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AWARENESS LEVEL AND BIOSECURITY COMPLIANCE OF EQUINE FARMERS AND OWNERS REGARDING EQUINE INFLUENZA IN KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Equine influenza remains a substantive threat to the equine industry and a deeply neglected area of research in Pakistan. To address this problem a cross-sectional study was conducted to explore the level of awareness and biosecurity compliance related to equine influenza among equine owners and farmers. 378 equine farmers and workers from four randomly selected districts of Northern Province (Khyber Pakhtunkhwa) were interviewed, irrespective of age and sex. Frequencies were used as outcome variables in the ordinal logistic regression statistical analysis. Variables on univariate analysis "having a P value of ≤0.2" were included in the Multivariate regression models built with manual stepwise approaches. Descriptive analysis of the data showed that 23% of the respondents had high awareness, 16% medium, and 27% low regarding equine influenza. "The majority of the respondent had" low biosecurity compliance. Amongst them, 43% had high, 19% medium, and 31% had "low biosecurity practices compliance". The age and geographical locations of the respondents were found to be significantly associated with low levels of awareness and biosecurity compliance. The comparative odds of lower biosecurity compliance were recorded decreasing as age increases. The overall awareness and biosecurity compliance level was found unsatisfactory, major reasons were low literacy rate and lack of training regarding the management of equines.

Key words: Equine influenza; biosecurity; ordinal logistic regression; Cross sectional, Pakistan.

INTRODUCTION

Equine influenza (EI) is one of the leading respiratory infections of equines caused by orthomyxo viruses of the genus influenza A virus. They are divided into subtypes based on the antigenic reactivity of their surface glycoproteins, hemagglutinin (H1-H16) and the neuraminidase molecules (N1-N9) they possess (Daly *et al*., 2011). Equine influenza viruses (EIV) have the ability to spread rapidly between the inhabitant animals with a low mortality rate in healthy equines, but they can cause severe problems to the breeding and racing industries. EIV causes severe clinical signs in equines with hyperadreno-corticism or which are under physiological stress (Alana *et al*., 2014). The potential of EIVs to infect mammalian hosts from multiple species and the recent crossover of EI from horses to canine species is troublesome with regard to the future virus mutations (Crawford *et al*., 2005).

Many equine diseases are enzootic, including salmonellosis and leptospirosis, regarding which some studies have reported that animal handlers and farmers are at ten times greater risk than the general public of being exposed to coxiellaburnetti, the infectious agent for Q fever. The spread of the infectious disease due to globalization and the more frequent movement of animals have recently made the improvement of biosecurity practices by equine owners and farmers more important to animal health authorities (Schemann *et al*., 2012). Because the infection control practices and animal husbandry of owners or farmers have direct influence on their animals' health (Delgado *et al*., 2012). Several studies from the last decade have linked the attitudes and perceptions of animal handlers to their biosecurity behavior and infection control (Palmer *et al*., 2009 and Gunn *et al*., 2008).

In the current study area, the national total of 5.6 million inhabitant equines is made up of 33% mules, 22% horses, and 13% donkeys (PES, 2013–15). An outbreak of equine influenza was reported to have infected all the equine species there, based on a seroprevalence study conducted in 2012–2013. This study reported a seroprevalence against H1 in horses (7.5%) and donkeys (9%) and a seroprevalence against H3 in horses (7.12%), donkeys (6.9%), and mules (6.0%), respectively (Sajid *et al*., 2013). Despite the significance of equestrian pursuits in Pakistan, the endemic infectious equine diseases (*e.g.*, strangle and glanders) and the occurrence of EI as an emerging infectious disease, the research to date on the biosecurity practices and awareness of Pakistani equine

owners or farmers has been limited. We conducted the present study to investigate the awareness level and the level of biosecurity practice compliance. Better understanding of these parameters will greatly assist in initiatives related to infectious disease control.

