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Research Article

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Seroprevalence of avian influenza in broilers of District Quetta, Balochistan, Pakistan

Arif M.[1], Rind R. U.[1], Shah M. G.[1], Nisha A. R.[2], Umer M.[2,4], Kaka U.[1,2], Zaman A.[3], Tariq M.[3], Rehman S. A.[5], Hasan S. M.[3] and Khan M. S.[2, 3]*

[1]*Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University Tando Jam, Pakistan*
[2]*Faculty of Veterinary Medicine, University Putra Malaysia*
[3]*Gomal College of Veterinary Sciences Gomal University, D. I. Khan, Pakistan*
[4]*Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Pakistan*
[5]*Disease Investigation Laboratory, Livestock and Dairy Development, Department Quetta, Pakistan*

ABSTRACT

A study on the seroprevalence of influenza virus in broilers was carried out. A total of 570 broilers were randomly collected from 52 poultry farms of Quetta and its surroundings to determine the seroprevalence and antibody titre of avian influenza virus in the sera of broilers. The seroprevalence of avian influenza virus in the sera of broilers was demonstrated by Enzyme Linked Immunosorbent Assay (ELISA). The sero-positive prevalence of avian influenza virus was recorded 14.03% (n=80) in broilers. All the positive sera of boilers determined by ELISA were further tested by using H_5, H_7, and H_9 specific strains antigen through Haemagglutination Inhibition and Haemagglutination tests, only H_9 was recognized from the sera of broilers. During present study the antibody titre of H9strain of avian influenza virus in the sera of broilers was investigated. The antibody titre of H9 strain of avian influenza virus was measured in the sera of broilers ranged from 1:8 to 1:164. It is concluded from the present investigation that avian influenza virus H_9 strain is prevailing in broilers and around Quetta.

INTRODUCTION

Avian influenza (AI) is a highly contagious viral disease, distributed worldwide. It affects the poultry and many other bird species, both wild and domestic of all ages (1). It is a respiratory disease of birds (poultry) caused by influenza virus A of the family Orthomyxoviridae (2). Economically it causes heavy losses in poultry industry worldwide(3). Avian influenza virus (AIV) infects poultry in two forms: the highly pathogenic avian influenza known as “fowl plague” and the low pathogenic avian influenza (4). Avian influenza viruses cause very high mortality that ranges from 50-89% and sometimes it reaches to 100% in poultry flocks (5). The surface of avian influenza virus is covered by two types of glycoprotein projections: the rod shaped trimmers of heamagglutinin (HA) and mushroom shaped trimmers of Neuraminidase (6). The heamagglutinin is the major antigen that elicits antibodies which protect the birds from clinical signs and death (7).

Avian influenza causes mild, acute and fatal disease of chickens and also in other avian species o(8). Especially ducks and waterfowls serve as natural reservoir and are important source of infection for domestic fowls and poultry(9). Birds excrete avian influenza viruses from both, the respiratory and digestive tracts. In poultry farms

bird-to-bird transmission is probably through aerosol route among flocks, and also infected poultry feces look like to be the most likely source of transmission for men associated in contacts with birds (10).

The significance of avian influenza is increased due to its zoonotic importance. The transmission of swine influenza virus to human has been reported more frequently than avian influenza virus (11). Avian influenza viruses (H_4 N_1) were limited to migratory birds but it spreads to domestic poultry and recently it emerged as an infective agent in mammals and the human population. It shows a distinct threat of a pandemic to the World Health Organization and also other organizations to make stringent efforts to control the disease (12). During the last decade, highly pathogenic strains of avian influenza virus, including the H_5N_1 subtype, crossed the barriers from birds to human and caused fatal disease. The H_5 N_1 subtype is characterized as pathogenic viral species to a larger number of animal species (13).

Based on antigenic properties of influenza viruses, these are categorized into 16 heamagglutinin (H) and 9 neuraminidase (N) subtypes (14, 15). They are grouped on the basis of pathogenicity for chickens into extremely high pathogenic avian influenza (HPAI) viruses, in which, mortality may possibly be as high as 100% and low pathogenic avian influenza (LPAI) viruses grounds considerable or milder respiratory disease (16). The HPAI viruses have been restricted to H_5 and H_7 subtypes, although not all viruses of these subtypes are highly pathogenic for chickens. Zoonotic implications and high risk of potential mutation enables the effective transmission of virus among humans and give rise to a high level of global alert, to make determinations to prevent human population from influenza pandemic. The requirement for executing effective disease surveillance structures have been emphasized as key measures in the management of this current threat to livestock, Poultry industries and humans (17).

