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**Risks of disease transmission between wildlife and livestock  
in the Chang Tang Nature Reserve in Tibet:  
Result of a visit in June 2009**



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July 2009

**Wildlife Conservation Society, New York, USA**

**Cover photo: Landscape in Dangxion County, Tibet, June 2009.**

Credits: Cover photo and photo 4 by Stéphane Ostrowski / WCS. Photos 1, 2 & 3 by Tse Brten Rgyal / WCS.

## Table of contents

Part I. Introduction .....	5
Summary of the visit to China in June 2009 .....	5
Background: the Chang Tang Nature Reserve .....	6
Wildlife-livestock competition in Chang Tang .....	6
Part II. Disease, wildlife, and human activities in the Tibetan plateau .....	7
Habitat fragmentation and disease risk .....	7
Risk of accidental introduction of pathogens from livestock .....	8
Global climate change and disease risk .....	9
Part III. Risk of disease spillover between domestic animals and wildlife.....	10
Introduction .....	10
Risk of disease spillover by direct contact .....	10
Risk of disease spillover via vector transmission .....	13
Risk of disease spillover by indirect contact .....	14
Risk of disease transmission between domestic and wild yak .....	15
Canine distemper in carnivores.....	17
Part IV. Recommendations .....	18
Responding to the vastness and remoteness of the Chang Tang .....	18
Acquiring baseline data on wildlife health .....	20
Building capacity of the animal health experts.....	20
Increasing the cooperation between health agencies.....	23
Committing into a regional strategy .....	24
Acknowledgments.....	25
Appendices .....	26
Appendix 1. Guidelines for baseline data collection in wild ungulates.....	26
Sampling methods.....	26
Passive sampling .....	26
Active sampling .....	27
High priority diseases.....	27
Foot and Mouth Disease.....	28
Peste des Petits Ruminants .....	29
Brucellosis .....	30
Blue Tongue and Epizootic Hemorrhagic Disease .....	30

Haemorrhagic Septicaemia .....	31
Mange.....	32
Low priority diseases .....	32
Infectious Bovine Rhinotracheitis.....	33
Bovine Viral Diarrhea .....	33
Parainfluenza-3 virus infection .....	33
Bovine Respiratory Syncytial Virus infection .....	34
Rinderpest .....	34
Canine distemper.....	35
Appendix 2. Utilization of veterinary diclofenac in Tibet. ....	36
Literature cited.....	37

## Part I. Introduction

### Summary of the visit to China in June 2009

During my three-week stay in China in June 2009, I was authorized to travel to Tibet. Although the duration of this permission was short (14 days), I was given the opportunity to visit Dangxion County (half-day drive north of Lhasa and south-east of Chang Tang) to learn from local veterinary workers and nomads about the veterinary organization in this county as well as health condition of livestock and occurrence of diseases in wildlife. Initially we planned to visit Shenzha and Nima Counties in order to assess the interaction between Tibetan antelopes (*Pantholops hodgsoni*) and livestock. This mission was unfortunately not agreed upon by the staff at the Veterinary Institute because of their concerns that recent snowfalls would not allow me to reach the distant site and perform an efficient investigation within the short time frame of my authorized stay in Tibet. The visit to Dangxion County proved however very rewarding. Although no interactions between livestock and wildlife could be witnessed, interviews of local veterinary workers, nomads and researchers at Nam Co Lake Research Station provided me with a general picture of environmental health challenges in the province (Photo 1).



Photo 1. An interview of veterinary professionals at the field veterinary unit in Umah township in Dangxion County. Mr Tsering Lhakpa, with the red jacket, is translating, 11 June 2009 (© Tse Brten Rgyal / WCS).

When I returned to Lhasa, I was kindly offered to visit the new facilities of the Veterinary Institute where I debriefed the mission's results with Mrs Sijiu and Mr Jiangyong Zeng, respectively vice-director and associate professor at the Institute, and provided two lectures to the academic staff and veterinary students at the Institute: 'WCS field veterinary projects in Continental Asia' and 'Diseases and Wildlife Conservation in Tibet'. In Beijing I also met and discussed the outcomes of the mission with Mrs Xie Yan, the current WCS country director, and with Mr Hongxuan He, director of the National Research Center for Wildlife Born Diseases (NRCWBD), a laboratory hosted at the Institute of Zoology in Beijing (Chinese Academy of Sciences) and under the administrative responsibility of the Ministry of Forestry. In theory any case of wildlife disease in China has to be reported to this laboratory for further investigations. Among other missions NRCWBD is committed to investigate alleged cases of avian influenza in wild birds and as such has been involved with the case of highly pathogenic avian influenza virus (HPAIV) H5N1 in Qinghai Lake in 2005, which killed more than 6000 water birds.

### Background: the Chang Tang Nature Reserve

Covering around 300,000 km<sup>2</sup> in the north-western part of the Tibetan Autonomous Region (TAR) of the People's Republic of China, the Chang Tang Nature Reserve (CTNR) is one of the largest protected areas in the world. It was established in 1993 to safeguard an ecosystem unique in the world. The Reserve especially upholds an exceptional community of large mammals, including six endangered ungulate species: the Tibetan antelope, the Tibetan wild ass or kiang (*Equus kiang*), the Tibetan gazelle (*Procapra picticaudata*), the wild yak (*Bos grunniens*), the blue sheep (*Pseudois nayaur*) and the Tibetan argali (*Ovis ammon hodgsoni*) (Schaller, 1998). Although remote and difficult to access, the CTNR still faces many threats, which endanger wild species, the rangeland ecosystem that supports them and the local pastoralists. Because of the high elevation of the CTNR (most of it lies between 4000 and 5000 m), the ecosystem there is fragile and difficult to restore once damaged.

### Wildlife-livestock competition in Chang Tang

Following increased controls of illegal hunting activities, changes in traditional pastoral production systems are currently the principle threats to wild ungulates of the Reserve. Increased livestock numbers, sedentarization of pastoralists, and rangeland fencing, all affect the range quality, pose problems of overgrazing, disturb migration routes of wild herbivores and increase competition for forage between domestic and non-domestic ungulates. Raising livestock, often the only economical resource for local people in Chang Tang, poses great challenges. Preserving autochthonous wildlife in a healthy ecosystem is essential to the long-term ecological

equilibrium of the Tibetan landscape and may also contribute to secure additional economical resources for the region in the future. One of the main challenges of decision makers is therefore to integrate the needs of local development with conservation goals. The project on Biodiversity Conservation and Sustainable Natural Resource Use in the Chang Tang Region of Tibet aims at fulfilling this challenge by developing coordinated planning and improving enforcement of plans and policies through cooperation with and between government departments.

#### Disease risk at wildlife-livestock interface

Until the 1980's, the Chang Tang's wildlife and ecosystem had been little studied. Then surveys led by George Schaller (1998) have provided information on rangelands, wildlife, and pastoral systems in the area. However, the health status of both wild and domestic ungulates which cohabit in this area, and therefore the risk of disease transmission between them, is still largely unknown.

## Part II. Disease, wildlife, and human activities in the Tibetan plateau

Quantitative and qualitative changes in natural habitats, accidental introduction of disease agents via livestock and global climate change, all linked to human activities, are known to affect wild ungulates worldwide. The three threats exist in the Tibetan plateau, and separately or combined, potentiate the risk of disease outbreak in wild herbivores.

#### Habitat fragmentation and disease risk

Habitat fragmentation, reduction and subsequent isolation of a continuous natural habitat into smaller patches, is a widespread phenomenon affecting most terrestrial ecosystems of the Tibetan plateau because of changing pastoralist practices. This process is expected to worsen with the increasing number of livestock and the recent introduction of new husbandry practices such as fencing. Habitat fragmentation directly affects wildlife populations by excluding individuals and reducing their resources but also indirectly through changes in community composition and interactions. From the perspective of disease risk, habitat fragmentation may paradoxically result in the short term to a decreased risk of disease outbreak in wildlife, as many subpopulations will escape disease epidemic that can devastate other infected patches. Also, if host populations are relatively small they may fall below the critical host threshold required for disease persistence. Moreover, fencing may contribute at avoiding close contacts between livestock and wildlife, and eliminate

locally the risk of introduction of new disease agents. Consequently, one possible result of habitat fragmentation may be the decline or even extinction of an infectious agent in wildlife hosts. Yet the majority of habitat fragmentation occurs without fencing and while decreasing the size of the range left available to wildlife it also increases the relative length of its edges. This process increases the likelihood of contacts between isolated populations and domesticated and other wild species living in adjacent habitats, facilitating the introduction of new diseases into previously unexposed populations. Furthermore in the case of the Tibetan plateau where hunting has been brought under better control in many areas, habitat fragmentation may also translate into crowding of naturally gregarious ungulate species, such as the Tibetan antelope, in shrinking territories. As a result, more individuals living in this habitat will be likely to encounter other host species living along the edge of this matrix, increasing also the probability of cross-species infection. Eventually on a longer term, isolation of populations could lead to a reduction of their genetic diversity and as a result lower immune capabilities to fight infectious aggressions.

