we have studied Wakhi livestock herds in Big Pamir since 2006, recording their species composition, numbers, ownership, range use, and transhumance patterns. We have also assessed their health status based on clinical examinations and questionnaire investigations. After analyzing the data collected during our earlier missions, we decided that we needed to further investigate the occurrence of FMD in the area, assess its epidemiological status and quantify its prevalence in livestock. This information is essential to understand the risk of cross-species dissemination of FMDV between livestock and wild ungulates and a prerequisite to develop disease control programs in the area.

Our results are presented in four chapters: 1/ Foot-and-mouth disease descriptive epidemiology in Wakhan Valley and Pamirs inferred from a questionnaire investigation; 2/ Results of livestock exposure to FMD measured in 2009; 3/ A preliminary trial of FMD vaccine on a subsample of cattle in Wakhan and results of livestock vaccination in Wakhan and Pamirs in 2009; and eventually 4/ A short discussion about foot-and-mouth disease in wildlife in Wakhan/Pamir ecosystem.

The work carried out since 2006 is starting to clarify the complex issue of livestock disease in the Afghan Pamir and hopefully brings some insights into the risk of disease spillover between domestic and wild ungulates in this fragile ecosystem.

Part I. Descriptive epidemiology of foot-and-mouth disease in Wakhan District

Introduction

Our earlier investigations have revealed several interesting epidemiological features for FMD in Wakhan, yet they were mainly based on anecdotal evidences and fragmentary interviews. For example in 2006 the veterinarians at the field veterinary unit of the Aga Khan Foundation in Ishkeshim mentioned to us that the disease occurred in Ishekeshim and Zebak districts mainly as winter epizootics. Although they did not investigate the cyclicity of

FMD epizootics in Wakhan Valley and Pamirs, they presumed that they likely followed a similar seasonal pattern in this neighboring region. The objective of the present study is to learn more of the recent history of the disease in the area in order to frame the timing and organization of future prophylactic activities.

Methods

Interviews

We collected most of the data by interviewing randomly selected Wakhi livestock owners among those possessing more than 20 livestock and using Pamirs during summer. At the beginning of each visit to a new village we identified the possible respondents by questioning an elder person. Out of 125 possible candidates we selected 95 cooperative respondents and interviewed 78 of them between 30 March and 7 April, and 17 on 28 and 29 June 2009. Two team members conducted the interviews in Dari (Persian language in Afghanistan) and one of them subsequently translated responses in English. Each interview lasted roughly 10–15 minutes and consisted in 20 predetermined questions about the background of the respondent, the number of livestock in his household, the timing and locality of the last episode of FMD ('Zikpod' in Wakhi language) affecting his herd, the observed symptoms, the number of animals clinically sick, the mortality rate, the usage of any modern or traditional treatment, the existence of prior vaccination, and the supposed origin of the disease in the area. The same questions were presented in the same manner and order to each subject. We tallied answers, calculated the percentages of various responses and carried out statistics with Statistix 8.1 software.

Results

Survey coverage

We collected information from respondents living in 32 villages of Wakhan between Sarhad-e-Broghil, the easternmost village in the upper part of the Wakhan Valley and Qazideh, one of the very first villages east of Ishkeshim at the 'entrance' of the lower part of the valley (Table 1). Respondents claimed a cumulated ownership of 4,820 sheep and goats, 510 yak and 411 cattle. Based on estimates of 14,500 sheep/goats and 1,700 yak summering in Pamirs (Ostrowski et al., 2007) the survey covered almost a third of Wakhi livestock in Pamirs. Because 80% of the respondents were surveyed before they moved their animals to Pamirs in spring 2009 we could examine their livestock. No clinical cases of FMD were noticed during the survey, in accordance with questionnaire results.

Time and location of the last foot-and-mouth disease outbreak

According to 68 (71.6%) respondents the last outbreak of FMD occurred in late summer/autumn 2008, while 21 (22.1%) respondents believed that the disease started earlier in mid summer 2008, 2 (2.1%) in spring 2008 and 3 (3.1%) in winter 2008/2009. One respondent did not know. Overall nearly 94% of the respondents agreed that the last outbreak of FMD started sometime between mid summer and mid autumn 2008.

Table 1. Village names and number of livestock owners surveyed by the WCS Ecosystem Health team between March and June 2009, Wakhan District, Badakhshan Province, Afghanistan.

Village name	Number of livestock owners surveyed
Digargung, Broghil	1
Qazideh, Pagish, Shikhaur, Pakuy, Pak, Qila-e Panja, Abgarch, Sast, Goz Khun, Baba Tungi, Karkat	2
Khandud, Ish Murg, Kandkhun, Wuzed, Rorung, Qila-e Wust, Kuzget, Sargez, Shelk, Kipkut, Nirs	3
Rachon, Archa, Deghulaman, Neshtkhwar, Kret	4
Sarhad-e-Broghil	5
Chilkand	6
Ptukh	7

This rather consensual response corroborated the clinical observations we made in Big Pamir in September 2008 of emaciated subadult domestic yak with quadrupedal lameness due to necrotic lesions of the feet following exposure to foot-and-mouth disease virus (Asia 1 serotype) (Ostrowski et al., 2009). Almost all interviewed people confirmed that in autumn 2008 the disease "appeared" simultaneously in livestock summering in highlands (Big Pamir, Little Pamir, Hindu Kush northern slopes) and in those residing in Wakhan Valley.