Keeping in view the above facts, the present study was therefore planned to survey the avian influenza virus in poultry birds in Quetta and possible risk factors to human health and also to recognize the species of avian influenza virus prevailing in the area.

EXPERIMENTAL SECTION

During present study, the district Quetta was divided into two regions and fourteen areas (Table 1). In these areas a total of 52 poultry farms were located, among these farms 38 were of broiler farms. The blood samples were randomly collected from 570 broilers (sick and normal) to determine avian influenza virus strains antibodies in the sera of birds. The blood samples were collected in sterile syringe and allowed to clot at 15^{o}C for 4 hours. The sera were then separated in clean sterilized 10 ml bottle or tube and transferred to the Disease Investigation Laboratory (DIL) Brewery Road Quetta. The serum samples were stored at -20°C in deep freezer for further investigation.

All serum samples were analyzed qualitatively through Enzyme Linked Immunosorbent Assay (ELISA) as adopted by Idexx Laboratory USA 2012 designed to determine positive or negative samples for avian influenza virus. The positive samples were further analyzed quantitatively by Hemagglutination Inhibition (HI) test (18, 19) to quantify the antibody titre in the sera while serum antigen were determined by Hemagglutination (HA) technique as adopted (20).

For Hemagglutination (HA) technique, 5ml of blood were collected from wing vein of commercial broilers by using a disposable sterile syringe. An anticoagulant (5mg quantity of ethylene diamine tetra acetic acid, EDTA) was added into empty syringes prior to collection of blood (19, 21). The contents of the syringe were mixed by gentle rotation to avoid rupture of suspended erythrocytes.

For RBCs, 10ml blood samples containing anticoagulant were centrifuged at 1500 rpm for 5 minutes. The plasma was separated without disturbing the sediment with the help of sterile Pasture pipette. A 99ml phosphate buffer solution (PBS) was added to the sediment and the cells were re-suspended by gentle shaking of the tube. The tube was again centrifuged at 1500 rpm for 5 minutes.

Avian influenza viruses, H_5, H_7 and H_9 were determined through antibodies in the sera of birds by Hemagglutination Inhibition test and 4-Haemagglutination Unit (4HAU) was calculated by using the procedure as adopted by Allan and Gough (1974).The HA titer is the reciprocal of the highest dilution showing heamagglutination and the end point titer and 4HA units of avian influenza viruses H_5 H_7 and H_9 were calculated as:

- HA of virus 1: 128
- HA units of virus 256/4 = 64

For obtaining 4HA units of virus, 1µl of the virus suspension was added to 63µl of the PBS and mixed well.

The Heamagglutination Inhibition (HI) test was conducted for each serum sample by using 4HA units of H_5, H_7 and H_9 serotypes of avian influenza virus (20).The following observations were recorded used for interpretation of Heamagglutination Inhabition test:

- The HI titre was expressed as reciprocal of the highest dilution of the serum inhibiting hemagglutination indicated by button formation.
- A small sharply outlined button of RBCs (bead formation) on the bottom of the well was considered as positive for HI test.
- The bottom of the well covered by a thin layer of finally clumped RBCs was recorded as negative HI test.
- Titer more than or equal to 128 was considered to be positive.
- The maximum dilution of each serum sample causing inhibition of hemagglutination was the end point. The HI titer expressed as reciprocal of the highest dilution of the serum showing Hemagglutination Inhibition (button formation). Moreover the serum samples showing Hemagglutination Inhibition activity was the serotype of AI causing infection in and around Quetta.

RESULTS

The aim of the present study was to record the prevalence of avian influenza in broilers in Quetta and its surrounding farms. The results regarding overall sero-prevalence of avian influenza virus randomly collected (sick and normal) from broilers in district Quetta and its surrounding farms are presented in Table 2, and 570 samples were examined from broilers by ELISA, 80 (14.03%) were found positive while 490 (85.96%) were recorded as negative.

Table 1: Fourteen Areas and Two Regions of District Quetta

S. No.	Areas	S.No.	Areas
1.	Akhtarabad	**8.**	Kharaotabad
2.	Chashma	**9.**	Baleli
3.	Siryab	**10.**	Kuchlak
4.	Masrqi bypass	**11.**	Samguli
5.	Hazaraganji	**12.**	ChashmaChozihi
6.	Magrabi Bypass	**13.**	Surpull Quetta
7.	Pushtunabad	**14.**	NowahKalli
I.	**Zagroon Town**	**II.**	**ChaltanTwon**

Table 2: The overall seroprevalence of avian influenza virus in broilers and layers at different farms of Quetta

Type of Farm	Total No. of samples examined	Positive samples		Negative samples	
		No. of samples	%	No. of samples	%
Broiler Farm	570	80	14.03	490	85.96