MATERIALS AND METHODS

Study design, sampling and data handling: In this study, four districts were randomly chosen from a list of the 26 districts of Khyber Pakhtunkhwa-province the northern part of Pakistan, as shown in the Figure. This province borders on Afghanistan (the Durand Line). Most of the equine species in Khyber Pakhtunkhwa-primarily donkeys-are imported on a large scale from Afghanistan and these animals are gathered together for purposes of sale and purchase in a single market located in the districts included in this study. Equine owners and farmers from these districts were enrolled via a consent form signed by them before taking the interview. A "cross-sectional" survey was conducted to pursue the objectives. Further details of the study design were adopted from (Schemann *et al*., 2012) to investigate the influencing factors associated with the spread of equine influenza (EI)-factors associated with biosecurity practice compliance (Schemann *et al*., 2012). Ultimately 378 equine farmers or owners were enrolled, the majority of whom (almost 80 %) had family background in keeping equines as a source of earnings for many decades.

Interviews were conducted "face to face" by four trained interviewers (veterinarians) between Nov 2015 and Jan 2017, based on a predesigned structured questionnaire. The researchers piloted the questionnaire on 25 technically sound government horse farm managers and 10 horse farmers/owners from the study area to test the questions for ambiguity, ensuring similar methods and response recording. Of the 38 questions included in interviews, 29 closed ended questions were used here in the present analysis.

The questionnaire was designed to collect information regarding demographics of respondents, level of awareness of EI, frequency of the biosecurity practices compliance and nature of farmers/owners current involvement with their equines. The questionnaire took about 25-30 minutes to complete and it contained total of 23 closed questions translated into Pashto (local language) at the time of interview to minimize confusion and maximize accuracy of the responses (Thursfield, 2007 and Dohoo *et al*., 2009). Microsoft Access 2010 database was created purposively for data entry. Statistical analysis and data entry were conducted using SPSS (Version 20.00). Ethical approval for this study was issued by Advance Studies and Research Board (ASRB) committee of the University of Veterinary and Animal Sciences.

Explanatory and Outcome variables: The outcome variables were based on questions, asking the equine farmers/owners to rate the level of awareness and frequency of their biosecurity practices compliance regarding equine influenza on a 3-point scale. Respondents selecting unsure category in observations for these questions were omitted. The resulting category outcome variables were used in the ordinal logistic regression statistical analysis to investigate statistical association of the explanatory variables with equine farmers/owners awareness level and biosecurity practices compliance frequency. The 29 (Table 1) explanatory variables investigated here in the present study are related with equine farmers/owners demographics, awareness level, biosecurity practices compliance.

Statistical Analysis: All statistical analyses of the data were conducted using SPSS (Version 20.00).

Outcome variable: Biosecurity compliance index i.e. high, medium, low, was created on the basis of equine farmers/owners responses to the 29 questions on their awareness level about equine influenza and on biosecurity practices compliance. Responses for the awareness level for each of the nine questions were scored on predefined scale of 1-5, as 1 for every time and 5 for never. Respondents were then categorized into 3 groups based on the median response to the 23 questions; high for ≤ 2, medium for >2 and ≤ 3 and low for >3 for both awareness level and biosecurity practices compliance. The awareness and biosecurity compliance index were used as outcome variables in the ordinal logistic regression models to identify the association between level of awareness at different levels and associated factors with biosecurity compliance.

Explanatory variables: A total of 29 explanatory variables were selected in this study. These variables were grouped accordingly as to the factor type they described into such as awareness level, biosecurity compliance and demographic factors. Variable age was forced into the Multivariate model because it was expected being as a confounder a priori. All the explanatory variables were ordinal or binary.

Descriptive and univariate analysis: The distributions of continuous and categorical explanatory variables were presented with histograms and frequency distributions, respectively. Furthermore, we examined contingency tables for the continuous explanatory and categorical explanatory variables for categories of biosecurity compliance index and awareness level outcome (high, medium and low).

