

Nomad-Animals Are Associated With Exposure To Emerging Diseases Pathogens

Farman Ali², Hamayun Khan¹, Shakoor Ahmed¹, Muhammad Athar Khan², Muhammad Hasan Raza², Muhammad Abuakar⁴, Nazir Ahmed Khan⁵, Zia Ullah Mahmood⁶, Naushad Khan⁷ and Sadeeq ur Rahman^{8*}

¹ Department of Animal Health, the University of Agriculture, Peshawar- Pakistan

²Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore- Pakistan

⁴National Veterinary Laboratory, Islamabad, Pakistan

⁵Department of Animal Nutrition, the University of Agriculture, Peshawar- Pakistan

⁶Department of Zoology, Islamia College University Peshawar.

⁷Institute of development study, The University of Agriculture, Peshawar-Pakistan

⁸College of Veterinary Sciences and AH Abdul Wali Khan University, Garden Campus, Mardan

sadeeq@awkum.edu.pk

Abstract: Infected nomad-animals may transmit pathogens of highly contagious diseases such as Foot and Mouth diseases (FMD), Pest des Petits Ruminants (PPR), and Contagious Caprine Pleuropneumonia (CCPP) *etc.* to remote areas. In the current study, we collected serosurveillance and retrospective data, between June 2013 and April 2014, of nomad-animals that migrate between North of Khyber Pakhtunkhwa and southern border areas of Afghanistan. Of these unvaccinated nomad-herds, 500 blood samples; 250 from sheep and 250 from goats, were analyzed for the presence of antibodies against PPR virus using Competitive enzyme linked immunosorbent assays; while, 100 blood samples; 50 from cattle and 50 from buffaloes, were analyzed for the presence of antibodies against FMD virus using 3-ABC-ELISA assay. Finally, nasal swabs from 60 pneumonia-suspected sheep and goats were processed for mycoplasma isolation. Our results indicated that 54.4 % of goats and 29.6 % of sheep samples were seropositive for PPR, 12 % of buffaloes and 20 % of cattle were seropositive for FMD and 18.33 % samples from sheep and goats were cultured positive for mycoplasma. Morbidity and mortality due to CCPP was as high as 92 % and 12.5 % due to CCPP, 52.5 % and 0 % in cattle due to FMD and 75.5 % and 0 % in buffaloes, respectively. Notably, our retrospective data revealed 0 % morbidity and mortality due to PPR. Altogether, data indicate that nomad-animals have been exposed to emerging diseases and that they might be a source of epidemic for areas they migrate.

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1. Introduction

Nomads of Khyber Pakhtunkhwa and the neighboring border region of Afghanistan are constantly roaming about for favorable climatic conditions and pasture for grazing of their livestock. In average, every nomad-family maintains more than 100 mixed animals including sheep, goats, cattle and horses *etc.*, which count for their main sources of income, food and transportation. With apparently low sources of income and lack of knowledge, they usually avoid methods of modern husbandry including vaccination of their flocks and thus when migrate can be infected with pathogens and infested with vectors and drop them off alongside their migration route (Gifford-Gonzalez, 2000).

Nomads that take advantage of the grazing areas of District Buner, located in the North of Khyber Pakhtunkhwa and bordering Swat-Kohistan, Dir Kohistan and the border-region with Afghanistan, often travel between the plain areas of the District Buner and up in the mountainous areas. S seasonal

migration of these nomads is spectacular phenomenon as they constantly looking for suitable climate and pasture for their animals. They come down to plains of District Buner from hilly areas of Dir-and Swat-Kohistan in the beginning of autumn and stay until the beginning of spring (October to April). During this off season they often practice short migrations and travel around 100 km after certain intervals in order to find enough grasses and pastures for their animals. In the mid or end of spring season they travel back to the mountains-pastures on high-hills of Swat- and Dir - Kohistan and border area of Afghanistan and stay there until the end of September (April to September).