Changing environmental conditions can influence the infectious characteristics of pathogens. In the continental semi-arid environments (<400 mm/year precipitation) of the Tibetan plateau, particularly in the western part, where wildlife is the most abundant, climatic constraints on vegetation have imposed relatively low wild ungulate densities in regard to the imposing land mass. In such circumstances, pathogens transmitted by a density-dependent process are likely to have evolved high transmission rates, low virulence and low host recovery in order to establish in host populations. An adaptation explained by the fact that pathogens that are highly virulent or have lower transmission rates are likely to require much higher host densities for establishment than those that are highly transmissible and relatively benign. Although these observations support that a majority of pathogens that have evolved in Tibetan wildlife are probably benign from an evolutionary point of view, the situation could change dramatically in the event of artificially increased densities such as observed during habitat fragmentation processes. In such circumstances, pathogens are theoretically more likely to evolve towards increased virulence, emerging to potentially more dangerous forms to hosts, adjacent wildlife and domesticated animals.

#### Risk of accidental introduction of pathogens from livestock

Exotic diseases and parasites are increasingly recognized as important factors driving population declines, geographical range contractions, or even extinction in many animal species (Osterhaus, 2001). The literature abounds with case studies that document catastrophic effects of introduced infectious agents in small populations.

Tibet Autonomous Region (TAR) shares more than 2000 km of international boundaries with India, Bhutan, Nepal and a disputed area between India and Pakistan. There is plethora of possibilities for free-ranging livestock such as domestic yaks to cross the border and vehicle disease agents from one population to another. For example in July 2007 in Ritu County (border area in western Tibet), local authorities observed an outbreak of Peste des Petits Ruminant a very contagious disease caused by a virus related to the devastating rinderpest morbillivirus (the latter was eradicated from China in the late 1950's following intensive vaccination campaigns). Authorities have suspected that the disease was introduced into this area through illegal importation of animals from India or even Pakistan. The apparent case fatality rate in the affected herd of goats reached 70%. Fortunately no cases affecting neighboring wildlife were reported although a number of nine domestic ungulates are known to be susceptible to the disease (Appendix 1). Although the outbreak seemed to have been efficiently contained and brought under control, it also suggests that the likelihood of introduction of pathogens in Tibetan livestock is not marginal and may in turn also pose a risk to its rich wildlife. Livestock infected with non-indigenous pathogens could also come into TAR from adjacent provinces in China. The spread of such diseases into wildlife populations would be further facilitated by the continual breakdown of barriers between livestock and wild animals in the nowadays highly fragmented steppes of the plateau. Today there is a wide spectrum of livestock populations in Tibet, all with varying degrees of dependency on humans that could come into contacts with wildlife. In the Chang Tang reserve free-ranging domestic yaks have been observed to come into contact with their wild relatives, and evidences exist of active reproduction between them. During our field visit, the Tibetan gazelles and the blue sheep were frequently cited by nomads and veterinary workers as coming occasionally into close contact (<100 m in horizontal distance) with sheep and goats.

### Global climate change and disease risk

The Intergovernmental Panel on Climate Change (IPCC) proposed models where global temperature in Tibet is to increase 4–5°C during the 21<sup>st</sup> century. Tibet is apparently among the areas on the planet which are particularly exposed to global climate warming. Indeed TAR has experienced an average temperature rise of 0.32°C/decade since 1961 whereas in the meantime temperature of mainland China rose on average 0.05–0.08°C/decade. Concomitantly the risk of species extinction is increased. While there is increasing evidence that the changes generated by human activities on global climate are affecting the distribution, prevalence, and severity of pathogenic organisms in natural ecosystems, to which extend they will affect Tibetan fauna remains largely unknown. Because the distribution and prevalence of infectious

agents often depends on environmental conditions, it is likely that changing weather pattern will also affect the prevalence of wildlife diseases. Two phenomena should be considered in priority. One is associated with the distribution shift of arthropod vectors to high-elevation refugia such as the Tibetan plateau, leading to the emergence of diseases only known at lower altitudes, or even leading to the extinction of endangered species surviving at higher elevations. The second is related to the increase in harmful UV radiation on organisms. In addition to changes in temperature and precipitation regimes throughout the world, greenhouse gases also contribute to the depletion of the ozone layer and to a concomitant increase in UV radiations. Such radiations can affect negatively many species of vertebrates, occasionally indirectly facilitating the spread of diseases via damages to skin and increasing susceptibility of integuments to fungi and other macroparasites.

### Part III. Risk of disease spillover between domestic animals and wildlife

#### Introduction

The transmission of infectious agents between individuals occurs either through direct contact, through indirect contact via a contaminated environment or via living vectors such as insects or acaria. In mountainous areas in Europe, the abundance of domestic herds and the increase of wild-living populations —partly due to strict enforcement of hunting laws and partly to human manipulation such as introduction or reinforcement— have led to increased cohabitations. As a matter of fact the spillover of diseases from domestic to wild-living ungulates has been largely reported in Europe during the last 20 years. In the Tibetan plateau, 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, no human manipulation has yet allowed a re-stocking of threatened populations of wild ungulates and although authorities have made great efforts to control it, illegal hunting is still present, particularly in the most remote areas where law is sometimes poorly enforced. We therefore hypothesize that the risk of disease spillover between wild and domestic ungulates is lower in the Tibetan than in the mountain ecosystems of Europe and North-America.

#### Risk of disease spillover by direct contact

The effectiveness of a direct contact depends on the brief survival of the infectious agent in the environment, particularly in aerosols, and on the distance between the ‘source’ and the ‘receptor’ individuals. Laboratory studies have shown that pathogens

with an enhanced ability to survive outside hosting cells and tissues (existence of an envelope or other protective structures) are more resilient in the environment and probably more infective than those fragile outside their cellular environment. The effectiveness of infectious aerosol according to the distance between the source and the target has rarely been studied. Dixon et al. (2002) however showed that strains of *Mannheimia haemolytica* (formerly known as *Pasteurella haemolytica*, the agent of hemorrhagic septicemia a disease well recognized in Tibet) nebulized into a wind tunnel can remain viable over a distance of ~20 m. Although this estimate applies to an infectious agent that is known to poorly survive in aerosols (Gilmour et al., 1990), the measurements were carried out in a horizontal setting, and distance effectiveness of such aerosol may prove even greater in mountain areas with significant vertical distances. One may therefore assume conservatively that a direct contact could occur when the simultaneous locations of a wild individual and a domestic are within a 100-m horizontal distance. The important consequence of this assumption is that in theory a number of wild ungulate species occurring in the Chang Tang are likely to come at some stage in direct contact with livestock. As a matter of fact nomads and veterinary workers we interviewed in an area relatively depleted of wild ungulates compared to the Chang Tang NR, admitted that they had seen occasionally Tibetan gazelles and blue sheep coming to close proximity (<100 m) with their tended herds of livestock. This is in contrast to the findings we made in Afghan Pamirs where our observations and interviews of shepherds and elders of communities showed that Marco Polo sheep (*Ovis ammon polii*) and Siberian ibex (*Capra [ibex] sibirica*) avoided direct contacts with tended herds of livestock (Ostrowski et al., 2009). In such location we have observed that marginal contact zones do exist spatially but are not effective during time, meaning that wild and domestic ungulate do not share them at the same time. The reason for this discrepancy compared to the situation in Tibet could be related to species specific avoidance behaviors but also to the fact that in Afghan Pamirs wild ungulates are very shy of men and herder dogs and do not approach tended herds closer than several hundred meters. Such avoidance behavior is probably linked to a significant level of persecution. This observation leads to the paradoxical hypothesis that hunting threat could contribute at decreasing the risk of disease transmission by direct contact between wild and domestic ungulates. A higher hunting pressure, would translate into an increased avoidance behavior from preys, and a decreased likelihood of disease spill-over between wild and domestic species. Such hypothesis could find some validity in a situation of range-use equilibrium between wild and domestic ungulates, yet in Tibet the rate of fragmentation of the habitat appears so high, and the 'edge-effect' so conspicuous that the risk of disease transmission at wildlife livestock interface could very well increase even when wild populations avoid contact because of hunting pressure. Moreover, like in Afghan Pamirs, direct contacts between

free-ranging domestic yaks and wild ungulates may occur in Tibet (see next part). In Afghan Pamir, our observations suggest that Marco Polo sheep tolerate domestic yaks in their immediate vicinity, such as Siberian ibex on the Afghan side of the eastern Hindu Kush mountain range.