Univariate ordinal logistic-regression analysis was conducted through SPSS to investigate unconditional statistical association of the explanatory variables with outcome variables by using cumulative logit model (Hosmer and Lemeshow, 2000). Based on the

unconditional association variables with likelihood ratio of ≥0.20 (Chi square P-value) were excluded from the Multivariate analysis in the final model. Variables with likelihood ratio in univariate analysis with p value ≤0.020 based on chi square were tested for the co-linearity in pair by calculating the Spearman's rank correlation coefficient for pairs of the ordinal variables and by Pearson chi square test for others. Strongly correlated explanatory variables were retained for further analysis.

Multivariate analyses: The Multivariate ordinal logistic-regression model was used with manual forward stepwise-approach to assess statistical association of the explanatory variables with outcome variables after adjusting them for each other. Variables with statistical significance of (P value ≤0.05) were retained in final model. Age group variable was considered as a confounder a priori and thus forced into final model irrespective of its P-value. Cumulative logit model proportional odds assumption was tested by score test. Biologically important interactions (two way) of explanatory variables in final model were observed and retained if found statistically significant (P ≤0.05). Hosmer-Lameshow technique was used to assess the goodness of fit of the final regression model (Hosmer and Lemeshow, 2000).

RESULTS

A total of 378 farmer/owners participated in this survey (response rate of 62.79%); however, some respondents did not own or partly own equines, some excused to participate due to short of time and were consequently excluded from the survey. Of the remaining respondents, 23% had high, 16% medium and 27% had low level cumulative awareness regarding equine influenza. While 31% had low, 19% medium and 33% respondents had high biosecurity compliance rate.

These respondents were selected only from the Northern Province known as Khyber Paktunkhwa of Pakistan from four distinct districts shown in Figure. Majority of (>90%) them were males. Approximately two thirds (232/378) of the respondents did not experience the equine influenza infection for the last two years in their equine species in this part of the country. The age distributions of the respondents are given in table 3. The frequency of farmers/owners in response to different explanatory variables regarding awareness is depicted in table 2 and for biosecurity practices compliance the results are depicted in Table 3. Significantly (P≤0.001) maximum number of the respondents (205/378) were aware of knowing equine influenza as a respiratory disease of equines. While others (173/378) perceived EI for some other problems i.e. effect of harsh weather, load of work and weakness.

Univariate analyses for awareness and biosecurity compliance index: Descriptive and contingency tables of 23 variables significant in the univariate statistical models (P ≤ 0.20) are given in Table 2 and 3 for all the explanatory variables. Both the tables compare the explanatory variables for awareness level and biosecurity practices compliance with the outcome variables.

Multivariate analyses for awareness and biosecurity compliance index: Of the total 29 variables selected for analysis, six variables had the univariate likelihood ratio of (Chi square P value ≥0.2) and were consequently excluded. Those variables which were previously excluded on the basis of having 10% missing value were added once again for resting in the final model, but none of these variables achieved statistical significance. The final model having 337 observations included eleven explanatory variables (details depicted in Table 4). Younger aged respondents ≤20 years of age reported poorest level of the biosecurity practices compliance. While Comparative odds of the lower biosecurity practices compliance appeared to decrease with increase in age. Age was found significantly associated with low biosecurity compliance. Analysis also revealed equine farmers/owners of district swat were more likely (Odds ratio=5.32, P value = 0.002) to be in lower biosecurity group as compared to farmers/owners of other districts. The highest odds significantly associated with lowest biosecurity compliance in farmers/owners were recorded in terms of vaccination against EI. The odds of biosecurity index were significantly higher for not quarantining newly arrived animals amongst equine farmers/owners. A statistically significant (P ≤0.001) greater number of farmers/owners were not practicing quarantine practice of newly arrived animals as well as diseased animals. An obvious trend of low biosecurity practices compliance was observed for all the explanatory variables regarding biosecurity compliance. The largest positive association with having the low biosecurity practices compliance was recorded for preventive practices. A positive strong association for the low biosecurity practices compliance was also recorded in relation with the hygiene practices.