Affected animal species and exposure level

All respondents confirmed that the disease affected sheep, goats, yak and cattle, but not horses and donkeys. Almost all of them (97%) said that cattle were the most susceptible to FMD. All interviewed owners said that during the last outbreak their cattle were affected, 84.2% that their yak were affected and 74.7% that their sheep/goats were affected. Yet it is possible that case fatality rate was higher in small ruminants since 47.3% and 30.5% of respondents admitted to have lost at least one sheep or a goat, respectively, whereas only 27.3% lost a cattle and 23.1% a yak. Overall in the surveyed cohort 26 cattle, 22 yak, 45 sheep and 29 goats have seemingly died of the disease or from secondary problems related to it, during or following the autumn 2008 outbreak. Reported to the population sizes of sampled yak and sheep/goats, it corresponds to a mortality rate of 4.3% for yak and 1.5% for sheep/goats. The vast majority of dead animals were either juveniles born in early 2008 or yearlings.

Lesions and symptoms

The lesions associated with the disease and symptoms were similar in different species and typical of what is known from the literature. They were those of a disease characterized by fever and blisters, which progressed to erosion in mouth, nares, muzzle, feet, or teat. Clinically affected cattle and yak seemed tired sometimes reluctant to move and presented excess salivation, lameness and decreased milk production. Salivation was apparently less common in sheep and goats which were usually only lame. According to respondents the majority of animals recovered within two weeks.

History and origin of of the disease in the region

Almost all respondents (95.7%) stated that the disease is present in Wakhan and Pamirs for more than 15 years, the majority of them saying that the disease was present in the area 'before they were born'. Remarkably only two interviewed livestock owners said that the disease was of recent occurrence and appeared in the area around 10 years ago during civil war times. Two respondents had no opinion on this matter. When asked about the suspected origin of the disease outbreaks during the last decade, 32 (33.6%) said that they did not know the origin of these outbreaks, 19 (20%) that the disease always comes from Pakistan, either via Pakistani traders or their livestock which, in summer, intermingle with Wakhi animals in the Hindu Kush range. On the opposite 16 (16.8%) said that the disease always comes from Badakhshan via Afghan traders (essentially from Badakhshan and Panshir), 16 (16.8%) that it comes either from Afghanistan or Pakistan, 3 (3.1%) from Kyrgyz livestock in Little Pamir, 3 (3.1%) from 'upper Wakhan', 3 (3.1%) that the disease is endemic in the area, and one (1.1%) that the disease 'does not come from Pakistan'.

Discussion

Mechanisms of transmission

Transmission of foot-and-mouth disease viruses between individual cattle and yak is likely to occur by two processes: *(1)* contact transmission between acutely infected and susceptible individuals and *(2)* transmission between carrier[1] animals and susceptible animals. Which of these two processes is likely to account for most infections in livestock in Wakhan and Pamirs is unclear. It is interesting to note that herders rarely report about occasional and sporadic cases of FMD infections but almost always about a sudden and massive outbreak of the disease. In contrast during winter 2006/2007 we observed an outbreak of FMD in cattle in the village of Karich, upper Wakhan. People interviewed at that time acknowledged that the disease was brought into the village via the arrival of clinically sick animals from another village. In this case contact transmission between acutely infected and susceptible individuals was the likely mechanism of transmission. On another hand, cattle and yak tended in Pamirs are not brought into contact with domestic 'newcomers' while pasturing highlands and yet seem to consistently develop the disease at the end of the aestivation. A likely possibility, for which the evidence still needs to be collected, is that domestic yaks or sheep could play the role of healthy carriers and are the principal source of transmission to susceptible livestock in Pamirs. The likelihood and mechanisms whereby carrier transmission could also occur in Pamirs between wildlife and livestock is discussed at the end of the report.

Susceptibility, seasonality and annual incidence

Respondents to the questionnaire consistently answered that the disease affects all domestic ungulate species but horses and donkeys and in priority yak and cattle calves in which it usually appears in Pamirs at the end of the aestivation (between late August and mid October). We suggest that this period corresponds to the start of maternal immunity decline in 3–5 month-old yak and cattle calves. The fact that calves are infected in priority may

[1] Foot and mouth disease carriers are defined as animals in which the virus persists, often at barely detectable levels, in the pharynx for at least 4 weeks (Salt, 1993). Carrier status appears to occur only in ruminants (Thomson et al., 2003).

suggest that adults have acquired immunity during previous outbreak but could also support that calves are either infected by their dams which would therefore play the role of healthy carrier, or possibly during minor epidemics among young animals in breeding herds, with carrier adult yaks ensuring that the viruses survive interepidemic periods. Both mechanisms would imply that domestic yaks can play the role of healthy carriers, a status that remains to be demonstrated. Since most of the calves in Wakhan/Pamirs are born in early spring, they become susceptible to infection more-or-less synchronously during late summer and autumn when passively acquired antibody wanes. Other susceptible livestock species in communal herds probably become exposed while infection is circulating among yak calves, possibly around water points where animal congregate, or through close contacts in common or neighboring night corrals of altitude settlements. If this transmission hypothesis is correct it could explain both the seasonality of the disease and the fact that yak calves seem to be infected first. It will also suggest that there is a time during each year when adult herds of yak are potent source of infection for their calves and possibly yearlings, which in turn will pose a threat to other livestock species that are likely to come into contact with them, even though there is no obvious clinical disease within adult yak herds.

It is also possible that sheep and goats which seem to be mildly affected by the disease could play the role of virus carrier and contaminate cattle and yak calves when immunity acquired passively from their dams declines.

Herders mentioned that the disease seems to reappear in the area every two or three years. Yet our personal observations support that the disease can be seen every year such as in late summers 2008 and 2009. The existence of an annual or pluriannual cyclicity would require further investigations and much longer monitoring periods.