An area wise investigation was carried out on the sero-prevalence of avian influenza virus in broilers at 14 different areas of Quetta to determine the intensity of infection in birds. The results regarding prevalence of avian influenza virus in broilers at 14 different areas were recorded and presented in Table 2. Of the 570 samples collected and examined, the highest prevalence of avian influenza virus was recorded at Mashriqi Bypass area of broiler farms as 40.0% while the lowest at Pashtunabad area farms and was observed as 2.77%. This variation in the sero-prevalence of avian influenza in broilers at different area may be due proper management and care provided to the farms otherwise there is no any clear reason that caused this variation among farms of broilers located at different areas. The detailed results about variation in the sero-prevalence of avian influenza virus recorded at 14 different areas of Quetta are also given in the same Table 3.

In general, it is concluded that avian influenza virus is prevailing in and around Quetta but it did not cause any serious problem before in broilers in the area. Furthermore, that the virus is prevailing could cause any serious

problem to the birds at any time in future. Therefore it needs a complete investigation to screen out the presence of avian influenza virus in Quetta in particular and province in general.

Table-3: The prevalence of avian influenza virus in broilers recorded from different area of Quetta

Areas	Broilers	Positive	%	Negative	%
Akhtarabad	37	2	5.40	35	94.59
Chashma	48	10	20.80	38	79.16
Siryab	47	3	6.38	44	93.61
Masrqi bypass	25	10	40.00	15	60.00
Hazaraganji	43	5	11.62	38	88.32
Magrabi Bypass	32	1	3.12	31	96.87
Pushtunabad	36	1	2.77	35	97.22
Kharaotabad	45	4	8.88	41	91.11
Baleli	36	10	27.77	26	72.22
Kuchlak	44	8	18.18	36	81.18
Samguli	43	6	13.95	37	86.04
ChashmaChozihi	50	6	12.00	44	88.00
Surpull Quetta	40	4	10.00	36	90.00
NowahKalli	44	10	22.72	34	77.27
Total	**570**	**80**	**14.03**	**490**	**85.96**

Table-4: The seroprevalence of avian influenza virus in broilers of different regions of Quetta

Regions	Broiler	Positive	%	Negative	%
Zargoon Town	225	28	12.44	197	87.55
Chiltan Town	345	52	15.07	293	84.92

Table 5: Determination of avian influenza virus specific antibody titre in the sera broilers

Farm No.	HI Titre	Farm No.	HI Titre
1	1.28, 1:64, 1:64	24	1:128, 1:64, 1:64
3	1:28, 1:64, 1:64	25	1:16, 1:32, 1:32, 1:32
4	1:8, 1:16	26	1:8, 1:16
5	1:164, 1:64, 1:28, 1:16, 1:16	27	1:128, 1:164, 1:64
6	1:128, 1:64, 1:64	28	1:8, 1:16
8	1:64; 1:64; 1:128: 1:16; 1:16	29	1:8; 1:16
11	1:128, 1:164, 1:64	30	1:16, 1:16, 1:128, 1:128, 1:128, 1:64
14	1:128, 1:164, 1:64	31	1:8, 1:16
15	1:8, 1:16	34	1:16, 1:32, 1:32, 1:32
18	1:162, 1:32, 1:32, 1:32	36	1:8, 1:16
20	1:64, 1:64, 1:28, 1:16, 1:16	37	1:8, 1:16
22	1:128; 1:64; 1:64	38	1:16, 1:32, 1:32, 1:32
23	1:64, 1:64, 1:28, 1:16, 1:16		