Table 1 Explanatory variables statistically analyzed for evaluation of awareness and biosecurity compliance amongst 378 equine keepers/owners regarding equine influenza in 2015-2016 in Khyber Pakhtunkhwa, Pakistan

Variable group	variables
Equine keepers/owners demographics	Age[a], Geographical zone[b] (Study zone).
Owners awareness/Perception regarding equine influenza	Do you know about equine influenza (EI), Does your animals ever infected with EI, EI is a seasonal epidemic disease in this area, occurs in newly purchased equines, EI occurs more in equines with birth origin of? Afghanistan; Iran or other provinces of Pakistan?, Do you Know Vaccine is available for EI, Keeping mix equine species promote EI, Foals are infected more with EI, EI is a Zoonotic disease, *involvement in equestrian event promote EI, EI is frequently respiratory disease of equines.
Level of biosecurity compliance amongst equine keepers/owners	Do you quarantine your newly arrived animals, do you quarantine the diseased animals, Cleaning animal gear before its use, Ensuring all the visitors using the disinfectant footbath, Disinfecting the horse floats before its use, washing your hands with soap, using clean bedding and feed, your equine contact with other equines, Sharing your animal gear with other equines.

[a]Ordinal variable, [b]Categorical variable.

*Equestrian events included showing, racing and sporting.

Respondents rated frequency of performing all of the measures according to a predefined scale from 1=every time to 5= never. Then median value for every respondent was determined and indexed into high = ≤ 2, Median = > 2 and ≤ 3, and low = >3, biosecurity compliance. The same conditions and coding were defined for awareness level such that a respondent knowing fully, enough or somewhat about the outcome asked for after that these

Table-2 Descriptive statistical analysis of data from 378 equine owners/farmers in the form of explanatory variables describing their level of awareness regarding equine Influenza in Khyber Pakhtunkhwa, Pakistan in 2015-2016.

Variables	Levels	Awareness/perception levels			Total	P-Value
		High level (Row %)	Medium level (Row %)	Low level (Row %)		
Do you know about *EI?	Yes	93(35.85)	39(19.02)	72(35.12)	205	≤0.001
	No	90(52.02)	30(23.12)	33(23.85)	173	
Does your animals ever infected with EI?	Yes	80(58.82)	27(21.32)	29(21.32)	136	0.006
	No	103(32.9)	52(21.38)	86(35.53)	232	
**EI occurs more in equines with birth origin of?	Pakistan	27(72.97)	07(18.91)	03(8.10)	37	≤0.001
	Afghanistan	59(73.68)	11(13.92)	09(11.39)	79	
	Iran	13(61.90)	03(19.03)	03(19.03)	21	
Do you know vaccine is available for EI?	Yes	17(58.62)	08(27.58)	05(17.23)	29	≤0.001
	No	167(37.8)	73(20.91)	109(31.23)	339	
Keeping mix equine species promote EI?	Yes	23(53.38)	18(31.86)	02(03.65)	33	≤0.001
	No	57(51.35)	37(33.33)	17(15.31)	111	
	Don't know	76(33.92)	67(29.91)	81(36.16)	223	
Foals are infected more with EI?	Yes	152(60.5)	52(20.71)	37(18.72)	251	0.003
	No	63(39.60)	28(22.03)	36(28.33)	127	
EI is a Zoonotic disease?	Yes	26(63.31)	03(09.75)	11(26.82)	31	≤0.001
	No	33(53.09)	17(27.86)	11(18.03)	61	
	Don't know	123(33.0)	103(36.91)	53(18.99)	279	
Involvement in equestrian event promotes EI?	Yes	101(51.0)	63(32.32)	33(16.66)	198	0.102
	No	73(30.55)	86(37.77)	21(11.66)	180	

EI is frequently respiratory disease of equines?	Yes	91(35.03)	32(20.79)	69(33.15)	202	0.001
	No	53(55.10)	19(19.38)	25(25.51)	98	
	Don't know	38(38.71)	29(37.17)	11(13.10)	78	

*EI=Equine Influenza

**Farmers in the current study area were reported purchasing their equines from Pak-Afghan border areas in Peshawar (KPK), Pak-Iran border areas in Baluchistan and from Punjab Province in Pakistan.