History and origin

In 2006 we reported that FMD could be a relatively new disease in Wakhan and Pamirs (Ostrowski, 2006). Seemingly the disease appeared in Wakhi livestock between 1992 and 1995 when large herds of small ruminants originating from Panshir and Badakhshan started using the Wakhan corridor to reach livestock markets of northern Pakistan. Kabul, the secular marketing outlet for this livestock, was no longer accessible, due to interethnic wars of succession. Contaminated herds moving through the corridor arguably disseminated the disease among Wakhi livestock. From an historical point of view, this hypothetical story was believable since Wakhan and Pamirs were renowned for centuries as livestock production areas where non-native domestic animals were seldom introduced (Dr Farman Ali, AKDN, pers. comm.). However, from an epidemiological perspective we questioned this theory since FMDV has reputedly been circulating for a very long time in neighboring areas of Pakistan, Afghanistan and Tajikistan. The present survey involving a much larger sample of interviews invalidates this earlier hypothesis and strongly suggests (95.7% of respondents) that the disease existed in Wakhan and Afghan Pamirs for more than 15 years, and long before the civil war in mid 1990's.

The origin of the FMD outbreaks was obviously a matter of confusion to respondents. One third of them did not even have a hypothesis on that issue, the rest incriminating Pakistani

livestock, traders and/or Afghan traders from Badakhshan/Panshir and their livestock. Yet the facts that in 2008 outbreaks of the disease were observed simultaneously in the Wakhan Valley and in Pamir, that it appeared throughout the valley within a couple of days and that animals relatively isolated in high altitude pastures also developed the disease strongly suggest that the disease is endemic in the area.

Summary 1 — According to a questionnaire survey carried out on 95 Wakhi livestock owners in April and June 2009 we determined that foot-and-mouth disease has been endemic in Afghan Wakhan and Pamirs for a long period of time (>15 years). The last clinical outbreak affected mainly young cattle and yak and occurred in late summer 2008 simultaneously and massively in animals aestivating in Pamirs and in those left in Wakhan Valley. We suggest that this period corresponds to the start of maternal immunity decline in 3–5 month-old yak and cattle calves. The lesions associated with the disease and the symptoms were similar among livestock species and typical of what is described in the literature. The disease had a very high morbidity, with cattle from all interviewed people affected during the 2008 outbreak, but a relatively low mortality (<5%). An in depth study of the carrier status of each species within the livestock community would be interesting to carry out to better understand the seasonal cyclicity of the disease and to better address the risk of disease spill-over from livestock to wildlife during aestivation periods in Pamirs. The worst epidemiological situation, for which the evidence still needs to be collected, is that free-ranging domestic yak could play the role of healthy carriers. Because of the existence of direct contacts between free-ranging domestic yak and Marco Polo sheep they would be the principal source of transmission to susceptible wildlife in Pamirs. With such perspective in mind, we recommend to reinforce the protective immunity of yaks against FMD with repeated and regular vaccinations.

Part II. Serological survey of Wakhi livestock

Introduction

Results of the questionnaire survey depicted in part I strongly support that foot-and-mouth disease is endemic in Wakhan and Pamirs. Earlier serological surveys of sheep and yak carried out in March–April 2008 showed that respectively 50% and 75% of them had antibodies to the disease. In the case of an endemic disease reappearing clinically consistently at the same time of the year, we should expect a similar serological prevalence in livestock sampled a year later in March–April 2009. The objective of the serological work carried out in 2009 is to confirm the high serological prevalence of antibodies against FMD in sheep, and to confirm the exposure of cattle (a species previously not tested).

In September 2008 we recorded the presence of antibodies directed against FMDV serotype Asia 1 in two clinically sick yaks in Big Pamir. In the present work we want to extend the virus neutralization testing on cattle affected by the disease at the same period in order to confirm the presence of serotype Asia 1 in this species and extend serotype screening to sheep. We hypothesize that serotype Asia 1 is also present in this species confirming the 'multi-species' circulation of Asia 1 serotype in Wakhan.

Plate 1. Blood sampling animals in remote Afghan Pamir Mountains implied a heavy logistical organization. Here a working camp of the ecosystem health team showing in the shade of one of the mission's tents three protective plastic boxes containing the liquid nitrogen dry shippers, Big Pamir, 19 September 2008.

Methods

Study area and animals sampled

We investigated livestock exposure in the upper Wakhan Valley between the villages of Qila-e Panja and Sarhad-e Broghil. Between 30 March and 8 April 2009 we sampled sheep in Sarhad-e Broghil (6), Karakat (6), Chilkand (5), Nirs (4), Ptukh (6), Neshtkwar (6), Archa (6), Deghulaman (5), Kandkhun (6), Karich (5), Baba Tungi (3), Kret (5), Kipkut (2), Sargaz (6), Shelk (3), and Qila-e-Wust (5), and cattle in Sast (6), Wuzed (10), Abgarch (12), Goz Khun (6) and Qila-e-Panja (8).

Clinical examination of livestock and interview of herders

We examined clinically all animals before sampling them. We excluded those presenting symptoms of sicknesses such as 'orf', diarrhea, heavy tick or sheep ked (*Mellophagus ovinus*) infestation, lameness, mouth ulceration, fever, skin lesions, and collected only blood from healthy adult (>18 month old) sheep and cattle. We interviewed the livestock owners and confirmed that the last outbreak of FMD in the district took place approximately six month ago in late summer/autumn 2008 (see part I) and that sampled animals had never been vaccinated.

Sample collections

Randomly selected livestock in the upper Wakhan Valley were sampled in the evening, upon their return to the night corral of settlements and villages. Between 5 and 10 ml of blood

were drawn aseptically in plain vacutainers (Terrumo®, USA) via jugular venipucture. Blood in tubes was allowed to clot at 15–22°C for 3–4 hours and centrifuged for 4–5 minutes with a manual centrifuge (Hettich, Germany). Sera (c. 1.5–2 ml) were pipetted and stored in cryovials at –196°C in liquid nitrogen dry shipper (Taylor–Wharton, USA) (Plate 1). The dry shippers were moved by car between villages and to Feyzabad, the provincial capital, and eventually by airplane to Kabul where samples were stored at –20°C until laboratory processing.