A variety of infectious agents from livestock are susceptible to contaminate wild ungulates in Tibet. In the absence of recent information on clinical and serological exposure of livestock to infectious agents in the Chang Tang NR it is difficult to infer any level of risk for wildlife. Yet results of our interviews suggest that brucellosis, hemorrhagic septicemia, foot and mouth disease (FMD) and sarcoptic mange, four diseases transmitted via direct or indirect contacts and known to affect wild ungulates at various locations in the world, are present throughout the plateau, and may pose a significant risk to the Tibetan wildlife of Chang Tang. Prevalence of brucellosis in livestock is apparently on the decline thanks to active vaccination campaigns (Tsering Dorji, pers. comm.), hemorrhagic septicemia and mange seem to be locally common in yaks, and the picornaviruses responsible of foot-and-mouth disease circulate epizootically despite active vaccination campaigns.

Blue sheep in Shimshal (north-east Pakistan) are known to suffer fatal cases of sarcoptic mange (Dagleish et al., 2007). The gregarious social organization of this species, with groups and individuals moving across the Tibetan plateau irrespective of national boundaries, is likely to contribute to the spreading of the disease at macro-regional level. The original source of the disease could have been from livestock from any one of the countries having territory within the Tibetan plateau. Besides the negative consequences on the survival of the blue sheep itself, the disease could have also an impact on the snow leopard (*Uncia uncia*), which relies on them as its primary source of food. A disease affecting massively blue sheep could be disastrous for snow leopards which may have to turn against livestock to feed their young. So far snow leopards only rarely attack livestock in Tibet, unlike grey wolves (*Canis lupus*) (Schaller, 1990), a situation which to some extent helps these rare big cats cohabit with human beings.

Foot and mouth disease is endemic in livestock in Tibet, as it is in countries having territory within the Tibetan plateau or neighboring mountain ranges. For example in Afghan Pamirs, 50 to 75% of livestock tested in summer 2008 and spring 2009 had antibodies against FMD (Stéphane Ostrowski, pers. obs.), suggesting that the virus actively circulated in domestic livestock at that time. As most cloven-hoofed mammals, Tibetan wild ungulates are certainly susceptible to FMD, with possible impacts at population level. Outbreaks of FMD have threatened the long-term persistence of the Mongolian gazelle (*Procapra gutturosa*), a keystone species on the

Mongolian Eastern Steppe (Nyamsuren et al., 2006). The Tibetan gazelle is a species genetically related to the Mongolian gazelle and they may have a similar susceptibility to FMD. Because Tibetan gazelles share range with domestic livestock (sheep, goats, and yak), there is a risk that together with other wildlife species they may transmit FMD to livestock. Consequently, such as for its Mongolian relative, there is a great need to understand the potential role of Tibetan gazelles in the FMD persistence throughout the Tibetan plateau.

#### Risk of disease spillover via vector transmission

The transmission of infectious agents to wild ungulates can also occur through living vectors, among which Diptera insects and a variety of Acaria are the most likely to occur in the Tibetan plateau. So far *Culicoides* midges responsible for bluetongue orbivirus (BTV) transmission have been found at altitudes lower than 4100 m, in the Tibetan plateau (Mellor et al., 2000). In the context of landscape transformation because of global warming these midges will likely adjust to even higher elevations. Currently because of the relatively low (<12°C) average temperatures recorded in Chang Tang (referring to data from two Chang Tang towns; Bangoin at 4700 m asl and Gerze at 4415 m asl, cited by Schaller, 1998), it is unlikely to observe BTV transmission by *Culicoides* at the altitudes, typically higher than 4400 m, frequented by wild ungulates in Chang Tang. BTV infection rates and rates of virogenesis within vector *Culicoides* have been shown to be temperature dependent (Mullens et al., 1995). At reduced average air temperatures ( $T_a$ ), infection and virogenesis rates fall, the time to earliest transmission is extended, and midges' survival rate is enhanced. Viral replication was not detected in midges maintained at or below 15°C, transmission was never recorded at these  $T_a$ s, and the apparent infection rate rapidly fell to zero. This information could be incorporated into a GIS elevation model, mapping areas of lower and higher risk of BTV transmission according to the 15°C isotherm. We suggest that BTV is unlikely to circulate in summer pastures in the Tibetan plateau and to spillover to wild ungulates. Yet progressive increase of ambient temperature, melting of permafrost as well as of perpetual snows could offer in the near future adequate environmental conditions for a number of Diptera insects to colonize the area from lower elevations and vehicle new infectious agents to immunologically naïve wildlife populations. In the course of our interviews, respondents mentioned that 'mosquitoes' once restricted to wetlands are currently spreading to other areas and houses, and that 'house flies' (*Musca domestica*?) unknown in the past at the surveyed elevations (4400–4600 m asl) are now common in summer.

Unlike *Culicoides*, Tabanid horseflies have been found active during summer at altitudes exceeding 4500 m. Tabanid flies can carry pathogens on their mouth parts

and potentially transmit them to wildlife. Likewise other flies can also contribute to the dissemination of pathogens via contaminated mouth parts. Among many, *Moraxella* spp. and *Mycoplasma conjunctivae*, known to be responsible for keratoconjunctivitis in mountain ungulates in Europe, are of greatest concern. Little, however, can be done to prevent such vectored transmission.

#### Risk of disease spillover by indirect contact

The transmission of many infectious agents to wild ungulates can also occur through indirect contact with a soil contaminated with excreta from domestic ruminants. *Toxoplasma gondii*, *Coxiella burnetii*, *Chlamydiophila abortus* and coccidial oocysts are extremely resistant in the environment particularly for the latter two in cold conditions. The relatively cool thermal environment of altitude plateaus appears ideal to the persistence of infectious agents outside their hosts. But persistence of infectious agents may also depend on other microclimatic factors such as humidity level and UV radiations, as well as upon land cover characteristics such as vegetation height. The Tibetan plateau is a relatively dry area, receiving little rainfalls during summer. Because of the altitude and overgrazing any infectious agent contaminating the soil would be exposed to intense UV radiations during summer. For these reasons we believe that the end of summer and autumn, before snow covers the ground, and when humidity level is increased and exposure to UV radiation reduced, are theoretically the best periods for the indirect transmission of infectious agents. In addition, when shed or excreted in autumn, microclimatic factors would favor pathogen survival perhaps until spring. Mountain ungulates commonly use summer livestock grazing areas during winter, a situation of concern in regard of the risk of indirect transmission of pathogens between livestock and wild ungulates.

*Brucella* spp., *Toxoplasma gondii*, *Coxiella burnetii* and *Chlamydiophila abortus* are able to survive months on the ground, but they are most often excreted with infected genital secretions or abortion products. Most abortions in Tibetan livestock occur at the end of pregnancies (interviewed veterinary workers, pers. comm.) in late winter and spring. At that time of the year the vast majority of sheep and goats are often at the lowest reaches of valleys that wild ungulates also visit but at higher elevations. Nevertheless interviewed herders told us that they let occasionally their yaks roam at high altitudes even in winter and early spring. In such circumstances the risk of disease transmission to wild ungulates via contamination of winter pasture with abortive tissues would be increased. Because yaks are likely to come into contact with wild ungulates and share altitude pastures across the Tibetan plateau their contribution as disease disseminators has often been questioned.

Table 1. Non-exhaustive list of major infectious diseases affecting domestic yak in Tibet.

Common name	Agent	Prevalence in Tibet	Reference
Anthrax	<i>Bacillus anthracis</i>	Variable but always high mortality	Lu and Ling (1985) <sup>a</sup>
Brucellosis	<i>Brucella abortus</i> / <i>melitensis</i>	1.8 – 56.3%	Local Disease Control Office (1983) <sup>a</sup>
Contagious Bovine Pleuropneumonia	<i>Mycoplasma mycoides</i>	1.9%	Lu and Ling (1985)
Chlamydiophyllosis	<i>Chlamydiophyla abortus</i>	Present	Wang (1990)
Hemorrhagic septicemia	<i>Mannheimia haemolytica</i>	<1% but locally common	Lu and Ling (1985)
Salmonellosis	<i>Salmonella typhimurium</i> / <i>dublin</i> / <i>newport</i>	5.2% (serology)	Lu and Ling (1985)
Tuberculosis	<i>Mycobacterium bovis</i> / <i>tuberculosis</i>	12.7% (IDR test)	Lu and Ling (1985)
Foot and mouth disease	Picornavirus	Endemic	Present mission, and Tsering Dorji pers. comm.
Viral diarrhea/mucosal disease	Pestivirus	Present	Geilhausen (2002)
Infectious bovine rhinotracheitis	Herpesvirus	35% (serology)	Qu and Li (1988)
Q fever	<i>Coxiella burnetii</i>	Present	Geilhausen (2002)
Hypodermosis	<i>Hypoderma lineatum</i> / <i>bovis</i> / <i>sinese</i>	Significant	Present mission, and Tsering Dorji pers. comm.
Coenurosis	<i>Coenurus cerebralis</i>	Very significant	Present mission, and Tsering Dorji pers. comm.