P-value based on Univariate ordinal logistic regression analysis.

Table-3. Descriptive results for explanatory variables significantly related with the low biosecurity practices compliance by 378 equine farmers/owners based on a study conducted in Khyber Pakhtunkhwa in 2015-2016 Pakistan.

Variables	Categories	Biosecurity Compliance			Total	P-Value
		High Freq (Row %)	Medium Freq (Row %)	Low Freq (Row %)		
Do you vaccinate your equines against *EI?	No	378(100)	0(0.00)	0(0.00)	378	≤0.001
	Yes	0(0.00)	0(0.00)	0(0.00)	0	
Do you quarantine your newly arrived animals?	No	231(73.5)	57(18.15)	26(8.28)	313	≤0.001
	Yes	23(35.93)	12(18.75)	29(35.3)	63	
Do you quarantine the diseased animals?	No	183(61.9)	72(23.23)	31(13.8)	297	≤0.001
	Yes	39(38.75)	17(21.25)	23(30.0)	80	
Cleaning of animal gear before its use.	No	152(56.5)	91(33.82)	26(9.66)	269	0.001
	Yes	19(17.33)	26(23.85)	63(58.7)	109	
Ensuring all the visitors using the disinfectant footbath.	No	311(100.0)	0(0.00)	0(0.00)	311	≤0.001
	Yes	0(0.00)	0(0.00)	0(0.00)	0	
	Don't know	51(76.11)	16(23.88)	0(0.00)	67	
Washing hands with soap.	No	177(63.1)	99(35.86)	0(0.00)	276	≤0.001
	Yes	60(58.82)	23(23.52)	18(17.6)	102	
Using clean bedding and feed.	No	93(33.86)	78(36.79)	31(19.3)	212	0.003
	Yes	71(32.77)	62(37.33)	33(19.8)	166	
Your equines do contact other equines?	No	57(35.23)	52(31.26)	17(13.3)	126	0.002
	Yes	107(32.3)	81(32.13)	63(25.3)	252	
Sharing your animal gear with other equines.	No	72(33.95)	53(25.72)	81(39.3)	206	0.172
	Yes	87(50.58)	51(29.65)	33(19.7)	172	

*EI=Equine Influenza.

P-value based on Univariate ordinal logistic regression analysis.

Table 4 Final Multivariate ordinal logistic regression model (P≤0.05) for the low biosecurity compliance variables based on data from 378 equines owners/farmers in Khyber Pakhtunkhwa, Pakistan in 2015-2016.

Parameters	Levels	B	SE(b)	Adjusted odds ratio (95% CI)	P-Value
Constant 1		-2.78	0.31	-	-
Constant 2		-1.60	0.30	-	-
Age	≤20 years	0	-	1	0.007
	21-33 years	- 0.73	0.31	0.31 (0.28-1.13)	-
	≤35 years	-0.52	0.33	0.59(0.23-0.88)	-
Geographical location	Peshawar	0	-	1	0.002
	Mardan	1.01	0.52	2.71(0.97-7.90)	-
	Charsadha	1.07	0.33	2.95(1.21-7.31)	-
	Swat	1.66	0.39	5.32(1.98-13.79)	-
Do you vaccinate your equines against *EI?	No	0	-	1	≤0.001
	Yes	3.33	70.23	31.32(2.61-7.02)	-
Quarantine of newly arrived animals?	No	0	-	1	≤0.001
	Yes	0.77	0.19	2.22(1.31-3.37)	-
Quarantine of diseased animals?	No	0	-	1	≤0.001
	Yes	1.33	0.36	3.86(1.96-7.59)	-
Cleaning of animal gear before its use.	No	0	-	1	0.021
	Yes	0.59	0.21	1.83(1.15-2.89)	-
Visitors using the disinfectant footbath.	No	0	-	1	0.003
	Yes	0.33	0.28	1.31(0.81-2.39)	-
	Don't know	0.81	0.25	2.37(1.35-3.17)	-
Washing hands with soap.	No	0	-	1	≤0.001
	Yes	0.81	0.23	2.26(1.33-3.53)	-
Using clean bedding and feed.	No	0	-	1	0.052
	Yes	-1.02	0.39	0.35(0.16-0.79)	-
Equines contacting other equines?	No	0	-	1	0.037
	Yes	0.38	0.27	1.36(0.89-2.31)	-
Animal sharing gear with other equines.	No	0	-	1	0.003
	Yes	-1.13	0.65	0.31(0.09-1.15)	-