Serological investigations

Sera were tested for foot-and-mouth disease antibodies with a competitive NS ELISA (Prionics AG, Switzerland) at Central Institute for Animal Disease Control, CIDC–Lelystad, The Netherlands. The ELISA test detects antibodies against the highly conserved non-structural (NS) protein of the FMD virus. This test can be used for all species. The ELISA test plates of the kit contain the FMDV NS protein 3ABC captured by the coated anti 3–ABC monoclonal antibody (mAb). A second mAb labeled with an enzyme that generates a color signal- the detection antibody- is then added. The reaction between the FMDV–NS antigen and the detection antibody is blocked by antibodies directed against the NS proteins present in the sample. Consequently, the sample is positive when no color develops. The ELISA test used has a high specificity and sensitivity. Its performance is comparable to or even better than that of the OIE index ELISA test, the NCPanaftosa test (Brocchi et al., 2006). Nine positive samples were also tested with virus neutralization test (VNT) ('in-house' prescribed test) against serotypes O1 Manisa, Asia 1 Shamir and A10–Holland at the same facility.

Results

ELISA screening

Results of serological screening with the NS ELISA test showed that 61% (95%CI: 51.6-69.8%) of sampled sheep and cattle had recently been exposed to foot-and-mouth disease, possibly during the reported outbreak of late summer 2008 (about 6 months before the present investigation). In such case and considering the natural kinetics of circulating antibodies, exposure level assessed via serology could have been even higher had we sampled animals earlier in the winter. When segregated by species, prevalence to FMD exposure was 52.6% (95%CI: 40.8–64.2%) in sheep and 76.2% (95% CI: 60.5–87.9%) in cattle, confirming the results of questionnaire survey (Part I), which suggested that the disease affected in priority cattle and yak.

Virus neutralization test

Results of virus neutralization tests performed on five sheep and four cattle positive with the competitive NS ELISA test confirmed the presence of reactive antibodies against Asia 1 serotype in the four cattle and one sheep, against both serotypes Asia 1 and O in three sheep and against serotype O alone in one sheep. In September 2008 we found reactive neutralizing antibodies only against serotype Asia 1 in two subadult domestic yaks displaying clinical foot-and-mouth infection (Plates 2a and b).

Plate 2a. An emaciated subadult domestic yak (*Bos grunniens*) with quadrupedal lameness due to necrotic lesions of the feet following exposure to foot-and-mouth disease virus (Asia 1 serotype), Big Pamir, 26 September 2008. Plate 2b (bottom). A close up of the interdigital and coronal necrotic lesions of the foot of a yak exposed to foot-and-mouth disease virus two weeks earlier, Big Pamir, 26 September 2008.

Discussion

Results of serological testing confirmed that the exposure level of livestock to FMD is relatively high in the Wakhan District, Afghanistan. Similar survey carried out in March–

April 2008 showed that nearly 50% (39/79) of sheep and 75% (18/24) of yak had antibodies against foot-and-mouth disease (Ostrowski et al., 2009). The present results are strikingly similar to those from last year confirming that the disease is endemic in sheep, cattle, yak and probably goats in the area. Assuming consistent antibody kinetic and similar epidemiological features between outbreaks it also suggests that there was a similar FMD "outbreak" in summer/autumn 2007, as confirmed anecdotically by Wakhis during informal discussions. In September 2009 we were told of clinical cases of FMD among non-vaccinated domestic yak in Pamir settlements. These observations and reports strongly support that the disease reappeared clinically in livestock every year between 2006 and 2009 and that the virus is carried asymptomatically by livestock or wild animals they come into contact with.

The high prevalence of exposure to FMD also supports that the disease is largely benign to livestock, and that the virus circulating in the population has evolved high contagiosity but low virulence. This was confirmed by the reported low mortality (<5%) of livestock during the last outbreak of clinical cases in summer/autumn 2008 (see part I). Although few livestock typically die of the disease it is however expected to affect negatively the livelihood of Wakhis. Affected young animals are said to have a poor growth during disease and throughout recovery (1–6 months) and adults have low milk production. This latter consequence is a salient food security concern in Wakhan as it is likely to impact children survival during winter months.

Results of virus neutralization test confirmed the presence of serotype Asia 1 in the area supporting the earlier results collected in yak (Ostrowski et al., 2009). We also found that serotype O is present in the area, yet it was detected only in sheep and not in cattle and yak. We found antibodies directed against multiple serotypes (serotypes Asia 1 and O) in sheep only. Because our sample size is small, further speculations should be made cautiously, but we suggest that serotype Asia 1 is currently responsible of most clinical cases of FMD in yak and cattle species in upper Wakhan and Pamirs. Serotype O is known to occur for long time in Afghanistan while the most recent appearance of serotype Asia 1 in Afghanistan dates back to 2004 (Valarcher et al., 2009).

Summary 2 — Results of serological testing confirmed that the exposure level of livestock to foot-and-mouth disease is relatively high in Wakhan District. Around 50% and 70% of tested sheep and cattle, respectively, were positive to the disease with a competitive NS ELISA test. Similar serological results retrieved from sheep and yak in 2008, as well as clinical observations confirmed that the disease is endemic in the area, and reappeared clinically in livestock in late summer/autumn in 2007, 2008 and 2009. The high serological prevalence recorded almost six months after the last documented outbreak of FMD, and the low mortality rate support that the picornavirus(es) circulating in the area has evolved high contagiosity and low virulence. We found antibodies against serotype Asia 1 (VNT) in clinically sick yak in Big Pamir in 2008 and in recovered cattle and sheep in April 2009. We also found antibodies against serotype O (VNT) in sheep in April 2009. Although both serotypes could be present concomitantly in the area, we suggest that serotype Asia 1 was responsible of most clinical cases of foot-and-mouth disease in yak and cattle in 2008.

