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Short Communication

Prevalence of H_9N_2 Avian Influenza Viruses in Hazara Region of Pakistan

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ABSTRACT

A surveillance of avian influenza viruses in Hazara region of Khyber Pakhtunkhwa Pakistan was carried out from April 2013 through September 2014. Pooled morbid material and fecal samples from 600 different locations across the region were analyzed for isolation. Isolation was carried out on embryonated chicken eggs. Identification of H protein was undertaken through hem agglutination Inhibition (HI) test while N typing was done using reverse transcriptase polymerase chain reaction (RT-PCR) procedure. Out of 600 examined locations twenty two (22) H_9N_2 subtypes isolates were recorded. The total prevalence recorded was 3.66% and out of 498 broiler operations yielded 17 (3.4%) positive isolates and 44 golden type native chicken farms yielded 05 (11.36%) isolates of AIV H_9N_2 and 58 captive pheasant's samples were negative. It was observed that frequency of H_9N_2 prevalence in poultry population was directly proportional to temperature, season and elevation.

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Authors' Contributions
MA designed the study as Principal Investigator. MAA isolated and identified the virus. P, YA and ZA were involved in sample collection. N conducted haemagglutination inhibition tests. NS supervised molecular analysis of viruses. KN as consultant virologist supervised the work.

Key words
H_9N_2 influenza viruses, LPAI viruses in Hazara, H_9N_2 in Hazara.

Avian influenza (AI) is a highly contagious viral disease caused by various subtypes of orthomyxoviridae family. AI infections are persisting in migratory birds particularly wild ducks. These birds harbor the virus and are incriminated to transfer the infection from one country to another (Sohaib *et al*., 2010).

In Pakistan during 1995-2003, several out breaks of highly pathogenic subtype H7N3 were reported which resulted in death of 3.2 million birds (Capua and Alexandar, 2004; Naeem and Hussain, 1995). For the first time in two commercial flocks of Khyber Pakhtunkhwa province during March, 2016 H5N1 infected birds were recorded (Naeem *et al*., 2007). Later on during 2007-08, several outbreaks of H5N1 were recorded specially in peacocks in the city of Mansehra and poultry population of Rawalpindi and Islamabad and in the city of Karachi (Siddique *et al*., 2012; Wasilenko *et al*., 2012).

AIV serotype H_9N_2 was first isolated in Northern part of Pakistan in 1998-99, continued to spread across the country and is now endemic in the country. The mortality up to 2% and egg drop with mild respiratory lesions (Naeem *et al*., 1999). Ayaz *et al*. (2010) reported widen spread circulation of H_9N_2 in Hazara region of Pakistan inflicting economic loss to the tune of Rs. 2.2 billion per annum to the commercial poultry. In 2013, circulation of AIV H_9N_2 in live bird markets of Lahore, Pakistan was reported by Sarwar *et al*. (2013).

AIV H_9N_2 caused high morbidity rates showing clinical signs of depression, diarrhea and low egg production in China. The husbandry based study from 1995-2002 in China revealed mortality rate upto approximately 5-30% Occurrence of H_9N_2 viruses recorded in Iraq during 2004-07 with 30-70% mortality in broiler flocks (Liu *et al*., 2003; Khamas, 2008). Molecular analysis of 29 AIV H_9N_2 viruses isolated during 2003-04 from India, shown resemblance (96% homology) with H_9N_2 isolated from Germany and Asian region other than China. Phylogenetic analysis of these isolates indicated a common ancestor Qa/HK/GI/97 which had contributed internal gene of H_5N_1 viruses circulating in Vietnam as reported by Nagarajan *et al*. (2009).

Materials and methods

An active surveillance had been done in Hazara region of Pakistan to record the prevalence of avian influenza viruses (H_9N_2) following the protocol as previously described by (Spackman *et al*., 2008). Sampling was carried out from random locations of poultry farms and live bird markets across the region spread over 18000 km^2. For this purpose swab samples (oral and cloacal) along with

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Table I.- Sample collected from various localities and area wise prevalence of H_9N_2.

Area and elevation	Commercial poultry farms	Live bird market	No of H_9N_2 isolates	Area wise prevalence (%)	No of H_9N_2 isolates	Area wise prevalence (%)
Abbottabad (4000 ft)	113	21	41	26	11	50
Mansehra (3600 ft)	96	11	38	8	6	27.3
Battagram (3400 ft)	36	4	21	7	1	4.5
Haripur (1700 ft)	76	8	26	14	3	13.7
Kohistan (1500 ft)	32	0	19	3	1	4.5
Total	**353**	**44**	**145**	**58**	**22**	

Table II.- Prevalence of H_9N_2.