<sup>a</sup>Cited by Dorji et al. (2003).

### Risk of disease transmission between domestic and wild yak

Once found in abundant numbers around the headwaters of the Yellow River, wild yaks populations have suffered dramatic decline due to hunting (Schaller, 1998). Nowadays most surviving wild yaks live in the Chang Tang reserve where they still remain under threat of illegal hunting, but also of hybridization with their domestic relatives. Because of the genetic proximity between wild and domestic yaks it is believed that wild specimens are very tolerant of their domesticated relatives and that most diseases recognized in domestic yaks can also affect wild animals. In such circumstances disease spill-over from domestic yaks appears as a significant threat faced by the last populations of wild yaks. Table 1 presents a non-exhaustive list of major diseases known to affect domestic yaks in Tibet.

However other diseases not listed in this table could also affect yaks and presumably have an impact on wild populations as well. Schaller (1998) reports of a poor survival of wild calves in the Aru Basin and suggests that this wild population cannot sustain itself even if all poaching were to cease. Such observation points out the possibility of a health factor currently affecting this population. Interviews that we have carried out among nomads and veterinary workers during the present mission suggest that domestic yaks have been affected for the last decades by a decreased reproduction rate. It is unknown whether this phenomenon is statistically significant, and if it is, whether it is caused by abortive diseases (e.g. brucellosis, chlamydia, Q fever, IBR, BVD/MD), poor nutritional status because of the overpopulation of livestock, other causes such as physiological impact of increasing ambient temperatures, or a combination of these reasons.

Schaller (1998) suspected that brucellosis could affect wild yaks in Tibet in a similar way it has been affecting bison (*Bison bison*) in North America. This is indeed possible but we lack data to support this disturbing hypothesis. Brucellosis, a disease documented world-wide in livestock as well as in a variety of terrestrial and marine wild mammals, is caused by intracellular bacteria of the genus *Brucella*. In China *B. melitensis* (biovars 2 and 3) is the most common among yak and its hybrids (yak×cattle) (Chen, 1983 in Dorji et al., 2003). Concern with brucellosis is made the greater by the fact that it is readily transmitted to humans. In Sichuan, Qinghai and Tibet, sample groups of yak were tested over the period 1952–1981, and an average of 17.4% (1.8–56.3%) tested positive (Local Disease Control Office 1983 in Dorji et al., 2003). Interestingly Wang and Yao (1984) reported that between 13% and 17% of yak in various groups on Haiyan pastures in Qinghai province had positive tests and that there was a significant reservoir of *Brucella* infection among wild animals (rodents, lagomorphs) as well as among sheep and dogs. In China, S2 vaccine (swine strain vaccine) has been widely advocated for *Brucella* control measures since 1982. In a study conducted to monitor the effects of the vaccine against brucellosis in growing yak, Chen and Zhang (1997) tested a total of 9,944 serum samples and 452 samples from aborted fetuses from Guoluo prefecture. The results indicated that the number of positive samples fell from 21% prior to vaccination to 0.4% and *Brucella* species were not isolated from fetal material. Abortion rate in pregnant yak cows also decreased from 18.1% before conducting the program in the Guoluo prefecture to 2.8%. In Tibet vaccination is also implemented in yaks with apparent good results (Tsering Dorji, pers. comm.), but it is unknown to which extent this practice is widespread. In addition the potential shedding of vaccine strains is of concern because of their residual virulence and resistance to antibiotics (Godfroid, 2002). The possibility of environmental contamination and infection of non target wild species is not negligible.

Unfortunately testing and slaughter of livestock are not considered to be viable options for control - at least not in the present circumstances of yak husbandry. Yet the implication of brucellosis in yak for human health is likely to require in the future more considerations for stamping-out methods of control of infected livestock.

### Canine distemper in carnivores

Canine distemper (CD) is a highly contagious, systemic, disease of dogs seen worldwide caused by a paramyxovirus closely related to the viruses of measles and rinderpest. This disease occurs also in members of six (Canidae, Felidae, Hyaenidae, Mustelidae, Procyonidae, and Viverridae) of the seven families of terrestrial carnivores. Wolves and large cats are reported to be very susceptible to the disease. In dogs typical clinical findings include transient fever 3–6 days after infection, followed by another episode of high fever accompanied by serous nasal discharge, mucopurulent ocular discharge, and anorexia. Intestinal and respiratory signs may follow and are usually complicated by secondary bacterial infection. Neurological signs are sometimes predominant as well as hyperkeratosis. Mortality is high in the presence of a neurologic syndrome.

When interviewing veterinary workers in Tibet, all of them reported the presence of a 'common' disease in dogs resembling canine distemper. The disease is said to affect both sexes equally and all age classes although animals less than two year old are more at risk. Fever followed by keratoconjunctivitis, mucopurulent ocular and nasal discharges, and anorexia are the main symptoms. Recovery is rare. In Tibet dogs of nomads are not vaccinated against CD.

Interspecies transmission of paramyxovirus has been well documented (Cleveland et al., 2001) and is an emerging threat to free-ranging carnivores (Williams, 2001). Domestic dogs represent the principle reservoir for this virus, but the potential for spill-over to free-ranging carnivore population worldwide is genuine and dog-born epizootics associated with population decline have been reported in free-ranging African lions (*Panthera leo*) (Roelke-Parker et al., 1996) and African wild dogs (*Lycan pictus*, van der Bildt et al., 2002). In Asia, Siberian tigers (*Panthera tigris altaica*) have also been affected by the disease in far east Russia (K. S. Quigley, pers. comm.). In the Tibetan plateau, veterinary workers concur in their observations that wolves (*Canis lupus*) are occasionally affected by a disease which features the main symptoms of canine distemper. Yet nothing is known about the epidemiology of the disease among wolves and other Tibetan wild carnivores. Although the brown bear is apparently less susceptible to the disease (Jacobson et al., 1988), it is likely that aside from wolves, the disease may affect the two fox species present in the Tibetan plateau, the red fox

(*Vulpes vulpes*) and the sand fox (*Vulpes ferrilata*), as well as two large to medium-size cats, the snow leopard and the lynx (*Lynx lynx*). To which extent these species are affected in Tibet and how it impacts their populations at landscape level is unknown, but deserves further investigations owing to the known negative effect of the disease on other species of free-ranging carnivores.

## Part IV. Recommendations

The challenges facing future health managers of one among the largest protected areas in the world are tremendous. According to my preliminary analysis of the situation, the current lack of knowledge and institutional responsiveness to disease risk in wildlife in the Chang Tang ecosystem could be due to five main causes:

- The area is remote, and exceptionally vast.
- There are no available baseline data for wildlife health in the Tibetan ecosystem.
- Available staff in the field lacks understanding about the importance of wildlife diseases in a context of increasing cohabitation with livestock, lacks capacity about wildlife health issues and logistical support.
- There seems to be a lack of cooperation and agreement between acting agencies about mandates of wildlife disease surveillance (i.e. Ministry of Forestry, Ministry of Agriculture, and Ministry of Health).
- Finally, there is no regional strategy concerning wildlife disease issues.

### Responding to the vastness and remoteness of the Chang Tang

Remoteness and vastness of the area is an essential factor that will affect every aspect of the strategy and logistical solutions developed in the future. Despite these geographical difficulties, TAR has succeeded to put in place and maintain a comprehensive network of veterinary workers, at township, town, county, prefecture and Lhasa levels (Photo 2). The system appears well structured and efficiently organized. Yet the pyramidal organization of epidemiological surveillance also suffers from the classical drawbacks of highly centralized systems when operating in very vast areas, i.e. slow detection rate, increased possibilities of miscommunication, slow reactivity to emerging issues, delayed and sometimes inadequate responses to emergency problems. In the perspective of an increasing number of veterinary

professionals, a progressive amelioration of their capacity and improving communication systems it is likely that this organization will progress towards significant improvement. Increasing the number of people with an understanding of wildlife disease problems, building-up their capacity in wildlife disease detection, diagnostic and sampling, and using their new expertise to put in place an epidemiological network of early ‘detectors’ will help integrate wildlife health problems in a modern ecosystem health perspective. In order to prioritize detection activities and research works, wildlife disease risk should be modeled thematically (wildlife per se, risk of transmission to livestock, zoonosis...), quantitatively (low, medium, high), temporally and spatially using database and geographical information system technology. This modern landscape management tool combined to powerful probabilistic models will help optimize timely and spatially work efforts, and provide spatial predictive models of wildlife/livestock disease transmission at the scale of Chang Tang.