Part III. Results of cattle and yak vaccination in 2009

Introduction

The decision to use vaccine prophylactically or as an aid to control and eradicate foot-and-mouth disease is complex and depends upon scientific, economic, societal and political factors specific to each country and each outbreak. In Afghanistan where the epidemiological surveillance system has suffered the effects of successive wars the use of vaccination responds almost exclusively to a desire of prevention and is largely driven by humanitarian considerations. Although the disease is known to be endemic in the country its distribution is sparsely known for a variety of reasons which include; insecurity and impossibility to access areas with war, lack of veterinary expertise and associated logistical capacities in more stable areas, remoteness of many districts in a largely mountainous country with poorly developed infrastructures, and uncontrolled movements of livestock through international borders. Consequently there have been no recent efforts to eradicate the disease but only prophylactic initiatives to support the livestock production sector and community livelihoods. In Afghanistan prophylactic activities are conducted by a range of actors involving state veterinary authorities, private and community paraveterinarians trained by the Dutch Committee for Afghanistan (DCA), a variety of NGOs, and in the recent past military forces. In general, vaccination efforts progress geographically in Afghanistan with increased efforts of official veterinary authorities, new paraveterinarians establishing in specific areas or targeted efforts of NGOs. In this regard, the Aga Khan Foundation promotes important veterinary activities in Afghanistan and particularly in the province of Badakhshan. Veterinary practitioners sponsored by the Aga Khan Development Network have actively vaccinated livestock against FMD in the vicinity of Wakhan, around Ishkeshim, in Zebak District and districts of northern Badakhshan. Yet, recently little and only sporadic efforts have been devoted to develop vaccination programs in the Wakhan District, mainly because of logistical difficulties, lack of modern animal health education among locals and the chronic difficulty at establishing a reliable cold chain essential to store vaccines before use. As a matter of fact the feasibility to vaccinate livestock populations in the Wakhan Valley and Pamirs has legitimately been questioned because of the difficulty to transport and store vaccines below 8°C in this distant and remote area, the general lack of understanding of vaccine benefits among local communities, and the lack of trained manpower necessary to carry out large scale vaccination programs.

Currently there is no universal vaccine for foot-and-mouth disease. FMD vaccines used prophylactically or as an aid to eradication must closely match the serotype and subtype of the prevalent FMDV strain, and with seven serotypes and more than 60 subtypes of FMDV reported worldwide this is not an easy task. In 2009 the DCA distributed in Afghanistan a vaccine produced in a regional production laboratory (Federal Centre for Animal Health, FGI "ARRIAH", 600901, Yur'evets, Vladimir, Russia) protecting against the three serotypes (A, O, Asia 1) and subtypes known to affect livestock in Central Asia (A Iran–05, O PanAsia 2, Asia 1). However the effectiveness of this vaccine when employed in remote livestock populations of Afghanistan largely exposed to endemic strains of FMDV is unknown.

In the present study, we evaluated the seroconversion of a sample of cattle in upper Wakhan Valley three weeks after being vaccinated with DCA vaccine. Then we organized, with the help of the two paraveterinarians whom we sponsored the activities since 2007, and in full cooperation with local communities, a large scale vaccination campaign of cattle and yak in the upper Wakhan Valley.

Methods

Preliminary vaccination trial

We selected 18 healthy adult cows in the villages of Kandkhun (N=9) and Abgarch (N=9) in upper Wakhan Valley in May 2009. The animals presented no symptoms evocative of infectious diseases, and had been exposed in autumn 2008 to an outbreak of foot-and-mouth disease, as all livestock in Wakhan. We took a blood-sample (initial), inoculated 14 of them subcutaneously in the middle third of the neck with 3 ml of vaccine taken from four different bottles of vaccine[2] and re-sampled blood 21 days after initial (post-inoculation). Two animals in each group were bled according to the same protocol but were not vaccinated and constituted unvaccinated controls. On both occasions blood was drawn aseptically in plain vacutainers (Terrumo®, USA) via jugular venipucture, it was allowed to clot at 15°C–22°C for 3–4 hours and centrifuged for 4–5 minutes with a manual centrifuge (Hettich, Germany). Sera (c. 1.5–2 ml) were pipetted and stored in cryovials at –196°C in liquid nitrogen dry shippers (Taylor–Wharton, USA) (Plates 4). The dry shippers were moved by car to Feyzabad and eventually by airplane to Kabul where samples were stored at –20°C until they were processed in the laboratory. Sera samples were tested with a competitive NS ELISA (Prionics AG, Switzerland) at CIDC–Lelystad, The Netherlands (see Part II for test description).

Mass vaccination of cattle and yak

In April 2009, during a preliminary visit to the Wakhi community we discussed with the elders of 26 villages in mid and upper Wakhan Valley, between the villages of Khandud and Sarhad-e Broghil, as well as with the main livestock owners, about the possibility to organize a mass vaccination campaign of cattle and yak in 2009. Because they responded positively to this offer we scheduled to start this prophylactic operation in late May 2009 with the help of Mr. Sarwar and Mr. Mohamed Gul, the two paraveterinarians based in Kandkhun and Abarch villages, respectively. In early May 2009 we came again into contact with the two paraveterinarians and requested from them to inform communities that the vaccination campaign was to start by the end of month of Sawr (third week of May). Shortly before starting vaccinations we travelled by car upstream along the Wakhan River informing