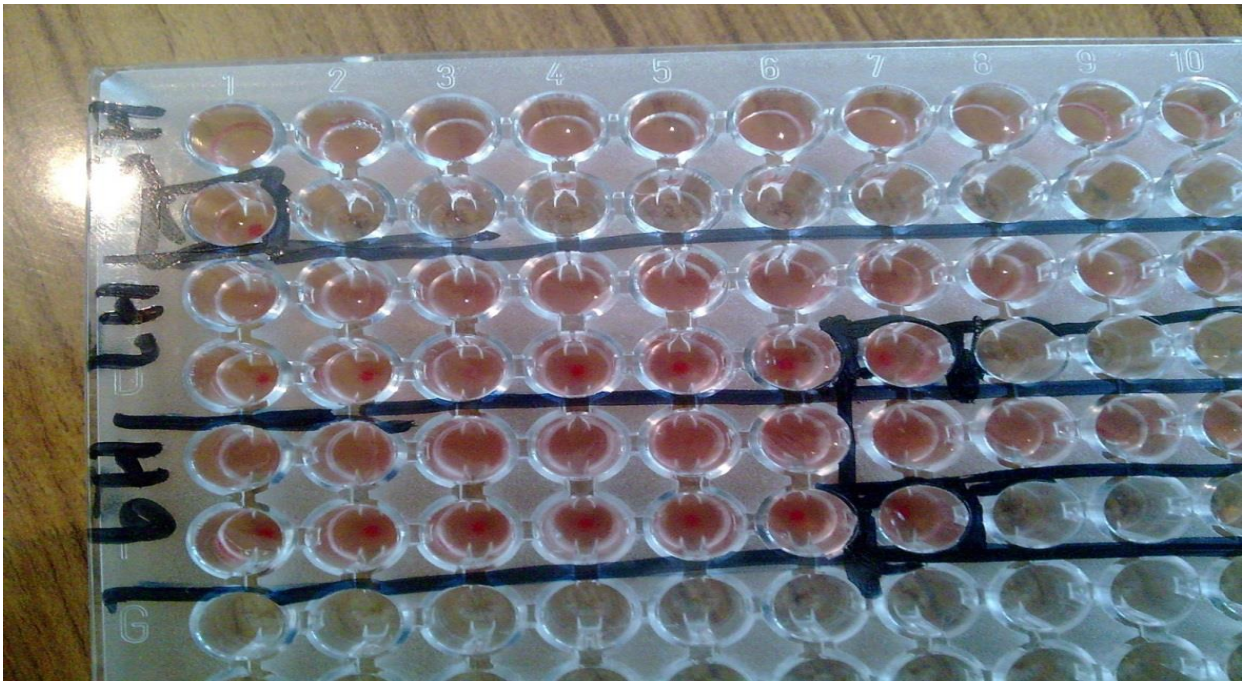
During present study a region wise investigation on the seroprevalence of avian influenza virus in broilers was carried out and the results are summarized in Table 4. From Zargoon region of Quetta, 225 samples were collected and examined by ELISA, 28 (12.44%) were found positive while 197 (87.55%) were recorded as negative. Similarly, 345 samples were obtained from Chilton region and tested for prevalence of virus, only 52 (15.07%) samples were detected as positive and remaining samples were observed as negative.

When comparison was made between two regions, the higher prevalence of avian influenza virus was determined in broilers of Chilton region of Quetta. No any clear reason was recorded during survey that increased the prevalence, however, the Chilton region was thickly populated and broiler farms were very close to each other. A sanitation problem was also observed there so that might be factor has contributed in higher prevalence of avian influenza virus in broilers of Chilton region. Furthermore, it was observed that the farm owners were non-technical and less educated. Whereas in Zargoon town region, the owners of the farms were somehow educated and technically sound and further that broiler farms were not close to each other and located at open atmosphere. The reason for lower prevalence of avian influenza virus in broilers at Zargoon region may be the factors that are mentioned for Chilton region are not found in Zargoon town region.

A survey on the seroprevalence and the presence of specific antibody of avian influenza virus species in the sera of broilers were carried- out through ELISA and Hemagglutination Inhibition tests respectively. Earlier to this, the

investigation was carried on the seroprevalence of the viral species in the sera of broilers. For the purpose, the blood samples were obtained from broilers of different farms of poultry birds in Quetta and it surroundings were initially tested by ELISA. All the positive sera were further tested for determination and confirmation of influenza virus species antibodies, therefore, the Hemagglutination Inhibition test was performed to record the specific antibodies of avian influenza virus in birds.

Plate 1: The micro titration plate with diluted serum for the specific identification of avian influenza virus (strains H5, H_7 and H_9) using Heamagglutination Inhibition test



The positive sera of the broilers were further processed and titrated with three different antigens, H_5, H_7 and H_9 by Hemagglutination Inhibition test (Plate 1). The data regarding HI antibody titre in the sera of broilers obtained from 38 different farms are summarized in Table 5. During investigation, only H_9 avian influenza virus antibody was detected from the sera of broilers. The highest antibody titre in the sera of broilers was recorded as 1:164 while the lowest was determined as 1:8. The variation in the antibody level in the sera of broilers at different farms was recorded and is placed in the same Table 5. Furthermore, except H_9, no any species was detected from serum samples. It is clear from the present study that, only H_9 avian influenza species is prevailing in Quetta and its surrounding area, further that, H_9 has been worldwide recognized as non-pathogenic or less pathogenic virus so there would be no threat to human population and birds as well but sometimes this virus can cause problem to poultry birds in future. During present study no serious problem from the virus to birds was recorded. It can be concluded from the survey that at least in this stage the poultry birds of the area are not at high risk of avian influenza virus infection.