*EI=Equine Influenza, Score test for the proportional Odds assumption, P= 0.53; the deviance goodness of fit value, P=0.933.

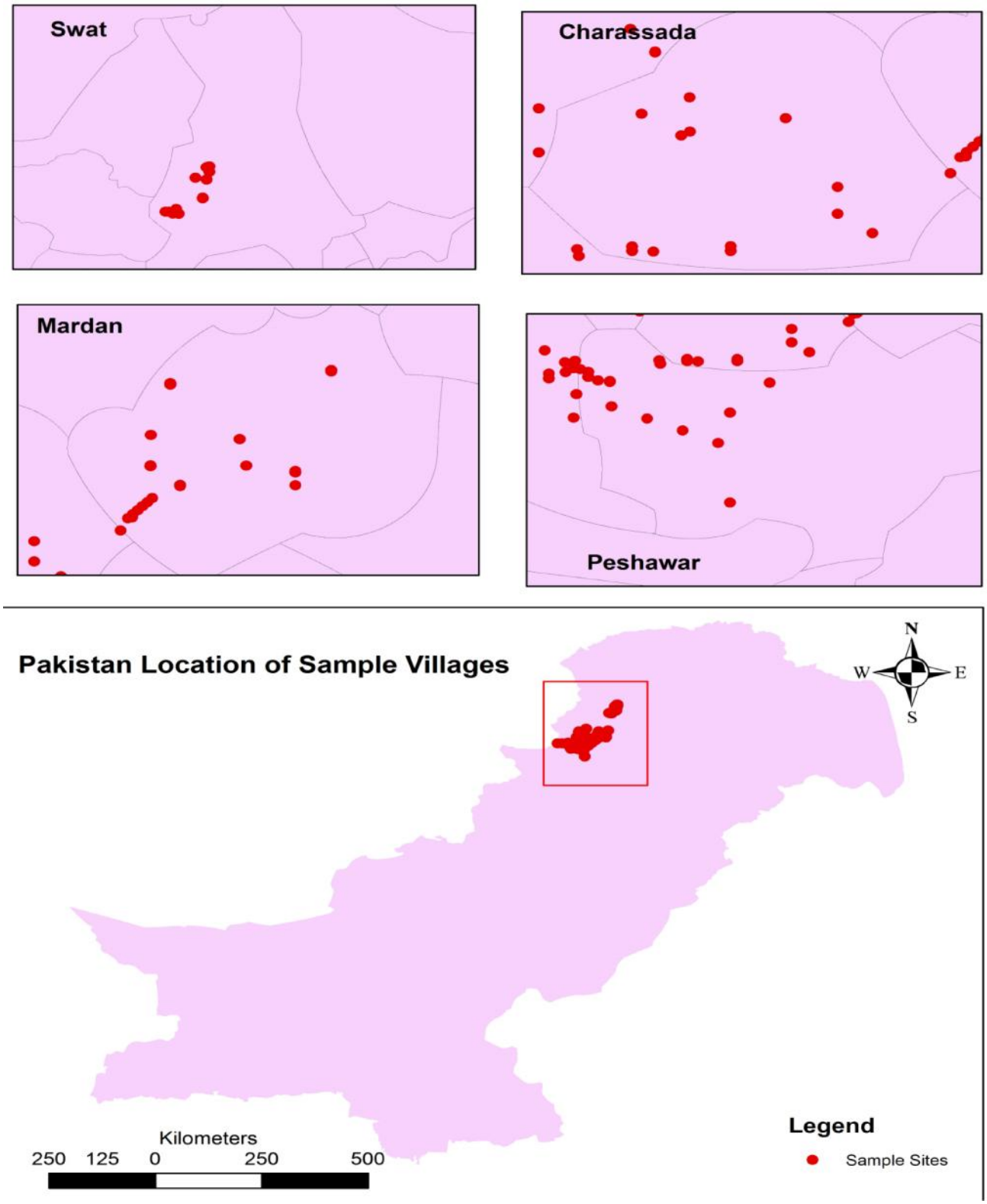

Figure: Geographical representation of the respondents included in the survey of biosecurity compliance and awareness level in of equine farmers and owners regarding equine influenza in 2015-16 in Khyber Pakhtunkhwa Pakistan.

DISCUSSION

Incursions of equine influenza and regular annual outbreaks of zoonotic diseases in Pakistan over the last decade have highlighted the need for good biosecurity practices. In wake of these disease incursions, effective epidemiological surveillance and control programs are necessary. An understanding the awareness of equine farmers or owners regarding EI and biosecurity practice compliance is very important in the establishment of effective control programs for future exotic infection incursions and for controlling endemic diseases.

We conducted this cross-sectional survey to determine the awareness level of equine farmers and owners and biosecurity practice compliance status using a predesigned piloted questionnaire. It was not possible to define a sampling frame due to reasons that included the lack of equine population and location records. Nonetheless we tried to reach a large number of farmers or owners through useful existing channels there mostly through selling and purchase markets, Brooke and civil hospitals. Thus, it is well acknowledged that our sample may not be representative for the entire equine farmer or owner population and that there might be selection bias, because only those respondents who brought their equines to market for sale and purchase or for treatment were contacted showing and care purpose respectively formed and sampling frame for the present study. Nevertheless, the present study sample represents respondents from a wide range of equine "sectors" including breeding, sporting/competition, recreational owners, and while the majorities were working equine farmers. Further sampling bias may be possible since 93% of the equine farmers or owners were males, but this could be due to the structure of Pakistani equine farming industry and management. It is totally different from Australian and American equestrian industries as reported by (AHIC, 2011 and AHC, 2005). These two countries have similar equine culture and activities to one another, but one far different from Pakistan. The results of the present study are validated as a reflection of what farmers and owners say they actually do, because the interviews were conducted "face to face" using the local language. These onsite surveys have proved substantially better than online surveys as reported by (Kirby *et al*., 2010). Regardless of the potential confines discussed, results of the present study are strengthened with its representativeness and large sample size of farmers or owners covering a wide range of equine industries. An additional strong point of the present study is the statistical analysis of the ordinal data collected regarding awareness level as well as biosecurity practices compliance, following (Dohoo*et al*., 2009). Compared to binary outcome analysis, this approach is highly informative and more powerful; obtaining information that would otherwise be lost. Such analysis proved pertinent to previous studies (Bish and Michie, 2010).