Photo 2. From left to right Mr Tsering Dorji, Director of the veterinary office at the Veterinary Institute in Lhasa, Mr Patentendu, head veterinarian of Dangxion County, and Mr Sunam, township veterinarian in the same county. These three veterinary professionals are in charge of three different levels of animal disease surveillance in Tibet. Any disease outbreak in livestock or occasionally in wildlife around Umah township would be signaled to Mr Sunam by nomads, in turn he will forward the information to Mr Patendu who will transmit it to Mr Tsering Dorji in Lhasa. Umah township, Dangxion County, Tibet, 11 June 2009 (© Tse Brten Rgyal / WCS).

## Acquiring baseline data on wildlife health

As previously explained, little is known about the presence and prevalence of infectious agents in non-domestic herbivores and carnivores of Tibetan highlands. However, in the context of cohabitation between domestic and non-domestic herbivores such as reported in the Chang Tang Reserve, transmission of diseases from one population to the other through direct or indirect contacts could have a great impact in term of economical and biodiversity losses. Because of that, one important objective for animal health authorities in Tibet will be to promote the acquisition of baseline data on infectious agents circulating in wild ungulates and carnivores in the Reserve. This could be done through an epidemiological surveillance system (Photo 3). Epidemiological surveillance is the systematic collection, analysis and dissemination of health data for the planning, implementation and evaluation of health programs. Sensitivity (probability of a positive test among infected individuals) and specificity (probability of a negative test among non infected individuals) are two essential attributes of quality of any epidemiological surveillance operation. Epidemiological surveillance is a very important aspect of disease monitoring in wildlife population. It is based on the ability to detect through a variety of sampling schemes the presence of an infectious agent in a population, either directly via antigen detection, isolation/identification and nucleic acids detection, or indirectly through the measurement of antibodies in the blood. Obviously epidemiological surveillance reaches maximum sensitivity when it is directed towards infectious agents that have a reasonable likelihood of being present in the population. Because clinical diseases are rarely observed in free-ranging animals, wildlife epidemiologists base their detections either on random sampling of live and dead animals, targeted sampling of harvested specimens, or more rarely on animals presenting clinical symptoms of a disease. In Appendix 1, I have outlined a list of the principal infectious agents and methods to be used in the epidemiological surveillance of wildlife in Tibet. The list is not exhaustive and will benefit from regular updating.

## Building capacity of the animal health experts

There are at least three strata in the veterinary academic system in China. The first and highest stratum consists of four universities with colleges of veterinary medicine in Beijing, Nanjing, Wuhan and Guangzhou, under the direct administration of the Chinese Department of Education. These four prestigious colleges provide the highest level of veterinary education and expertise available in China. In addition to these lead colleges, there are veterinary institutes, colleges and departments under the administration of provincial governments and also similar structures under the administration of city governments.



Photo 3. Interviewing nomads and inhabitants of Tunga township, Dangxion County, about symptoms of fluorine intoxication in their livestock, and the extent to which it could also affect wildlife living in the same area, 11 June 2009 (© Tse Brten Rgyal / WCS).

Although less prestigious these institutions deliver a serious education background in veterinary science that is adjusted to the specificity of local demands. In the past decade the veterinary academic system has markedly improved at all levels of the educational organization. As a consequence Chinese public veterinary system has been very efficient at developing preventive policies and putting in place disease eradication campaigns. Yet, little to no educational programs have been developed in the field of wildlife diseases aside of those that have a zoonotic impact (e.g. plague in marmots or outbreaks of anthrax). Such situation is very unfortunate in regions, such as TAR, where wildlife species are still relatively abundant and cohabit with domestic animals and humans, posing a risk of disease transfer between wildlife, livestock and humans. Because the current national policy is to strengthen the public health system including the role of veterinary medicine in the early detection of emerging zoonosis, provincial authorities in TAR will certainly find very useful to integrate in their educational programs a wildlife disease component. On the long term this could be achieved by developing the capacity of their veterinary academic staff and field workers at recognizing diseases in wildlife and developing efficient mechanisms of early detection and useful research projects.



Photo 4; The Institute of Zoology of the Academy of Sciences in Beijing has been hosting the National Research Center for Wildlife Born Diseases (NRCWBD) since 2003 (© Stéphane Ostrowski / WCS).

Upon visiting TAAAS in Lhasa, I have noticed the tremendous efforts to increase and modernize the ‘in-house’ diagnostic facilities and capabilities of the Veterinary Institute. Efforts to upgrade the equipment and staff’s expertise will soon follow. In particular modern state-of-the-art methodologies (for example molecular tools) should be mastered to detect the most common diseases of livestock but also those that could pose disease risks to wildlife. It is at the present stage of development that wildlife diseases relevant to livestock health professionals should be included in the capacity building programming of future investigators.

In 2003, China has created the National Research Center for Wildlife Born Diseases (NRCWBD), based at the Institute of Zoology of the Academy of Sciences in Beijing (Photo 4). The creation of this structure was directly related to the emergence of diseases allegedly originating from wildlife reservoirs such as severe acute respiratory syndrome (SARS) and highly pathogenic strains of avian influenza viruses.

NRCWBD is also concerned by diseases affecting endangered species such as the giant panda (*Ailuropoda melanoleuca*) and the Tibetan antelope (*Pantholops hodgsoni*). Those two flagship species of Chinese conservation are under the scrutiny of national authorities. Any diseases affecting them must therefore be investigated in priority.

Prof. Hongxuan, the very competent leader of the structure, has the capacity to run a large variety of laboratory investigations for an early detection of a range of infectious agents in wildlife hosts. For the handling of dangerous infectious agents, such as HPAIV, the NRCWBD collaborates with P3-confinement laboratories and sister research institutes in China.

Raising capacity in wildlife diseases of the talented staff at the Veterinary Institute of TAAAS could be done through study trips, targeted visits, and specific teaching activities organized by NRCWBD in Beijing and facilitated by WCS. Ultimately such cooperation could be framed in an educational cursus. It would certainly help improve the level of expertise of a selected group of veterinary academics at TAAAS who are willing to acquire regional expertise on wildlife disease issues. In turn the newly trained staff will be able to propagate the knowledge among students and the rest of academic staff at TAAAS. We deeply recommend to develop such approach as soon as possible. In the meantime WCS could also help build the capacity and interest of the veterinary personnel at Lhasa via disease-specific lectures delivered by health experts, produce a brochure that will introduce essentials in wildlife disease recognition and diagnostic for field veterinary workers at township and county levels in Tibet and train Forestry personnel in wildlife disease detection.

#### Increasing the cooperation between health agencies

The NRCWBD is mandated to investigate any wildlife disease issue in the country. The structure benefits from a satisfactory level of expertise and good capacity in disease diagnostic. It also works in collaboration with sister institutes and research centers in other ministries. Yet it is located in Beijing, far from TAR and Chang Tang, and has no regional antennas that would help improve its involvement in regional issues. All public veterinary services in Tibet are under the administrative supervision of TAAAS which has no mandate to investigate diseases in wildlife, a task dedicated to forestry authorities. Yet the latter have little capacity in health matters and can only rely to the far-located NRCWBD in case of disease outbreak in wildlife. Eventually human health authorities are involved in zoonotic wildlife diseases (e.g. anthrax), sometimes exclusively of any other partners, such as in suspected cases of plague in marmots via their network of 'epidemics stations'. These structures all supported by heavy administrations cooperate poorly between each others. In an ideal situation, personnel from Department of Forestry would be trained at detecting wildlife diseases when reported to them by nomads, in collaboration with TAAAS veterinary workers they would investigate and sample the cases that would be further investigated in laboratory by a NRCWBD antenna working cooperatively with TAAAS diagnostic laboratory at Lhasa. In case of suspected zoonotic diseases, all

cases will be jointly (and not exclusively) investigated by human health authorities. There is an obvious requirement for more cooperation and communication between the different health actors involved. Reasons for this poor communication are numerous and certainly not specific to China or Tibet, but apprehending health problems within a global ecosystem perspective would certainly help recognize and resolve conflicts between health professionals and increase the efficiency of detection of emerging diseases from wildlife origin as well as the quality of the sanitary response. An innovative decision would be to create an Ecosystem Health Committee for Tibet that would gather on a regular base local and regional health partners (human/domestic animals/wildlife) to discuss research and respond to local health challenges. More cooperation is also required at macro-regional and national levels between administrations supervising agriculture/livestock, and wildlife/range. Joint seminars, data sharing, and cross capacity building could improve the current administrative clustering.