	Broiler operations	Native golden type	Captive pheasants
No of H_9N_2 isolates	17	05	0
Prevalence	3.41	11.36	0

morbid material (trachea, lungs and spleen) from 5 birds each from each selected location were collected as described in Tables I and II. The swabs and morbid material was pooled separately from each location and were brought to laboratory on ice. While these samples along with the standard samples collection information were transported to National Reference Lab for Poultry Diseases (NRLPD), NARC, Islamabad through special messenger in sample shipment boxes provided by NRLPD for further evaluation. A copy of the sample information form was also kept for record in the laboratory future reference.

The virus isolation and identification was carried out at NRLPD. Briefly swabs were re-suspended in sterile PBS, centrifuged and filtered through 0.2 µm disposable syringe filters (Sartorius Minisart Cat# 16534K, Germany). The morbid material was also processed for a final homogenate 10% w/v through tissue homogenizer Stomacher-80 (Seward, UK), centrifuged at 1000 rpm for 1 min in refrigerated centrifuge, and the supernatant was collected with 0.2 µm syringe filter (Sartorius Minisart Cat# 16534K, Germany). The processed material was inoculated in 9-11 days old embryonated chicken eggs. The virus identification from the positive allantoic fluid was done through hemagglutination inhibition test and the confirmation was done through RT-PCR (Siddique *et al.*, 2008).

Results

Of the 600 avian locations examined, 22 yielded H_9N_2 avian influenza subtype isolates with an overall prevalence of 3.66%. Out of 498 broiler operations tested against H_9N_2 presence, 17 broiler locations yielded H_9N_2 viruses and the prevalence percentage being 3.41%, likewise five H9N2 viruses isolated from 44 locations of native type golden chicken farms showing the prevalence being 11.36% (Table I). Fifty eight captive pheasants examined for the type found negative. Area wise prevalence was highest in Abbottabad (50%) followed by Mansehra (27.3%), Haripur (13.7%), Battagram (4.5%) and Kohistan (4.5%). Altitude was also considered and it was found that prevalence of H_9N_2 virus was higher at high altitude (Table I). The month wise study of the prevalence indicated higher in cold months of the year (Fig. 1).

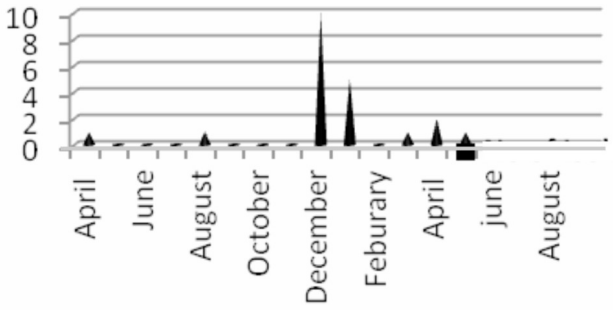

Fig. 1. Seasonal prevalence of H_9N_2. The figure shows number of isolates.

Discussion

Hazara region, an area of 18000 sq.km is located north of Islamabad. A big population of poultry, as estimated in economic survey of Pakistan 2014-15, rural poultry 3 million, parent stock 4 million, layer 1.5 million and broiler 5 million exist in the region. Two fly ways (East African-West Asia and Central Asia) traverse over the region specially the Indus river route termed as green flyway being followed by migratory ducks having stopover in Tarbela Lake on river Indus which runs at the western boundary of the region (Shirazi, 2005).

Suitable geo-ecological situation of Hazara region had been popular among commercial poultry producers of the country. Parent breeding farmers were attracted in the region in early 1980, soon after, broiler farming also started and within ten years the region was heavily

populated by commercial poultry. Haphazard poultry establishment made the biosecurity measures difficult to implement at individual farm which paved the way for easy spread of infectious diseases like avian influenza. The first ever outbreak of H_7N_3 was recorded in the region in 1995 (Naeem and Hussain, 1995). A low pathogenic avian influenza outbreak of H_9N_2 was recorded in 1999 inflicting drop in egg production and low mortality up to 2% (Naeem *et al*., 1999). From 2003-04 an epizootic of high pathogenic avian influenza H_7N_3 and later on H5N1 during 2006-08 affected the major poultry of the region (Naeem *et al*., 2007; Abbas *et al*., 2010; Siddique *et al*., 2012). This was accompanied by an outbreak of low pathogenicity AIV H_9N_2 in commercial chicken population which continued during 2005. The present findings indicated the prevalence of H_9N_2 spread across the Hazara region (Table II). Importantly frequency of this subtype is increasing noticeably in countries like Iran, Pakistan, United Arab Emirates and India (Nili and Asasi, 2002; Iqbal *et al*., 2009; Capua and Alexander, 2004; Amir *et al*., 2007). The above findings are in line with our present study.