The overall low awareness level and low biosecurity compliance among all the farmers or owners might be due their low income; most of the respondents were earning their livelihood through their working equines. Previous studies reported that respondents receiving their primary income via equine a related industry were found alow biosecurity compliance level (Taylor *et al*., 2008). Also, most of the respondents were of the perception that these practices do not affect the occurrence of the disease less due to awareness of EI, but because they perceived their current biosecurity practices to be ineffective. We suspected that these farmers acknowledged their poor practices but do not feel sufficiently aggravated to adopt biosecurity measures. It may also be due to the low awareness level of these farmers regarding equine influenza and its potential to spread over a large population. These results are in line with the findings of (Watkins *et al*., 2007), reporting Influenza pandemic preparedness; motivating smallholder farmers for protection for influenza pandemic in Australia. Where low awareness level of the farmers were reported as unsatisfactory which was found as a potential risk factor in spread of the infection. The low biosecurity compliance may also be attributed to low self-efficacy, cost in terms of time or inconvenience and high response cost. All of these have been identified determinants of health protective behaviors. They are also considered core elements in several health behavior models (Bish and Michie, 2010). Age was found significantly associated with the biosecurity compliance and awareness level with an odd ratio of ≤1. Which describes that awareness level and biosecurity compliance increases with the increase age. While geographical location studied as a demographic categorical variable was found significantly ($P \leq 0.001$) associated with biosecurity compliance and awareness level. The farmers of district Swat (Odd ratio =5.32(C.I=1.98-13.79)) were having unsatisfactory awareness level and biosecurity compliance as compared to the other districts of Khyber Pakhutnkwa regarding equine influenza. It could be due to the education status of the farmers from this region. Because, farmers of district Swat were reported to be uneducated mostly as compared to the other districts farmers participating in the study. In other factors influencing on the geographical factor to be a risk factor could be the remoteness of this region where veterinary services are not well available to the farmers as compared to the other regions.

A significantly low ($p \leq 0.001$) biosecurity practice compliance frequencies were recorded regarding washing hands with soap, visitors using the disinfectant footbath, cleaning of gear before use, and using clean bedding. These variables are highly protective in terms of reducing infection risk (Rogers *et al*., 2010). Filed

evaluations, however revealed a clear difference between awareness level and applications of biosecurity compliance. A significant difference ($p \leq 0.001$) in awareness level and biosecurity practices compliance regarding the above variables was recorded. Some biosecurity measures were practiced at a relatively acceptable level; some were rarely practiced, while others were poorly implemented. Considering the quantitative assessment of these results, our findings were supported by the same assertion reported by (Cross *et al*., 2009). These factors play a vital role in the control and eradication of any infectious disease because half-hearted compliance and partial implementation biosecurity practices will limit endeavors to control or eradicate epidemics of rapidly spreading trans-boundary equine infectious diseases i.e. equine influenza The literacy level of respondents might also be among the major reasons for low levels of awareness and biosecurity compliance, as most of the interviewed respondents were illiterate (>80%) or had only primary level education status (8-10%). Contingent evidence points to the fact that literacy level deeply influence adherence to compliance with biosecurity practices. Previous studies have also reported such relations (Yu *et al*., 2013 and Kuo *et al*., 2001). In the present study respondents with high levels of awareness or perception regarding EI were also those with high levels of biosecurity compliance consistent with previously-reported findings from the field (Eastwood *et al*., 2009).

Intensive training and motivation for adherence to biosecurity practices will be needed in the field to guarantee reduction in burden of equine influenza spread (Kurschied*et al*., 2015). Still these gaps in awareness level and biosecurity compliance raise concerns and suggest that future campaigns, if delivered, should craft extra effort to specially target equine farmers and, beyond the studied variables, focus on more specific behaviors that are for control, prevention and eradication of equine influenza infections. More research is still required to identify the effective strategies to reach them and those sectors within the equine industry in which they are well represented.

Study strengths and limitations: In this study selection bias was faced due to the random selection from Brooke hospitals, civil hospitals, equines sale and purchase markets and filed, reduces external validity, and thus generalizability of results (Firestone *et al*., 2011), yet no supplementary sampling frame for Pakistani equine farmers or owners was available owing to the absence of any registration or membership legislation from the government side for them or for their equines.

A common restraint of the epidemiological studies carried out using questionnaires is their subjective nature of explanatory and the outcome variables. To avoid the resulting misclassification bias in the present study, only the closed responses (categorical) were allowed to exploit accuracy of these categorical responses. Furthermore, most of the questions included an option for the interviewers to respond that "the measure wasn't not applicable" or "they didn't know" the answer. The purpose for provision of these options were to address the possible misclassification bias, where the respondents can select an answer randomly, while such observations were considered as missing data in the analysis. Also, "face to face" interviews were carried out in local language for the current study to increase rapport, cooperation, reliability and consistency of the responses, as well as the completeness of data.

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

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