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Research Article

Sero-prevalence of Influenza a Antibodies in Pigs of Bhaktapur, Kavre and Banke Districts of Nepal

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Abstract

Pigs play a key role in inter-species transmission of influenza virus, because they have receptors to both avian and human influenza viral strains. A study was conducted in three different districts namely Bhaktapur, Kavre and Banke with face to face type of questionnaire survey and serum sample collection**.** Indirect Enzyme Linked Immunoassay was utilized for the collected 231 samples for serologic evidence of influenza A. Of the total 231 samples tested, 11 were positive for Influenza virus A with an overall sero-prevalence of (4.76%; Cl95%: 2.68-8.324) Kavre district had highest (5.88%; Cl95%: 2.539-13.04) sero-prevalence, followed by Bhaktapur (5.13%; Cl95%: 2.012-12.46) and Banke (2.94%; Cl95%; 0.8104-10.1) with no significant difference (p=0.685). Rearing swine along with poultry was the most significant risk factor (p=0.03); all positive cases were from the farms that adopted integrated farming system with little to no bio-security measures, especially poultry and swine. Present finding depicts that Influenza A is prevalent in pig farms of Kavre, Banke and Bhaktapur. Further research is needed to sub-type the influenza virus and also determine the effect of commercial poultry and migratory birds on the outbreak of influenza A in swine.

Keywords: Sero-prevalence; Influenza A; ELISA; Risk factor; Pig; Nepal.

Introduction

Influenza (flu) is a contagious respiratory illness of birds and mammals caused by enveloped, segmented, negative sense single stranded RNA virus of the family Orthomyxoviridae. Influenza is transmitted through aerosols containing the virus, direct contact with infected droppings or nasal secretions or through contaminated surface. These viruses can undergo genetic drift upon the gradual change of individual gene through mutation over time or genetic shift where different influenza viruses exchange entire gene segments.

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Four types of antigens are present in influenza virus among which Hem-agglutinin (HA) and neuraminidase (NA) are found in the outer surface of the particle and the matrix (M) and the nucleoprotein (NP) are found in the inner surface of the particle. The type of the nucleoprotein (NP) identifies the type of the virus. Based on this property, influenza viruses have been classified into three serotypes of which type A strains cause infectious disease in swine commonly called as Swine Influenza (SI). Swine plays a crucial role in influenza A virus ecology, being sensitive to both avian and mammalian influenza viruses. Pigs serve as major reservoirs of H1N1, H3N2 and H1N2 influenza viruses, which are endemic in pig populations world-wide and are responsible for one of the most prevalent respiratory diseases in pigs (Brown, 2000). Epidemics caused by these viruses recur every 1-3 years. Influenza A viruses (IAV) are important pathogens responsible for economic losses in the swine industry and represent a threat to public health (Dibarbora et al., 2013).

Pigs have receptors for both avian and human influenza viruses (Ito et al., 1998) and serve as a mixing vessel for emergence of new virus strain that may cause influenza pandemics. Similarly, the segmented genome and its ability to grow in many human and animal species, including birds and pigs cause genetic diversity of influenza A virus. Moreover, antigenic shift and antigenic drift are responsible to generate new strain of virus. Most of the pigs in Nepal are reared under integrated farming systems with poultry, fish and swine together. This data testifies the dependence of human in pig for animal protein which verifies the chances of zoonotic transmission of influenza from pig to humans. Humans exposed to pig on regular basis, may transmit influenza A virus to pig and also humans might get infected from pigs. Influenza A virus in pigs has been widely reported from various parts of world e.g. China (Peires et al., 2001), Malaysia (Suriya et al., 2008), India (Sabale et al., 2013), Vietnam (Hein et al., 2008), and Spain (Simon-Grife et al., 2011). But, the literature remains silent about presence of this virus though only one study has been done so far in Nepal. Thus present study was carried out to determine the sero-positivity of influenza A virus in pigs of Bhaktapur, Kavre, and Banke Districts. The present study also tries to describe the possibility of acquiring Influenza A virus infection to pigs raised in Bhaktapur, Kavre and Banke district attributing to their housing system, biosecurity and presence of poultry in pig farms.