[2] A liquid inactivated sorbed foot and mouth disease vaccine (virus grown in BHK-21 cells) against A Iran-05, O PanAsia-2 and Asia 1 types, produced by the Federal Centre for Animal Health, 600901, Yur'evets, Vladimir, Russia. At DCA headquarter in Kabul the vaccine was stored at +1°C–+8°C according to manufacturer's recommendations and monitored throughout transportations by a temperature range indicator. After purchasing vaccines we stored them within the same temperature range at WCS headquarter in Kabul, air-shipped them to Feyzabad, the provincial capital of Badakhshan, and transported them by car to upper Wakhan in cool boxes with ice packs. Eventually they were stored in solar-powered refrigerators in Abgarch and Kandkhun field veterinary units

villagers that the vaccination team will start the work in Sarhad-e Broghil (the easternmost village) and progressively move downstream, in order to vaccinate as many cattle and yak as possible in villages and appended pastures (Plates 3 & 4). Because we failed to vaccinate the majority of yak during this first prophylactic operation we carried out a second mass-vaccination in September 2009, targeting yak in Little Pamir (Mr. Sarwar and Mr. Mohamed Gul), Big Pamir (Dr AliMadad Rajabi and Dr Hafizullah Noori) and upper Wakhan Valley (Dr AliMadad, Dr Hafizullah, and Mr. Mohamed Gul). Aestivation pastures were reached on horsebacks in 2–3 days and all vaccinations were performed in 5 days. Vaccines were transported in cool boxes with ice packs and kept below +8°C throughout the campaign. Vaccinations involved the active participation of people from local communities who gathered, captured and restrained the majority of their animals (Plate 5). Only healthy cattle and yak were vaccinated, those less than two month-old were not vaccinated as we assumed that they were still protected by antibodies passively acquired from their mothers. No injuries or fatalities related to handling or vaccination occurred during these operations. We recorded the total number of vaccinated cattle and yak in each village/settlement discriminated by age (adult versus less than 18 month-old) and sex for adults.

Plate 3. Dr AliMadad Rajabi vaccinates an adult cow for foot-and-mouth disease with the help of two handlers, 29 May 2009, near Abgarch village, Wakhan District, Badakhshan Province, Afghanistan.

Plate 4. A paraveterinarian trained by WCS veterinary team vaccinates a cow for foot-and-mouth disease, May 2009, Wakhan District, Badakhshan Province, Afghanistan.

Plate 5. Mass vaccination campaign for foot-and-mouth disease involved the participation of people from local communities who gathered, captured and restrained the majority of animals, May 2009, Wakhan District, Badakhshan Province, Afghanistan.

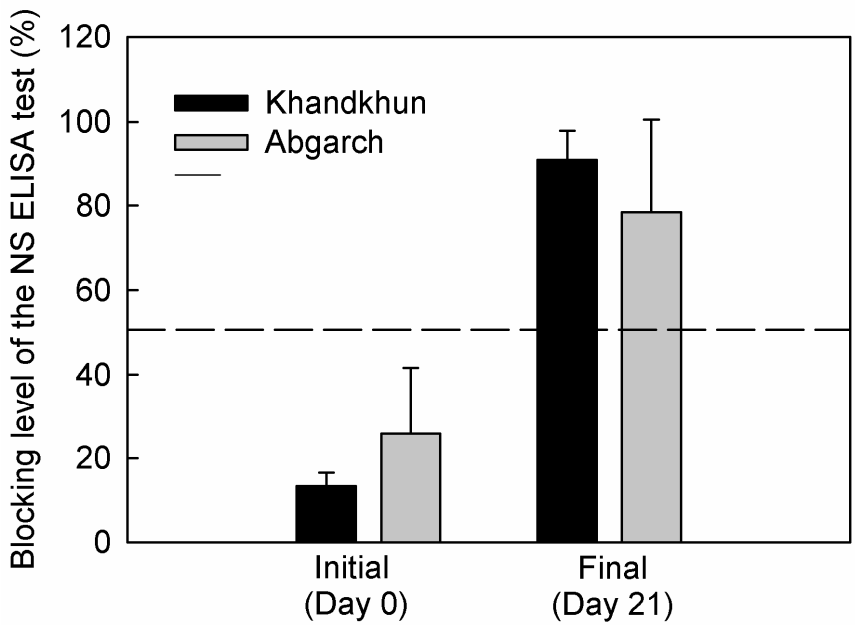

Figure 1. Antibody levels measured with a semi-quantitative ELISA test in 14 adult cows in the villages of Kandkhun (*N*=7) and Abgarch (*N*=7), before and after vaccination against foot-and-mouth disease. The dashed line shows the cut-off value of positivity for the test corresponding to 50% blocking, May 2009, Wakhan District, Badakhshan Province, Afghanistan.

Results

Preliminary vaccination trial

Twenty-one days after vaccination, 13 of the 14 (93%) vaccinated cattle had an increase in antibodies above the positive cut-off value of 50% blocking in the ELISA test (Figure 1). One vaccinated animal in Abgarch had an increase of antibodies (i.e. 16% to 44% blocking), yet not above the positive cut-off value. There was no significant difference ($P>0.05$) in seroconversion intensity between cattle in Kandkhun and Abgarch. The four unvaccinated control animals had no significant seroconversion. We observed no clinical side effects related to vaccination.

Vaccination of cattle and yak

We did 5,043 vaccinations against foot-and-mouth disease on cattle and yak east of Qila-e Panja village in the upper end of Wakhan Valley and Pamir pastures. Based on earlier estimates of population sizes for Wakhi livestock we posit that at least 90% of the yak and cattle populations in this vast and remote area received one vaccination shot for FMD.

We did 2,904 vaccinations on cattle, including 1,737 in May 09 and 1,167 in September 09 (Table 2). Because a large majority of animals vaccinated in September had already been vaccinated in May, and livestock in Wakhan are not identified individually, it is difficult to know the accurate number of vaccinated cattle. Yet at least 1,737 received one injection and likely close to 1,132 received two. The number of cattle in upper Wakhan is not clearly known but was estimated at 1,500–2,000 heads in 2006–2007 (Ostrowski et al., 2007).

Table 2. Number of cattle vaccinated in Wakhan District (Wakhan Valley/Big Pamir/Little Pamir) in May and September 2009 discriminated by age and sex, Badakhshan Province, Afghanistan.