### Committing into a regional strategy

Efforts to improve cooperation and data-sharing between Tibetan health partners should translate into the evaluation of several hypothetical strategic directions that would define the goals of a holistic health policy. In the case of Chang Tang Natural Reserve, addressing health problems that may affect wildlife should be a priority owing to the decision taken by authorities to singularize this landscape as protected. Livelihood and landscape transformation are two anthropogenic processes likely to have a significant effect on the health status of Chang Tang's native fauna. In the present document I have emphasized the risk of disease spill-over from livestock to wildlife and vice versa, yet other activities and processes, such as resource extraction activities and global warming may also pose significant threats to the health of Tibetan wildlife. The long-term effectiveness of a strategic planning is likely to benefit from the holistic understanding that a healthy ecosystem is a prerequisite to healthy human beings, and that developing projects aiming at maintaining healthy ecosystems will ultimately benefit inhabitants of Tibet on the long term. Unhealthy wildlife may be at the origin of an increase in emerging diseases in livestock and human beings. Yet per se it is rarely the seminal cause of such problems, but more frequently the revelator of a disturbed environment. Destroying unhealthy wildlife that may pose a risk to human beings and their livelihood shuts down temporarily an alarm signal but seldom addresses the source of the problem.

## Acknowledgments

I wish to thank the Tibet Academy of Agricultural and Animal Sciences (TAAAS) for allowing me to carry out this mission, and particularly Mrs Sejiu, and Mr Tsering Dorji, Director of the Veterinary Office at the Veterinary Institute, who was an invaluable expert during the field trip. Mr Jiangyong Zeng mentored the visit of the new facilities of the Veterinary Institute in Lhasa, and I thank him for that. Thanks and appreciations go also to Mr Tendah, director of the Animal Husbandry Unit in Dangxion County who greatly facilitated our stay, to Mr Patentendu, the senior state veterinarian in the county for his great knowledge and wise approach of health issues, and Mr Sunam, Mr Nianda, Mr Pemasamjug, and Mr Tile, veterinary workers at Umah township, Nam Co Lake township, Nyingtrung township and Yanjpachen town, respectively, for their kindness, availability and invaluable experience. I extend also my gratitude to all staff at WCS and in particular to Mr Tse Brten Rgyal who took care of me during all my stay in Tibet. Mr Lhakpa Tsering was a conscientious and dedicated translator.

## Appendices

### Appendix 1. Guidelines for baseline data collection in wild ungulates

The following guidelines have already been presented in Ostrowski, 2007. Considering the large variety of potential infectious agents in any wild population, and order to save time and money, we recommend to prioritize detection efforts according to the following criteria:

- 1- the recognized susceptibility (RS) of wild herbivores to the infectious agent;
- 2- the recognized presence (RP) of this infectious agent in livestock in Tibet;
- 3- the likelihood of transmission (LT) of the infectious agent through direct or indirect contacts between domestic and non-domestic herbivore populations.

Diseases that fulfill those criteria should receive priority allocation of resources, logistics and manpower. The detections should be organized so as to evaluate the presence of these diseases in surveyed populations assess their prevalence, and distinguish between a spillover event of infection from domestic animals, a sustainable infection in wild species or a state of asymptomatic carriage. Recent investigations carried out by WCS in Mongolian gazelles in the eastern steppes of Mongolia have shown how important it is to understand livestock/wildlife interface issues (Nyamsuren et al., 2006).

#### Sampling methods

##### Passive sampling

It is the opportunistic sampling from multiple sources, especially related to morbidity and mortality events. Pre-existing programs such as regulated hunting and culling, as well as scientific captures, may also provide occasions to perform sampling. Samples will have to be collected according to a rigorous and standardized protocol during each of such missions. Because these surveillance operations are complicated and require a coordinated planning, an active and careful collaboration between the different partners and biologists involved in these missions will be essential. While capture or harvest operations will provide access to fresh material (blood, fresh tissues), post-mortem examinations, for example in the context of a mass-mortality event, may also provide valuable information about disease ecology such as sex and age ratio of dead animals and probable death causes. In the case of infectious agents resisting post-mortem conditions, sampling of infected tissues can confirm the cause of death (for

example bone marrow sampling in suspected *Mannheimia* sp. infection or FMD or scab sampling for detection of parapoxvirus infection).

#### Active sampling

It is the focused and targeted sampling of a species with predetermined level of accuracy and detection. It is especially designed to collect samples from animals but can also be combined with other objectives (e.g. population productivity assessment). One such mission has been proposed in summer 2006 by Dr Schaller and would consist in sampling blood of newborn calves of Tibetan antelopes to assess the exposure status of their mothers. Ruminants acquire no antibodies from the mother before birth. Because maturation of the immune system is incompletely achieved at birth, antibody production develops slowly in the newborn ruminant. The newborn would rapidly be overwhelmed by invasive organisms if it did not receive antibodies from the mother. Antibodies acquired in this way come from the colostrums via the neonatal intestine. In most ruminants antibodies contained in the colostrum of the mother are maximally absorbed through the digestive tract within the first 2 hours of life. After 24 hours, a 'closure' phenomenon occurs which prevents absorption of additional antibodies from the milk. However antibodies in the late colostrum and in the milk still contribute at protecting the calf at the level of digestive tract lumen. In the meantime, maternal antibodies that now circulate in the newborn calf are an essential part of its early humoral immunity, a protection capability fully originating from the mother. In theory sampling serum from a less than one-week-old calf would allow the detection of maximal levels of maternal antibodies and provide a qualitative understanding of the diseases the mother has been exposed to. Antibody titer in the newborn will decrease at a rate that varies according to initial antibody level, species, quantity of antibodies transferred and type of antibodies. Depending of the initial titer maternal antibodies could be detected for several months after birth. It is therefore not abnormal to find in young calves a high titer of antibodies against a specific pathogen shortly after its birth and no track of such titer a couple of months later. Tibetan antelopes aggregate in specific locations and at specific time of the year to give birth. Dr Schaller rightfully proposed to organize a mission at this time of year to collect samples from newborn calves. In addition freshly dead calves as inevitably reported during calving time and even weak females may also provide the opportunity for more sampling and better understand the exposure of Tibetan antelopes to the targeted infectious agents.

#### High priority diseases

We propose to evaluate in priority the exposure of non-domesticated herbivores in the Chang Tang ecosystem to: Foot and Mouth Disease (FMD), Peste des Petits Ruminants (PPR), Brucellosis, Blue tongue (BT) /Epizootic hemorrhagic disease

(EHD), Hemorrhagic Septicemia (HS) and mange. These diseases clinically affect wild ungulates, are present in livestock in Tibet, and are likely to be transmitted from livestock to wild herbivores and possibly vice versa for a number of them.

#### Foot and Mouth Disease (RS, RP, LT)

- *Agent:* Foot and mouth disease virus (FMDV) is a member of the *Aphthovirus* genus in the Picornaviridae family. Seven distinct serotypes are recognized: A, O, C, SAT-1, SAT-2, SAT-3, Asia-1, the O serotype being the most common.
- *Occurrence:* FMD is widely distributed throughout the world. It is endemic in parts of Asia, Africa, the Middle East and South America. Since 1999, FMD outbreaks (O and Asia-1 serotypes) have been recorded in livestock in several provinces of China including Tibet.
- *Hosts:* FMD is an infectious disease affecting cloven-hoofed animals, both domestic (cattle, zebu, domestic buffalo, yak, sheep, goats, and swine) and wild (ruminants and Suidae). Camelidae (Bactrian camels, dromedaries, llamas, vicunas) have low susceptibility.
- *The disease in wildlife:* In Africa, natural reservoirs of FMDV are domestic cattle and African buffalo (*Syncerus caffer*). Although other domestic and wild species become infected, they seem to be unable to maintain the infection for more than a few months in the absence of cattle or African buffalo. Elsewhere in the world cattle are usually the main reservoir, although in some instances the viruses involved appear to be specifically adapted to domestic pigs or sheep and goats. Wildlife outside Africa has not, so far, been shown to be able to maintain FMD viruses. The evidence indicates that infection of deer in the past was derived from contact, direct or indirect, with infected domestic animals. Circumstantial evidence indicates, particularly in the African buffalo, that carriers are able, on rare occasions, to transmit the infection to susceptible animals with which they come in close contact: the mechanism involved is unknown. The carrier state in cattle usually does not persist for more than 6 months, although in a small proportion it may last up to 3 years. In African buffalo individual animals have been shown to harbor the virus for at least 5 years, but it is probably not a lifelong phenomenon. Within a herd of buffalo, the virus may be maintained for 24 years or longer. There is no information on the duration of the carrier state in another domestic buffalo, the swamp buffalo of East Asia. Domestic buffalo, sheep and goat do not usually carry FMD viruses for more than a few months. Understanding the role of wild ungulates in the epidemiology of FMD in Tibet is critical to developing efficient FMD control strategies.