Materials and Methods

The Study Area

The pig blood samples were collected from Bhaktapur, Kavre and Banke from the different commercial pig farms and backyard pigs. The study was carried out at Virology unit of Animal Health Research Division (AHRD), Khumaltar, and Kathmandu from August 2016 to February 2017.

Study Design and Data Collection

This is a cross-sectional study. A total of 231 pigs were included in this study regardless of age and sex. The samples collected were serum and the individual pig was sampling unit.

Sample Size Determination

At 10.87% prevalence (P), 95% confidence interval for Z statistics (which is 1.96) and at 5% precision (D), sample size was determined to be $N = Z^2 P (1-P) /D^2$ (Daniel, 1999). This sample size obtained is 148 for Bhaktapur and Kavre. A total of 163 samples (78 from Bhaktapur and 85 from Kavre) were taken from the farms of Bhaktapur and Kavre to increase the power of study. At 21.43% prevalence, using the same formula, sample size obtained was 261 for Banke. But only 68 samples were collected.

Questionnaire

The farmers were interviewed using a predesigned questionnaire. The questions were focused on determining the correlation of influenza A in pigs with poultry keeping, farming system, housing system, biosecurity etc. Questions related to general record, livestock husbandry in the area, type of water consumption waste management were also included.

Blood Collection and Serum Separation

Blood samples from the pigs were collected from the jugular vein immediately after questionnaire survey and were transported to AHRD, Khumaltar maintaining cold chain. There they were centrifuged at 2000 rpm for 20 minute for serum separation. The serum was transferred to a serum vial and stored in a freezer (-20 ^{0}C).

Serological Study

Sera of all pigs were assayed for Influenza A antibodies using commercially available competitive enzyme immunoassay kit)for detection of anti-influenza A antibodies. Antibodies were assayed using a ‘‘ID screen Influenza A antibody competition multi-species” with 100% sensitivity and 100% specificity. All the tests were performed following the instructions of the manufacturer. The diagnostic kit (ID Vet, France, Lot No. 884; Code FLUACA-10P) detect antibodies directed against the internal nucleocapsid of the influenza A virus in pig. It can be used with the pig serum or plasma. The test uses technology developed by FAO reference laboratory (CIRAD-EMVT, Montpellier, France).

Statistical analysis

Results were analyzed through software SPSS 16.0 and MS-Excel 2010. For comparison of the frequencies among the groups, the chi-square test was used and Fisher exact test was employed when indicated. Values were considered to be statistically significant when obtained p-value was less than 0.05. Crude (unadjusted) odds ratio (OR) and 95% confidence interval (CI) were calculated.

Result and Discussion

Of the total 231 serum samples, 11 samples were positive for Influenza A virus, thus the sero-prevalence was 4.76% in the studied three districts (Table 1). Kavre had highest (5.88%) prevalence followed by Bhaktapur (5.13%) and Banke (2.94%) with no significant difference (Table 2). Similarly, there were no significant differences among the locations (Table 2) or housing systems (Table 3). However, there were significant differences in prevalence among the animals mixed with other species (Table 5) and with/without biosecurity measures (Table 4). The association between prevalence of Influenza A with breed types, vaccination status and segregation of infected pigs is non-significant (Table 6). This study suggests that Influenza A is prevalent among the pigs of Bhaktapur, Banke and Kavre district. The positive rate obtained in this study (4.76%) is lower than the figures of the previous studies conducted by Pokhrel et al. (2016) in Eastern, Central and Western region of Nepal in which sero-prevalence was 21.73% by ELISA.

Table 1: The overall sero-prevalence of Influenza A in swine

Species	Number of sample tested	Number of positive samples	Prevalence (%)
Pig	231	11	4.76%; Cl95%: 2.68-8.324

Data shows the sample size, number of positive samples and prevalence percentage

Table 2: Location wise prevalence of Influenza A

	Positive	Negative	Total	p value
Bhaktapur	4	74	78	p= 0.685
Banke	2	66	68	
Kavre	5	80	85	
Total	**11**	**220**	**100**	**p> 0.05**

Data shows the negative and positive cases per district.