Elevation may also be another cause of difference in incidence as Abbottabad is at higher altitude (above 4000 ft.) followed by Mansehra (above 3500 ft.) and Haripur (above 1700 ft.) above sea level. The highest incidence was again in non-descript chicken (11.4%) as compared to previous study. The seasonal spread of incidence was highest in cold months of December and January (Fig. 1).

Conclusion

The present study indicated the existence of H_9N_2 avian influenza in Hazara region of Pakistan. The higher prevalence in golden type native chicken is alarming. This type of chicken is kept as domestic layer, hence endemicity of the virus in rural area should be further studied in other parts of the country.

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Statement of conflict of interest

Authors have declared no conflict of interest.

References

Amir, U.B., Wernery, U., Ilyushina, N. and Webster, R.G., 2007. *Virology,* **361**: 45-55. https://doi.org/10.1016/j.virol.2006.10.037

Abbas, M.A., Spackman, E., Swayne, D.E., Ahmed, Z., Sarmento, L., Siddique, N., Naeem, K., Hameed, A. and Rehmani, S., 2010. *Virol. J.,* **7**: 137. https://doi.org/10.1186/1743-422X-7-137

Ayaz, M., Naeem, K., Qayyum, A., Sajid, M., Ahmad, I., Hussain, A. and M., H., 2010. *J. Anim. Hlth Prod.,* **21**: 21-23.

Capua, I. and Alexander, D.J., 2004. *Avian Pathol.,* **33**: 393-404. https://doi.org/10.1080/03079450410001724085

Iqbal, M., Yaqub, T., Reddy, K. and McCauley, J.W., 2009. *PLoS One,* **4**: e5788. https://doi.org/10.1371/journal.pone.0005788

Khamas, E.J., 2008. *Iraqi J. Vet. Med.,* **32**: 223-230.

Liu, M., He, S., Walker, D., Zhou, N., Perez, D.R., Mo, B., Li, F., Huang, X., Webster, R.G. and Webby, R.J., 2003. *Virology,* **305**: 267-275. https://doi.org/10.1006/viro.2002.1762

Naeem, K. and Hussain, M., 1995. *Vet. Rec.,* **137**: 439. https://doi.org/10.1136/vr.137.17.439

Naeem, K., Siddique, N., Ayaz, M. and Jalalee, M.A., 2007. *Avian Dis.,* **51**: 189-193. https://doi.org/10.1637/7617-042506R.1

Naeem, K., Ullah, A., Manvell, R.J. and Alexander, D.J., 1999. *Vet. Rec.,* **145**: 560. https://doi.org/10.1136/vr.145.19.560

Nagarajan, S., Rajukumar, K., Tosh, C., Ramaswamy, V., Purohit, K., Saxena, G., Behera, P., Pattnaik, B., Pradhan, H.K. and Dubey, S.C., 2009. *Vet. Microbiol.,* **133**: 154-163. https://doi.org/10.1016/j.vetmic.2008.06.013

Nili, H. and Asasi, K., 2002. *Avian Pathol.,* **31**: 247-252. https://doi.org/10.1080/03079450220136567

Sarwar, M., Muhammad, K., Rabbani, M., Younus, M., Sarwar, N., Ali, M.A. and Ahad, A., 2013. *J. Anim. Pl. Sci.,* **23**: 388-392.

Shirazi, S.A.J., 2005. *International visitors: Birds come flying in.* Avaialable at: http://www.wildlifeofpakistan.com/PakistanBirdClub/birdcomeflyingin.html

Siddique, N., Naeem, K., Abbas, M.A., Ahmed, Z. and Malik, S.A., 2012. *Virol. J.,* **9**: 300. https://doi.org/10.1186/1743-422X-9-300

Siddique, N., Naeem, K., Ahmed, Z. and Malik, S.A., 2008. *Int. J. Poult. Sci.,* **7**: 1122-1127. https://doi.org/10.3923/ijps.2008.1122.1127

Sohaib, M., Muhammad, S., Khushi, M., Rabbani, M., Altaf, M. and Hanif, A., 2010. *Pakistan J. Zool*., **42**: 325-329.

Spackman, E., Suarez, D.L. and Senne, D.A., 2008. *Avian influenze diagnostics and surveillance methods*. Blackwell Publishers, Ames, Iowa.

Wasilenko, J., Pantin-Jackwood, M., Khan, T., Ahmed, A., Rehmani, S., Lone, N., Swayne, D. and Spackman, E., 2012. *Virus Genes*, **44**: 247-252. https://doi.org/10.1007/s11262-011-0689-2