- *Proposed actions:* Targeted and random detection. Evaluate the level of exposure to FMD in wild ungulates in contact with livestock as well as in animals captured, harvested, culled or found recently dead with no reported contact with livestock. Methods: virus neutralization, competitive solid phase ELISA, or liquid phase blocking ELISA.
- *Carrier stage:* Evaluate the presence of the viral agent in individuals presenting positive antibody prevalence (methods: antigen detection test, virus isolation, nucleic acid detection).

#### Peste des Petits Ruminants (RS, RP, LT)

- *Agent:* Like rinderpest virus, Peste des Petits Ruminants virus (PPRV) is a member of the *Morbillivirus* genus in the Paramyxoviridae family.
- *Occurrence:* Once thought to occur only in Africa, PPR is now present in the Middle East, Pakistan, Afghanistan, Nepal, Bhutan, Bangladesh and India. It has been reported in Tibet in 2007.
- *Hosts:* Goats and to a lesser extent sheep. Cattle and pigs develop unapparent infections.
- *The disease in wildlife:* To date the disease has only been diagnosed in captive dorcas gazelle (*Gazelle dorcas*), Nubian ibex (*Capra [ibex] nubiana*), gemsbok (*Oryx gazella gazella*) and Laristan sheep (*Ovis orientalis laristanica*). Experimentally the white tailed-deer (*Odocoileus virginianus*) is fully susceptible. No case has been reported so far in free-ranging wildlife and the susceptibility of Tibetan ungulates is unknown. To date no wildlife reservoir has been identified but the fact that cattle can carry and disseminate the virus without clinical symptoms suggest that the virus could have co-evolved to a commensal relationship in other hosts.
- *Proposed actions:* Targeted and random detection. Evaluate the level of exposure to PPR in wild ungulates in contact with livestock as well as in animals captured, harvested, culled or found recently dead with no reported contact with livestock (methods: virus neutralization test or competitive ELISA).
- *Carrier stage:* Evaluate the presence of the viral agent in individuals presenting positive antibody prevalence (methods: antigen detection test, virus isolation, RNA detection).

#### Brucellosis (RS, RP, LT)

- **Agent:** Coccobacilli (short-roded Gram-negative bacteria) of the genus *Brucella*. There are several different species of *Brucella*, with different host specificities.
- **Occurrence:** It has a worldwide distribution although several countries have succeeded to eradicate it. It is present in Tibet.
- **Hosts:** In the Artiodactyla order, *B. abortus*, *B. melitensis*, and *B. ovis* affect cattle, sheep and goats.
- **The disease in wildlife:** *Brucella* infections have been documented worldwide in a great variety of terrestrial and marine wildlife species. *B. abortus* has been isolated from a range of non-domestic artiodactyls including the bison (*Bison bison*), the elk (*Cervus elaphus*), and the Cape eland (*Taurotragus oryx*). Arabian oryx (*Oryx leucoryx*), chamois (*Rupicapra rupicapra*) and alpine ibex (*Capra ibex*) are susceptible to *B. melitensis*, with clinical signs, while reindeer (*Rangifer tarandus*) can be infected by a biovar of *B. suis*. An important consideration with regard to terrestrial brucellosis in wildlife is to distinguish between a spillover of infection from domestic animals and a sustainable infection in wild species.
- **Proposed actions:** Targeted and random detection. Evaluate the level of exposure to *Brucella* in wild ungulates in contact with livestock as well as in animals captured, harvested, culled or found recently dead, with no reported contact with livestock (methods: at least two serological tests such as Rose Bengal and competitive ELISA).
- **Carrier stage:** Evaluate the presence of the bacterial agent in individuals presenting positive antibody prevalence (methods: antigen detection test, virus isolation, nucleic acid detection).

#### Blue Tongue (RS, RP, LT?) and Epizootic Hemorrhagic Disease (RS, RP?, LT?)

- **Agent:** Blue Tongue virus (BTV) and Epizootic Hemorrhagic Disease virus (EHDV) are members of the *Orbivirus* genus in Reoviridae family. A total of 24 serotypes have been identified worldwide for BTV and 10 for EHDV.
- **Occurrence:** BT and EHD are transmitted by midges of the genus *Culicoides*, which are biological vectors. The central role of the insect vector in BT and EHD transmission makes distribution of those two diseases strongly related to the distribution of *Culicoides*. Hence BT and EHD distribution is governed by ecological factors, such as rainfall, temperature, humidity and soil characteristics. BTV exists in North, Central, and South America; Africa; and

parts of Asia; Europe; the Middle East; and the South Pacific; EHDV is probably similarly distributed. BT has been diagnosed in China for the first time in 1979. BTV seems to have recently reached Tibet (Culicoides have been found in Tibet at altitudes up to 4,200 m). It is unclear if EHDV is currently present in Tibet but in the context of global temperature increase, the disease is likely to emerge on the Tibetan plateau in the future.

- *Hosts:* BT is an infectious, non contagious disease of sheep and other domestic and wild ruminants, such as goats, cattle, deer, bighorn sheep, most species of African antelope and other Artiodactyla.
- *The disease in wildlife:* The outcome of BTV infection ranges from unapparent in the vast majority of infected animals to fatal in a proportion of infected sheep, deer and some other wild ruminants. In non domestic ruminants, clinical symptoms can vary from an acute hemorrhagic syndrome with high mortality, as observed in white-tailed deer (*Odocoileus virginianus*), to non existent as seen in the North American elk (*Cervus canadensis*). EHDV can produce a disease in wild ruminants with clinical manifestations identical to those observed in BT. BTV and EHDV have been relatively little investigated in wildlife outside North America.
- *Proposed actions:* Targeted and random detection: Evaluate the level of exposure to BTV and EHDV in wild ungulates in contact with livestock, as well as in animals captured, harvested, culled or found recently dead, with no reported contact with livestock (methods: agar gel immunodiffusion and competitive ELISA)
- *Carrier stage:* Evaluate the presence of the viral agent in individuals presenting a positive antibody prevalence (methods: virus isolation and serotyping, nucleic acid detection).

#### Haemorrhagic Septicaemia (RS, RP, LT?)

- *Agent:* *Pasteurella multocida*, a Gram-negative coccobacillus bacterium. Two serotypes, the Asian B:2 and the African E:2 (Carter-Heddleston system), are mainly responsible for the disease. They correspond to serotypes 6:B and 6:E (Namioka-Carter system). In wild animals, serotype B:2,5 is predominant. The association of other serotypes, namely A:1 and A:3 with a HS-like condition in cattle and buffaloes in India has been recorded.
- *Occurrence:* Worldwide occurrence. Present in Tibet, particularly in yak.

- *Hosts*: Cattle, water buffaloes and bisons are natural reservoirs of the disease, buffaloes being the more susceptible. *Pasteurella multocida* is a commensal agent in the upper respiratory tract of many animals.
- *The disease in wildlife*: Recent HS cases have been reported in Asia and Europe in wild mammal species, including water buffaloes, deer, elephants and yaks. In Asian countries, disease outbreaks mostly occur under the climatic conditions typical of monsoon (high humidity and high temperatures).
- *Proposed actions and carrier stage*: Targeted, random detection. Evaluate the level of exposure to *Pasteurella* in wild ungulates in contact with livestock as well as in animals captured, harvested, culled or found recently dead, with no reported contact with livestock (methods: tonsillar biopsy or swabbing culture, isolation and typing).

#### Mange (RS, RP, LT)

- *Agent*: Mange results from infestation by either astigmatid mites belonging to different genus including *Chorioptes*, *Psoroptes*, *Sarcoptes*, *Otodectes*, *Knemidokoptes*, and *Notoedres*, or prostigmatid mites such as *Cheyletiella*, *Demodex* and *Psorobia*.
- *Occurrence*: Several of these mites have been recorded in Tibetan livestock.
- *Hosts*: A very large variety of hosts. Livestock are infested worldwide.
- *The disease in wildlife*: Reported in a large variety of non domestic species. Noteworthy there was a recent occurrence of sarcoptic mange infesting blue sheep (*Pseudois nayaur*) in northern Pakistan that may have reached Tibet.
- *Proposed actions and carrier stage*: Targeted, random detection: Evaluate the level of exposure to mange ectoparasites based on the recovery and identification of the mite from the affected hosts (hair clipping, skin scraping, ear canal swabbing).