Vaccination session	Adult males	Adult females	Young[1] unsexed	Total
May 09	498 / 0 / 0	989 / 0 / 0	250 / 0 / 0	1737 / 0 / 0
September 09	333 / 15 / 11	592 / 9 / 0	207 / 0 / 0	1132 / 24 / 11
Total	831 / 15 / 11	1581 / 9 / 0	457 / 0 / 0	2869 / 24 / 11

[1]Less than 18-mo-old when vaccinated

Table 3. Number of domestic yak vaccinated in Wakhan District (Wakhan Valley/Big Pamir/Little Pamir) in May and September 2009 discriminated by age and sex, Badakhshan Province, Afghanistan.

Vaccination session	Adult males	Adult females	Young[1] unsexed	Total
May 09	220 / 0 / 0	104 / 0 / 0	27 / 0 / 0	351 / 0 / 0
September 09	3 / 201 / 379	2 / 340 / 498	3 / 193 / 169	8 / 734 / 1046
Total	223 / 201 / 379	106 / 340 / 498	30 / 193 / 169	359 / 734 / 1046

[1]Less than 18-mo-old when vaccinated

Consequently at least 90% of the cattle population of Wakhis in upper Wakhan, Big Pamir and Little Pamir received one vaccinal shot for FMD in 2009.

We did 2,139 vaccinations on domestic yak in 2009 including 351 in May and 1,788 in September (Table 3). Relatively few animals were vaccinated in May because most of them had already been moved to their aestivation pastures in Pamirs. The majority of yak vaccinated in May were re-vaccinated in September while in Pamirs. At least 1,788 animals received one vaccinal shot and 351 (19.6%) likely received two. In 2006–2007 we estimated the yak population of Wakhis at c. 1,700 animals (Ostrowski et al., 2007). Based on this estimate we believe that more than 90% of the yak population of Wakhis received at least one vaccination shot against FMD in 2009.

Discussion

The results of the vaccination trial on a subsample of cattle in upper Wakhan showed that the vaccine we used was effective at triggering an immune response in inoculated animals. Antibodies capable of protecting the host against FMD can only be measured in virus neutralization tests (VNT) and not ELISAs. Because we observed on a small sample size (see part II) that animals testing positive with the NS ELISA were also positive with VNT it is possible that production of virus neutralizing antibodies could parallel antibody production against the highly conserved non-structural (NS) protein of the FMD virus, and that the vaccine we used provides protection against subsequent infection of cattle with a virulent strain of FMDV. We did not observe any post vaccination side effects that could have adversely affected the health of vaccinated animals. Consequently we concluded that the vaccine proposed by DCA in 2009 was safe and effective to protect cattle and yak against specific serotypes and subtypes. The results of the vaccination trial also strongly suggested that the vaccine had been properly stored in Kabul and that the cold chain, which is a logistical issue of concern in hot tropical countries such as Afghanistan, was not significantly

disrupted during transportation from the site of production in western Russia to the remote site in Afghanistan where it was ultimately used. They also support that the mass-vaccinations of livestock we have carried out in the challenging environment of Pamirs in 2009 were likely effective and could be consistently repeated in the future, under similar logistical conditions, in the remotest areas of Afghanistan.

We successfully vaccinated against FMD more than 90% of the cattle and yak populations owned by Wakhis in upper Wakhan and Pamirs with at least one vaccination shot. It shows that even in very remote areas of Afghanistan, such as in Pamirs, it is feasible to conduct large-scale vaccination campaigns that ensure significant protection of the livestock populations against the currently circulating strains of FMDV. In May we trained the two paraveterinarians at organizing and implementing mass-vaccinations. In September they were able to undertake half of the vaccinations by their own, including in the remote Little Pamir. We are confident that they are now capable, with the help of community elders, to repeat the operation in the future. We also believe that livestock owners will be more inclined to request and pay the two paraveterinarians to conduct FMD vaccination in the future. Indeed the first results suggest that the September 2009 epizootic of FMD in Wakhan affected only non-vaccinated animals particularly in lower Wakhan Valley, a powerful piece of evidence for locals who, as it is often the case among uneducated people, only trust what they see. Ideally a similar vaccination effort should be carried out in 2010 and extended to cattle and yak in lower Wakhan. Then in 2011 livestock owners should be requested to participate at funding the vaccines, a progressive involvement that will need careful communication and the establishment of permanent cold-chain structures to be successful on the long term.

Ideally two vaccination shots six months apart would provide the highest level of protection for adult cattle. This was indeed achieved in almost 65% of the vaccinated cattle but in only 20% of the vaccinated yak. One reason for this relatively low number of yak double-vaccinated is that most of them had already been moved to Pamir and Hindu Kush pastures by the time we started prophylactic campaigns in late May. We recommend in the future starting vaccinating yak earlier in the year, probably not later than late April. In such circumstances a larger proportion of the yak population will likely receive two vaccination shots per year.

Summary 3 — Results of the vaccination trial on a subsample of cattle showed that the vaccine we used was effective at triggering an immune response in inoculated animals. We did not observe any post vaccination side effects that could have adversely affected the health of vaccinated animals. Consequently we concluded that the vaccine proposed by DCA in 2009 was safe and effective to protect cattle and yak against specific serotypes and subtypes. We successfully vaccinated for FMD, with at least one vaccination shot, more than 90% (c. 4,000 animals) of the cattle and yak populations owned by Wakhis in upper Wakhan Valley and Pamirs. This achievement would not have been possible without the cooperative involvement of the two paraveterinarians on site and the active participation of communities. Ideally a similar vaccination effort should be sponsored in 2010 and extended to cattle and yak in lower Wakhan. In the future communities will be committed to support the annual vaccination effort against foot-and-mouth disease.