Table 3: Housing system wise prevalence of Influenza A

	Positive	Negative	Total	P value
Intensive	4	74	78	p= 0.685
Free range	2	66	68	
Semi-intensive	5	80	85	
Total	11	220	100	p>0.05

(**Intensive: small space with large number of pigs kept in a single pen with no mobility outside the pen, free range: Back yard, scavenging pigs, Semi-intensive: amount of space available is limited but animal are allowed for outside run)

Table 4: Biosecurity and Influenza A

	Positive	Negative
No-Bio-security	11	147
Biosecurity	0	73

Data shows the number of positive cases found with biosecurity measures taken at the farm.

Table 5: Other species in relation with prevalence of Influenza A

	Positive	Negative	Odds ratio
Other species	10	121	8.182; Cl95%:1.03-65.01
No-other species	1	99	

Data shows the odds ratio with and without others species at the farm

Table 6: Analysis of risk factors of Influenza in pigs with Odds ratio 95% confidence interval and p value

Characteristics	Group	Crude odds ratio	95% Confidence interval	p value
1.Presence of other species	Yes	8.182	1.03-65.01	*0.03
	No any	1		
2. Segregation	Yes	1		
	No	1.944	0.5733-6.595	0.320
3. Vaccination	Yes	1		
	No	1.458	0.4314- 4.93	0.539
4. Breed	Cross	1.806	0.3796-8.591	0.732
	Indigenous	1.00		

* = Statistically significant at 5% level

The prevalence we found in Nepal is lower than in study in Vietnam (47%) by Hein et al. (2008), in Poland (9.8%) by Daniel et al. (2009), in Maharashtra and Gujrat states, India (37.8, 29 and 0.3 percent serum samples were positive for antibodies against H1N1 pandemic, H3N2 and seasonal H1N1 viruses out of 925 samples) by Sabale et al. (2013). The difference may be due to variation in sample size, geography, climate and differences in test procedure.

By location, sero-prevalence evidenced in Banke, Bhaktapur and Kavre were 2.94%, 5.13% and 5.88% respectively. Although prevalence is higher in Kavre, it is not statistically significant, which indicates that all three housing system have equal probability of getting Influenza A. This may be attributable to purposive sampling technique, presence of other species like poultry or fish at farm. This is supported by Pensaert et al. (1981) which demonstrated the transmission of an avian virus was associated with outbreak of swine influenza in Belgium in 1979.

Housing system was categorized into Free-range, Intensive and Semi-intensive, and prevalence was assessed in each type. Our study demonstrated higher prevalence in Semi-intensive (5.88%) followed by Free-range (2.94%) and Intensive (5.13%) although the data are not significantly different implying equal chances of getting influenza irrespective of housing system. However, there was a significant difference in sero-prevalence with the introduction of other species at the poultry farm. Statistical analyses indicated a significant difference in sero-prevalence with the presence other species at the farm (poultry) ($p< 0.05$). It was also found that pig reared along with other avian species (i.e. ducks, backyard chicken) were 8.182 times more likely to be sero-positive than the pig not raised with other species. This is in agreement with report by Yassine et al. (2011) where H9 virus were continuously isolated from pigs in Asia plus sporadic infections with highly pathogenic H5-avian influenza viruses. Ponds or lakes situated nearby farm which was also the case in our study, can be habitats for different avian species. The contact between pigs and poultry birds may lead to influenza transmission between avian and swine species (Peiris et al., 2001).

All positive cases were obtained from farm with no biosecurity measures which are in accordance with the result of Simon-Grifé et al. (2011). But, it is difficult to say whether poultry transmitted virus to pigs or already circulating in pigs or introduced from humans. For this, sub-typing and sequencing of influenza virus would be helpful. Increased surveillance and improved virological and serological testing to characterize the viruses that are circulating in both pigs and poultry in the region.

Conclusion

This study demonstrates that the Influenza A is prevalent in pigs of Kavre, Bhaktapur and Banke district that were grown in close association with poultry with poor biosecurity practice. In nutshell, our results serve as a source of useful information to assess the criteria for the vaccination of pigs against SIV and preliminary epidemiological steps for the control of the disease. Further research need to be done to identify the strains of virus i.e avian, human or pigs. Much research also needs to be done to contemplate the effects of migratory birds on Influenza A.

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