#### Low priority diseases

Other diseases could be investigated, depending on the availability of resources, expertise and timeliness: Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea (BVD), Parainfluenza-3 virus (PI-3) infection, Bovine Respiratory Syncytial Virus (BRSV) infection, and Rinderpest (RS, LT). Exposure of wild herbivores to these agents may have been reported, but seldom with clinical symptoms of infections (IBR, BVD, PI-3 infection, BRSV infection), or the disease is likely to be absent from

China (Rinderpest). We also propose to investigate canine distemper in domestic dog and wild carnivore populations.

Infectious Bovine Rhinotracheitis (RS?, RP, LT?)

- *Agent:* Family Herpesviridae, genus *Varicellovirus*, 4 recognized subtypes.
- *Occurrence:* Worldwide. Present in Tibet.
- *Hosts:* Cattle.
- *The disease in wildlife:* Exposure was detected in a very large variety of wild herbivores but no clinical signs or significant findings associated with the diseases have so far been reported in wild herbivores.
- *Random detection only:* Evaluate opportunistically the level of exposure (for example with competitive ELISA) of any wild ungulate captured, harvested, culled or found recently dead, with or without likely contact with livestock.

Bovine Viral Diarrhea (RS?, RP, LT?)

- *Agent:* Family Flaviviridae, genus *Pestivirus*, 2 recognized types.
- *Occurrence:* Worldwide. Present in Tibet (domestic yak).
- *Hosts:* Cattle.
- *The disease in wildlife:* Exposure was detected in a very large variety of wild herbivores but no clinical signs or significant findings associated with the diseases have so far been reported in wild herbivores.
- *Random detection only:* Evaluate opportunistically the level of exposure (for example with competitive ELISA) of any wild ungulate captured, harvested, culled or found recently dead, with or without likely contact with livestock.

Parainfluenza-3 virus infection (RS?, RP?, LT?)

- *Agent:* Family Paramyxoviridae.
- *Occurrence:* Worldwide. Probably present in Tibet.
- *Hosts:* Widespread in cattle.
- *The disease in wildlife:* Exposure was detected in a very large variety of wild herbivores but no clinical signs or significant findings associated with the diseases have so far been reported in wild herbivores.

- *Random detection only:* Evaluate opportunistically the level of exposure (for example with competitive ELISA) of any wild ungulate captured, harvested, culled, or found recently dead, with or without likely contact with livestock.

Bovine Respiratory Syncytial Virus infection (RS?, RP?, LT?)

- *Agent:* Family Paramyxoviridae, genus *Pneumovirus*.
- *Occurrence:* Worldwide. Probably present in Tibet.
- *Hosts:* Widespread in cattle.
- *The disease in wildlife:* Exposure was detected in a very large variety of wild herbivores but no clinical signs or significant findings associated with the diseases have so far been reported in wild herbivores.
- *Random detection only:* Evaluate opportunistically the level of exposure (for example with competitive ELISA) of any wild ungulate captured, harvested, culled or found recently dead, with or without likely contact with livestock.

Rinderpest (RS, LT)

- *Agent:* Virus family Paramyxoviridae, genus *Morbillivirus*, three recognized lineages, lineage 1 and 3 most probably eradicated thanks to vaccination en mass.
- *Occurrence:* In Africa it has been eradicated from several countries and sub-regions, and is normally absent from the northern and southern parts of the continent. Lineage 3 of the virus used to occur in the Middle East, southwestern and central Asia. This lineage may be close to eradication as it has not resurfaced in the region since 2000 (Pakistan). In China, the disease has been eradicated through vaccination since 1955.
- *Hosts:* Cattle, zebus, water buffaloes, sheep, goats are susceptible as well as a very large number of wild animals within the Artiodactyla order, such as the African buffalo, the Cape eland (*Taurotragus oryx*), kudu (*Tragelaphus* spp.), wildebeest (*Connochaetes* spp.), various antelopes, etc.
- *The disease in wildlife:* Even though infections with lineage 2 (possibly the last remaining lineage in the world) may pass unnoticed in cattle, the virus is highly infectious in wildlife. Among species commonly regarded as highly susceptible (buffalo, eland, and lesser kudu), it causes fever, a nasal discharge, typical erosive stomatitis, gastroenteritis, and death. Buffaloes infected with lineage 2 showed enlarged peripheral lymph nodes, plaque-like keratinized skin lesions

and keratoconjunctivitis. Lesser kudus were similarly affected, but whereas blindness —caused by a severe keratoconjunctivitis— was common, diarrhea was unusual. Eland showed necrosis and erosions of the buccal mucosa together with dehydration and emaciation. Therefore, a diagnosis of rinderpest in any of these species points to the likelihood of a simultaneous transmission of the virus, even at a subclinical level, to neighboring cattle. There are reasonable evidences that the disease may have been eradicated in Asia and as such the relevancy of searching for the presence of the virus in Tibet may be questionable. However the virus might occur in a mild form and remain unnoticed in remote areas and there are some reasons to look for its presence in wildlife.

- *Random detection only:* Evaluate opportunistically the level of exposure (with competitive ELISA) of any wild ungulate captured, harvested, culled or found recently dead, with or without likely contact with livestock.

Canine distemper (RS in carnivores, RP in carnivores, LT in carnivores)

- *Agent:* Family Paramyxoviridae, genus *Morbillivirus*.
- *Occurrence:* Worldwide. Present in Tibet.
- *Hosts:* Domestic/feral dog.
- *The disease in wildlife:* Exposure was detected in a very large variety of wild carnivores with negative impact on wild dogs and African lions. Wild Siberian tigers also susceptible.
- *Proposed actions:* Targeted and random detection. Evaluate the level of exposure to CD in population of domestic dogs in the Chang Tang and wolves, killed, captured, culled or found recently dead (methods: at least two serological tests such as indirect immunofluorescent assay and competitive ELISA).
- *Sick and carrier stage:* Evaluate the presence of the viral agent or viral antigens in tissues/blood in individuals clinically sick or clinically healthy but presenting positive antibody titre (methods: histochemistry, antigen detection test, virus isolation, nucleic acid detection).

## Appendix 2. Utilization of veterinary diclofenac in Tibet.

The oriental white-backed vulture (*Gyps bengalensis*), the long-billed vulture (*Gyps indicus*) and the slender-billed vulture (*Gyps tenuirostris*), three species once widespread across the Indian subcontinent, have suffered dramatic decline at least for the last decade. They are currently categorized as Critically Endangered (BirdLife International, 2001). Renal failure, an organismal impairment found to be caused by toxic effects of diclofenac residues in livestock carcasses, was advocated as the main cause of decline (Oaks et al., 2004). Other species of *Gyps* vultures, such as the Eurasian griffon vulture (*Gyps fulvus*) are also susceptible to diclofenac, a non-steroidal anti-inflammatory drug (NSAID) (Swan et al., 2006), and it is probable that most vulture species are susceptible to this drug. In 2006 and 2007, the governments of India and Pakistan took an important step in reversing the decline of vulture species, by ordering a ban on the production and sale of the veterinary formulations of diclofenac. The Himalayan vulture (*Gyps himalayensis*), the lammergeier (*Gypaetus barbatus*) and marginally the monk vulture (*Aegypius monachus*) occur in Tibet. The first two are important scavenger species of the Tibetan fauna, contributing to maintain healthy ecosystems by removing potentially hazardous carcasses. They are also culturally important since they participate as scavengers in sky burial rituals practiced by Buddhist Tibetans.

In an attempt to learn more about the extent of utilization of diclofenac in Tibet, we interviewed veterinary workers (the head State Veterinarian for the County and four paraveterinarians in three townships and one town) in Dangxion County, on the utilization of diclofenac to treat domestic animals. They all responded that diclofenac ('Shuang Lü Fen Suan Na', transcript from Chinese ideograms) is used routinely as a human medicine in Tibet but not as a veterinary drug, at least in this county. To support his claim one of the paraveterinarians invited us to visit the pharmacy of his field veterinary station where no diclofenac was present, the only NSAID available was amydopyrine in injectable formulation. Drs Tsering Dorji and Jiangyong Zeng at the Veterinary Institute of TAAAS in Lhasa both confirmed that veterinary diclofenac was not distributed through the State Veterinary system. Currently the NSAID drug recommended for use in livestock and distributed by the Veterinary Institute is metamizole (better known under the names Dipyrone, Novalgin, or Analgin). Because State veterinarians are not the exclusive providers of veterinary drugs to nomads and farmers, we also investigated the occurrence of diclofenac in three private veterinary drug retailers and their wholesalers in Lhasa. None was distributing diclofenac. Based on these investigations and on our understanding of therapeutic customs of farmers and nomads in Tibet, we conclude that the likelihood of finding diclofenac residues in livestock carcasses in Tibet is very low and that the

**toxic risk on vulture population is probably largely limited to sky burial practices, diclofenac being widely available in Tibet as a human medicine.**

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