Infectious diseases are transmitted between hosts by a variety of mechanisms, including direct, airborne and vector-born transmission. Control of animal-to-animal transmission of pathogens agents is key concept in infectious disease epidemiology. Foot and mouth disease (FMD) and Peste des petits ruminants (PPR) are viral and highly contagious infections that spread quite fast amongst the animals (Anjum *et al.*,

2004; Jamal *et al.*, 2010), while contagious caprine Pleuropneumonia (CCPP) is caused by bacterial pathogen that often disseminate very fast through aerosol and contacts (Sadique *et al.*, 2012). Importantly, though, these diseases are endemic in Pakistan, but to constrain them vaccination is common at domestic and commercial level (Anjum *et al.*, 2004; Zahur *et al.*, 2006; Sadique *et al.*, 2012). The seroprevalence data regarding FMD, PPR and CCPP has been mostly derived from domestic and commercial farm animals from the North of Pakistan (Zahur *et al.*, 2006; Azizi 2010). However, despite the fact that nomad animals could be potential source of pathogen spread, limited data is available regarding the presence and load of emerging pathogens associated with nomad animals. We thus, investigated whether nomad-animals can be a source of pathogen spread. Our results indicate that indeed nomad-animals are intensively infected with contagious diseases and they might serve as reservoir of these pathogens.

2. Material and Methods

Study area

The study was carried out on the livestock belonged to nomads that migrate from Kohistan of District Swat and Dir and far Northern Eastern border area of Afghanistan to the grazing areas of District Buner, in North of the Khyber Pakhtunkhwa province of Pakistan. The study involves sampling from animals and was therefore approved first from ethical committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan.

Surveillance and retrospective data

The serosurveillance were conducted in early summer season from June 2013 to early April 2014. June-September was considered as summer, October to November as autumn, December to February as winter and March to April as spring. For retrospective data collection, 100 nomads that maintained big flocks (more than 100) of sheep and goat and herds of cattle (more than 10) were randomly sampled. Data was first collected based on the interviews on a preformed standard questionnaire (not shown) in the local language "Pashto" and included major clinical signs and symptoms, duration, morbidity, mortality, and treatment, features of these diseases. We also recorded the number of animals infected, their age, sex, and clinical history *etc.*

Blood collection and screening for emerging diseases

A total of 500 blood samples, 250 from sheep and 250 from goats were collected for detection of antibodies against PPR virus and 100 blood samples, 50 from cattle and 50 from buffaloes for antibody

detection against FMD were processed at National Veterinary laboratory (NVL) Islamabad, Pakistan.

Serosurveillance of FMD and PPR

For FMD virus serosurveillance indirect 3AB C-ELISA technique (Shen *et al.*, 1999), while competitive ELISA technique was applied to determine seroprevalence against PPR (Singh *et al.*, 2004; Khan *et al.*, 2007). Indirect 3 ABC-ELISA (IDEXX Laboratories, Inc., United States) were performed on microtitre plates (NUNC Maxisorp) at 492 nm wavelength as instructed by manufacturer. Similarly, PPR-competitive ELISA kits (Institute of Animal Health, Pirbright Laboratory, Surry, UK) were implied through ELISA plate (NUNC, Maxisorp). Other chemicals were purchased from Sigma, IL.

Isolation and growth of Mycoplasma from infected animals

A total of 60 nasal swab samples, 22 from sheep and 38 from goats, were processed at veterinary research institute (VRI), Lahore-Pakistan for isolation of Mycoplasma by culturing onto selective media as described earlier (Manual., 2005). Plate cultures were examined after every three days through a microscope and were refreshed. The samples were examined for fourteen days if not positive on initial growth. Cultures suspected of L-forms of bacteria were examined for reversion to bacterial form by five passages on solid Mycoplasma medium. To confirm the presence of Mycoplasma on pleura pneumonia like organism (PPLO) broth, we performed biochemical tests such as glucose fermentation, tetrazolium reduction and arginin hydrolysis, and analysed the colony phenotypically under the microscope for its specific appearance (Manual., 2005).