DISCUSSION

Avian influenza has emerged as a major disease of poultry birds. It caused great economic losses during the last decade in Pakistan and other countries of the world. Avian influenza infects the poultry and causes mortality in birds and also in human beings. The zoonotic importance of avian influenza attracting attention of the scientists to work against the species. Avian Influenza is a viral disease and causes asymptomatic infections in various hosts. It causes an acute and fatal disease in chickens, turkeys and many other avian species (22). The role of the poultry workers in the transmission cannot be ignored. Similarly marketing services may also share in the transmission of avian influenza virus (4).

During present survey, a total of 570 sera were collected from broilers and examined through ELISA, 80 (14.03%) sera were found positive while 490 (85.96%) were recorded as negative. Nili H and Shahid MA (23, 24) conducted

an investigation on the prevalence of avian influenza in broiler chickens, they recorded that the prevalence of H_9 in broiler chickens that ranged from 20-60%. The findings of the present study are in close agreement to the findings of the above researchers. An investigation against the seroprevalence of avian influenza in 38 broiler breeder flocks was carried out by (2, 25) who recorded more than 20% sero-prevalence in broilers by ELISA in Jordan. The seroprevalence of H_9 avian influenza virus recorded for boilers in the present survey is in close agreement to the above authors who detected the same prevalence as recorded in the study for broilers. The sero-prevalence of avian influenza in broilers were same at different area may be due to no proper management and care provided to the farms also not adopted the vaccination schedule.

An area-wise study on the seroprevalence of avian influenza virus was also conducted during present survey. The highest prevalence (40.0%) of avian influenza virus was recorded at Mashriqi Bypass area while the lowest (2.77%) at Pashtunabad area farms. This variation in the sero-prevalence of avian influenza in broilers at different area may be due improper management and care provided to the farms also not fallow the vaccination schedule, otherwise there is no any clear reason for this variation among farms of broilers located at different areas.

Furthermore, the region-wise seroprevalence of avian influenza virus in broilers was also investigated. At Zargoon region of Quetta, 225 samples were tested, 28(12.44%) were found positive while 197(87.55%) were detected as negative. Similarly, 345 samples from Chilton region were examined, 52 (15.07%) were detected as positive while others were observed as negative (84.92%).

The results regarding seroprevalence of avian influenza H_9 strain in the sera of broilers were recorded in the present study are in line to the findings of Alkhalaf AM *et al* and Awad M *et al* (26, 27) who recorded the seroprevalence of avian influenza from different regions and areas of Saudi Arabia (Al-Qassim, Hail, Al- Jouf and Northern Border regions) for H_9 strain using Enzyme Linked Immunosorbent Assay (ELISA) and Hemagglutination Inhibition (HI) tests.

The ELISA detected the highest AIV prevalence in Northern Border regions (45.71%), followed by Al-Jouf (29.65%), Al-Qassim (23.98%) and Hial (20.94%). Therefore, the results about the seroprevalence of H_9 avian influenza virus noted in the present in different areas and regions of Quetta districts are in agreement to the above author, who also recorded similar patternr of seroprevalence in different regions of Saudi Arabia as recorded in the present survey for different regions and areas of Quetta, Balochistan, Pakistan. Capoa I *et al* (28) also conducted similar investigation on the seroprevalence of avian influenza viruses in poultry birds which caused very high mortality ranged from 50-89% and sometimes it reached to 100% in poultry flocks was also higher than the present study. So the results of the present study do not agree at all to the findings of the above workers, they recorded the higher prevalence while we recorded lower prevalence. In General, the results of the present survey are not in line to the above authors who recorded higher seroprevalence as compared to the present study.

The specific antibody titer of H9 strain of avian influenza virus in the sera of broilers was also demonstrated during study. The specific antibodies titer of H9strain of avian influenza virus was measured in the sera of broilers that ranged from 1:8 to 1:164. No variation in the antibody titre in the sera within broilers was observed during present investigation. During the present study H_9 species was detected from serum samples. It is clears form the present study that, only H_9 avian influenza is prevailing in Quetta and its surrounding area ,further that H_9 has been worldwide recognized as non-pathogenic or less pathogenic virus so there would be no threat to human population and birds as well but sometimes this virus can cause to poultry birds in future. During present study no serious problem from the virus to birds was recorded. It can concluded from the survey that at least in this stage the poultry birds of the area are not at high risk of avian influenza virus infection

CONCLUSION

It is concluded from the present study that avian influenza virus H9 strain is prevailing in broilers in Quetta and its surroundings while H_5 and H_7 viruses were not detected from the sera of the broilers.

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