Part IV. Final discussion and conclusion

Horizontal inter-species transmission is a central mechanism in the emergence of diseases in wild-living populations (Ostherhaus, 2001; Richomme et al., 2006). The probability for a pathogen to cross the species barrier from a 'source' to a 'receptor' species depends on the type of pathogen, on the susceptibility of the receptor and on the rate of efficient direct (from animal to animal) or indirect (via environmental contamination or vector transmission) contacts between the species. The contact rate between the source and the receptor is intimately linked to the relationship between these species and the likelihood of sharing the same habitat (Cleaveland et al., 2001; Woolhouse et al., 2001). We have shown in earlier work (Ostrowski et al., 2009) that free-ranging domestic yak and Marco Polo sheep (*Ovis ammon polii*) occasionally share common pastures at the same time of the year in Big Pamir. The risk of disease transmission between this livestock species and argalis in Pamirs is therefore reasonably high, especially in the case of FMD, a highly contagious disease that has been recorded to affect clinically a large variety of wild ungulates (Hedger, 1981).

In mountainous habitats, the abundance of domestic animals leads to forced cohabitation between them and their wild counterparts. The spillover of contagious diseases from domestic to wild-living ungulates has been largely reported during the last 25 years (Frölich et al., 2002; Hudson et al., 2002) with sometimes detrimental effects at population level in vulnerable species (Callan et al., 1991; Dagleish et al., 2007).

Transmission of infectious agents between individuals takes place either through direct contact, contact with a contaminated environment or via living vectors such as insects and acaria. In mountainous areas in Europe, the abundance of domestic herds and the increase of wild-living populations —partly due to human manipulation such as introduction or reinforcing—, have led to increased cohabitations. As a matter of fact the spillover of disease from domestic to wild-living ungulates has been largely reported in Europe during the last 20 years (Richomme et al., 2006). In Afghan Pamirs, domestic and wild-living ungulates are competitors for food, which should result in pasture sharing and, thus, to the possible transmission of infectious agents. However unlike what is observed in Europe and North-America, wild ungulates are heavily and unsustainably hunted and no human manipulation has ever allowed a re-stocking of these persecuted populations. We therefore hypothesize that features of disease spillover between wild and domestic ungulates are likely to be different in an area such as Afghan Pamirs compared to what has been described in mountain ecosystems of Europe and North-America. It seems reasonable to hypothesize that despite being endemic in livestock in the area, FMD infrequently infects Marco Polo sheep which very rarely come into close contact with a clinically infected domestic animal, usually kept at the settlement.

There is however concern that Marco Polo sheep could come into contact with asymptomatic domestic carrier of FMDV. Carrier status happens to occur only in ruminants. In domestic animals, cattle and sheep have been documented to be persistently infected. Fortunately in the context of Pamirs, these two species are unlikely to pose a significant risk of FMD spill-over to wild ungulates since cattle are extremely few to aestivate in Pamirs and

sheep almost never come into close contact (<400 m) with their wild relatives. The case of the domestic yak is however of greater concern since the species is known to come into close contact with wild ungulates in aestivation areas in Pamirs. Unfortunately there have been to our knowledge no researches investigating the existence of persistent FMD infection in this species. Such study would however be extremely useful to conduct since domestic yak are known to intermingle with wild ungulates across all highlands of Central Asia, and conclusions would interest sanitary authorities of the largest nations in Asia.

Transmission of FMD to livestock by persistently infected wild ungulates has been debated for years. Recently Valarcher et al. (2009) who investigated the molecular epidemiology of FMDV serotype Asia 1, which caused outbreaks of the disease in Asia during 2003–2007, questioned the possible role played by 'large antelope' populations in Central Asia in the virus transportation across international borders. Several elements however do not support the hypothesis that wild ungulates could play a significant role in carriage and spreading of FMDV to livestock in Central Asia. First, although many susceptible wild ruminants may presumably become carriers, only African buffaloes (*Syncerus caffer*) have so far been shown conclusively to transmit FMD while in that state (Dawe et al., 1994). Second, the only locality in which overt FMD has been reported regularly in wildlife over the last 60 years is the Kruger National Park in South Africa, where there have been 31 recorded outbreaks in impala (*Aepyceros melampus*) between 1938 and 2002 (Thomson et al., 2003). No such situation has ever been recorded in Asia. Third, Valarcher et al. (2009) have shown that the last outbreaks of FMDV Asia 1 in Central Asia (Group II) were closely connected and that the virus which circulated in Central Asia spread a long distance, presumably from Pakistan, in a short period of time. The small population sizes and high level of persecution supported by wild ungulate populations in Central Asia added to the lack of major migratory movements in the species occurring in northern Pakistan, Wakhan and Tajikistan do not support the hypothesis of wild ruminants being at the origin of this fast and explosive FMDV circulation. In contrast the frequent movements of livestock across largely permissive international borders in Pamirs, the lack of epidemiological surveillance in the area and the high state of endemicity of the disease in local livestock support that domestic hoofstock, moved illegally and frequently through international borders could play a key role in the means by which foot-and-mouth disease viruses circulate within the region.

Although there are reasonably strong reasons supporting that wild mountain ungulates in Central Asia do not play a significant role in the maintenance and dissemination of FMDV, we still lack solid evidences to confirm this hypothesis. We therefore recommend pursuing researches in this topic. Studies should include opportunistic and systematic serological screening of accessible animals, including with virus neutralization test in order to evaluate exposure level and serotypes involved. They should also include isolation and detection of antigen or nucleic acids of FMDV in all animals with clinical signs. Eventually sampling of pharyngeal cells (Probang test) from healthy animals would help assess the existence of persistent infection. There are opportunities to conduct active epidemiological investigations in wild ungulates in collaboration with trophy hunting operations, especially in Tajikistan. These investigations are needed as prerequisites for further development of control programs

in a region located at the crossroad of Pakistan, China and India, three countries which host the largest livestock populations in Asia.

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