3. Results

Nomad-animals migration, demography and distribution size of their livestock

In the current study we included nomads taking care of at least 200 animals and these will be here and after called nomad-animals. These Nomad-animals were found either (i) Short-range migrants, who were not settled in a defined grazing area long enough and moved back (Kohistan of Swat and Dir, Afghanistan *etc.*) at the end of winter and stay for spring and summer, or (ii) long-range-migrants, who once come down to the grazing area of district Buner in autumn; stay at a particular defined grazing area, where they establish their temporary residence for the whole winter season. However, at the end of the winter, all of them including their animals do not always go back to the mountains for summer. A part of the herd may leave behind to serve those who stay and the rest (majority) of the animals migrate to the mountains. To reach at spots of District Buner, these nomads take one of the give five routes and covers an average of

200 km distance (i) upper Swat-Kohistan to kalam-Madyan-Shangla and finally district Buneer (ii) Chitral-Dir-Gilgit-Besham and finally Buneer (iii) Bajawar Agency and district Dir through Barikoot to district Buneer (iv) Torkhum Pak-Afghan border area in adjacent to Khyber Agency and in-route to Peshawar-Sawabi-Buneer (v) Konarh and Helazi mountains, and boarder area of Mohmand and Bajaur Agency through Dir and Sawabi to Buneer. Remarkably, when we analyzed closely their knowledge, attitude and practices about application of vaccination for prevention and control of infectious diseases in their flocks, it turned out that majority of them do not follow modern approaches including vaccination.

Retrospective epidemiological aspects of CCPP, FMD and PPR

Our data indicated that CCPP was recorded during autumn and winter season (Table-1). The clinical features associated with CCPP include coughing, first watery and then mucopurulent nasal discharges, pneumonia, fever and abortions in the months of autumn and winter seasons (from September to February) in the relatively hilly areas. Clinical signs were severe in goats than in sheep and morbidity rate was higher than in goats than in sheep. The mortality rate was however negligible in both sheep and goats and those died were mainly because of prenatal and postnatal nature. Interestingly, no clinical case of CCPP was observed in summer season in sheep and goats (Table-1). The overall morbidity and mortality rates of CCPP in the autumn season were 82 % and 10.5 % in sheep and 92 % and 12.5 % in goats, respectively. Similarly, the mortality and morbidity rate in sheep during winter season was 6.5 %, and 66 %, respectively, however, 8 % and 89.5 %, mortality and morbidity rates, respectively, were observed in goats in winter season. Similarly the case fatality rate of 12.8 % and 9.8 % were recorded in nomadic sheep in autumn and winter seasons, respectively. While the case fatality rate of 13.5 % and 8.97 % were recorded in nomadic goats in autumn and in winter seasons, respectively. The prevalence in both species was higher in autumn than winter and zero prevalence was recorded in rest of the seasons (Table-1).

In the case of FMD, which they called “Tabaq” in their local language, we observed 0% mortality in the cattle herds, mixed with buffaloes, of all ages and both genders, while morbidity rate was 52.5 % in cattle and 75 % in buffaloes, respectively. The case fatality rate of 1.41 % was recorded in buffaloes in summer season while zero % in cattle (Table-1). Gender and age wise disease incidence rate and death rate were not recorded because the nomads were

unable to memorize the past events with so specificity. The signs were severe in buffaloes than in cattle. Major clinical observations were; decreased appetite, lethargic movement, weakness, stomatitis, lameness and high temperatures. Interestingly, it was observed that during the disease period the adult showed signs of FMD but the young ones less than one year were died asymptotically (Table-1). Of note, our retrospective data analysis indicated that no outbreak of PPR in the current summer season was observed (Table-1).

Seroprevalence of FMD and PPR

Our descriptive epidemiology indicated incidence of FMD and CCP during the last year seasons, but no PPR was observed. Thus, we wanted to know whether these animals have circulating antibodies against these diseases (FMD and PPR) to confirm their exposure. Our results indicated that FMD was 32 % prevalent in cattle and buffaloes, 12% in buffalos and 20% in cattle. Notably, we knew that nomad-animals are devoid of vaccination, but to be on the safe side, we preferred 3ABC ELISA technique to distinguish infected animals from vaccinated. Our statistical analysis indicates no association between prevalence of FMD antibodies and the type of animal infected (P-value 0.086; not shown). Out of 100 animals, 24 % adults and 8 % young were seropositive for FMD virus (Table-2). Statistical analysis indicates significant (p-value $0.008 \leq 0.05$ and not shown) association between the ages of cattle and buffaloes and antibodies against FMD. Interestingly, our results indicated 20% female and 12% male were seropositive showing no significant association between the gender of animals (cattle and buffaloes) and presence of antibodies against FMD.

Notably, results of competitive ELISA (C-ELISA) indicated, by contrast with the retrospective data of no incidence of PPR, 27.2 % (136/250) of goats and 14.8 % (74/250) of sheep samples were seropositive for PPRV (Singh *et al.*, 2004). Furthermore, our data indicated that 38.8 % (194/500) of female and 3.2% (16/500) of male animals were positive for the presence of PPRV. Similarly, 37.8 % (189/500) of adults and 4.2 % (21/500) of young were found seropositive (Table-3) and were showing a significant association between antibodies of PPR and ages of both species of migratory sheep and goat (statistical data not shown).

Prevalence of CCP based on the isolation of *Mycoplasma* pathogen

Our data from CCPP culture indicated that 18.33% (11/60) were positive amongst 15% (9/60) goats and 3.33% (2/60) sheep. Out of 18.33 % positive samples 10 % were young and 8.33 % were adult, while, 6% were male and 11 % were female (Table-4).

Table-1 Retrospective epidemiology of FMD, CCP and PPR

Disease	Animals	Season	Mortality %	Morbidity %	Case fatality %
FMD	Cattle n= 379	Summer	0	52.5	0
		Autumn	0	0	0
		Winter	0	0	0
		Spring	0	0	0
	Buffaloes n = 94	Summer	1.1	75.5	1.41
		Autumn	0	0	0
		Winter	0	0	0
		Spring	0	0	0
CCPP	Sheep n =7817	Summer	0	0	0
		Autumn	10.5	82	12.8
		Winter	6.5	66	9.8
		Spring	0	0	0
	Goats n = 11848	Summer	0	0	0
		Autumn	12.5	92	13.5
		Winter	8	89.5	8.97
		Spring	0	0	0
PPR	Sheep and goats n =19658	In all seasons no clinical signs resembling PPR was observed.			

Above table indicates retrospective data that was deduced from the predesigned questionnaire that was filled in by interviewing from 100 different nomad-families

4. Discussions

Nomads and nomad-associated animals (nomad-animals) migration is central to the epidemiology and dissemination of contagious pathogens such as FMD, PPR and CCPP. Most of the nomads including those we followed in the current study do not follow vaccination and modern tools for prevention and control of infectious diseases for their animals, and;

therefore, are more prone to infections. And hence, during their route of migration, they can disseminate pathogens, which they acquired from a distant area. Our retrospective and serosurveillance data indicated that indeed, nomad-animals are infected with FMD, PPR and CCP and as such these animals may act as reservoir and source of infections for the local animals and environment.

Table-2 Seroprevalence of FMDV antibodies in cattle and buffaloes

Results	Species of the animal		Total No. (%)
	Number of Buffaloes (%)	Number of Cattle (%)	
Positive	6/50 (12)	10/50 (20)	16/100 (16)
	Female	Male	
Positive	10/50 (20)	6/50 (12)	16/100 (16)
	Adult	Young	
Positive	12/50 (24)	4/50 (8)	16/100(16)

Above table indicate the number of animals that was tested either seropositive or seronegative by 3-ABC-ELISA technique for the detection of antibodies against FMD virus

Our retrospective data analysis indicated that nomads who stayed at district Buner during autumn and winter, travel all the way from different mountainous areas of Swat-Kohistan, Dir-Kohistan, Gilgit-Chitral and Afghanistan *etc.* during the early autumn season and go back to the same areas in spring or early summer. Our retrospective as well as seroprevalence data indicated the incidence of FMD in nomad-animals with higher incidence and case fatality rate in buffaloes was found higher than in cattle during summer season as reported by Jamal *et al.*,

2010 in local domestic animals. Our retrospective data indicated that incidence of FMD occurred in different areas of district Buner including Shangla, Besham and Chitral of Khyber Pakhtunkhwa Province during the last summer (Results not shown), and particularly, when they were travelling (July-September-2014). Other studies have also revealed high incidence of FMD in Pakistan during summer season as conditions like high moistness, low temperatures, neutral pH and lack of ultra-violet rays might favor FMD virus existence and transmission (Zahur *et al.*, 2006; Klein

et al., 2008). FMD virus is highly contagious and spread very fast amongst the animals through aerosols and direct contact with infected animals and contaminated environment (Zahur *et al.*, 2006). Nomad-animals are mixed herds, overcrowded during the night, which favor the spread of virus among other animals. When these animals travel in-route to other areas, they definitely pose threats to the local animals and environment.

The infected animals secrete PPR virus in tears, nasal discharge, and secretion from coughing and in faeces (Ezeibe *et al.*, 2008). Therefore, close contact with infected animals is associated with acquiring infections, particularly, through inhalation of the fine drops that animals release during coughing (Chauhan *et al.*, 2009). Surrounding environment like water, grasses, feed troughs and bedding can also be contaminated during coughing and sneezing. Since animals excrete the virus before showing signs of the diseases, as observed in our retrospective data analysis, the virus can thus be spread by movement of infected animals. Although, our retrospective data revealed no incidence of PPR during the last year, however, by contrast, antibodies were found in animals that were thought to be free of PPR. This indicates the threat and potential of these nomad-animals to spread latent infections such as PPR

infection. In addition, the presence of antibodies and absence of clinical signs might also be due to earlier exposure during the recent past or presence of the latent infection in convalescent period. In the latter case animals do not reveal usual clinical picture of the disease but, rather seems healthy even in the presence of pathogen in the body. It should be noted that these animals were never vaccinated against PPR virus (Özkul *et al.*, 2002; Rashid *et al.*, 2008).

CCPP is spreads through inhalation of infected respiratory droplets, close contact with infected animals or infected environment. The disease is spread from one area to another through infected animals. Our retrospective analysis shows that CCPP incidents were recorded more frequently in autumn both in sheep and goats as compared to winter season. Since, nomads usually travel in early autumn seasons towards their next destination for winter. Therefore, seasonal factors such as low temperature (< 20 to 15⁰C) fluctuating humidity and travelling stress might have played role in the establishment of CCPP infection, which is also suggested by the findings of (Hussain *et al.*, 2012). In agreement with this, our data also indicated incidence of CCPP throughout regions, which were supposed to be in route or on the way of nomad-migration (results not shown).

Table-3 Seroprevalence of PPRV antibodies of sheep & goats

Results	Species of the animal		Total Number (%)
	Number of Goats (%)	Number of Sheep (%)	
Positive	136/250 (54.4)	74/250 (29.6)	210 (42)
	Female	Male	
Positive	194/500 (38.8)	16/500 (3.2)	210 (42)
	Adult	Young	
Positive	189/500 (37.8)	21/500 (4.2)	210 (42)

Above table indicate the number of animals that was tested either seropositive or seronegative by c-ELISA technique for the detection of antibodies against PPR virus.

Table-4 Isolation and identification of Mycoplasma pathogen from CCPP suspected animals

Isolation of CCPP	Species of the animal		Total No (%)
	Number of Sheep	Number of Goat	
Positive	2/60 (3.3)	9/60 (15)	11 (18.33)
	Female	Male	
Positive	7/60 (11.67)	4/60 (6.66)	11 (18.33)
	Adult	Young	
Positive	5/60 (8.33)	6/60 (10.00)	11 (18.33)

Above data indicate the number of animals from which Mycoplasma bacterium was cultured positive or negative.

Overall, our data indicated that nomad animals are carrying antibodies or pathogens of highly contagious and emerging disease such as FMD, PPR and CCPP and that can be spread to remote areas when they are migrating along the way of their destinations. Altogether, there are complex

interrelationship that exists between nomads, their animals (nomad-animals), local animals and the route and pattern of their migration. Nomad-animals nevertheless got an important role in the transmission and dissemination of highly contagious diseases as described in this report. It is possible that they are also

important source for other Zoonotic infections. However, there is much to be learned about the diseases they spread in Pakistan and the mechanism of their transmission. A better understanding of nomad-animal migration pattern, their containment, effective vaccination and monitoring would be useful.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding Author:

Dr. Sadeeq ur Rahman,
College of Veterinary Sciences and AH, Abdul Wali Khan University, Mardan, Khyber Pakhunkhwa-Pakistan. Sadeeq@awkhmu.edu.pk

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