Seroprevalence and risk factors of avian influenza H9 virus among poultry professionals in Rawalpindi, Pakistan

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ARTICLE INFO

ABSTRACT

Background: Avian influenza H9 is endemic in commercial and backyard poultry in Pakistan and is a serious occupational health hazard to industry workers. This study aimed to determine the seroprevalence of avian influenza H9 infection in people working with poultry in Rawalpindi, Pakistan and assess the measures they took to protect themselves from infection.

Methods: A cross-sectional study was conducted from December 2016 to May 2017 of 419 people working with poultry in Rawalpindi Division, including farm workers, vaccinators, field veterinarians, butchers and staff working in diagnostic laboratories. Potential participants were randomly approached and gave written consent to participate. Data were collected using a standardized questionnaire and serum samples were processed to detect H9 antibodies using the haemagglutination inhibition test.

Results: Of the 419 participants, 406 (96.9%) were male. The mean age of the participants was 36.4 (SD 10.86) years. A total of 332 participants agreed to a blood test, 167 of whom were positive for A(H9) antibodies, giving an overall seroprevalence of 50.3%. Laboratory staff had the highest seroprevalence (100%) and veterinarians the lowest (38.5%). Vaccinators, butchers and farm workers had a seroprevalence of 83.3%, 52.4% and 45.5% respectively. Personals who used facemasks had significantly lower (P < 0.002) seroprevalence (29.6%) than those who never used them (90.6%). Similarly, those who always used gloves and washed their hands with soap had a seroprevalence of 32.8% compared with 89.0% in those who never took these precautions. Of the participants who handled antigens, 92.3% were seropositive.

Conclusion: Laboratory staff and vaccinators are exposed to viral cultures and influenza vaccines respectively which may explain their high seroprevalence.

Introduction

Poultry is one of the biggest sectors of the livestock industry in Pakistan with current investment of more than 200 billion Pakistan rupees (about US$ two billion). This sector directly or indirectly provides employment to over 1.5 million people [1]. Poultry, being readily available and cheaper in price, is a leading animal protein source. The poultry sector has been growing at a constant rate of 8–10% over the past few years. This growth has posed some important economic and public health challenges. Zoonotic pathogens put those working in the poultry industry and those using poultry products at risk of acquiring the diseases they transmit. Avian influenza is one of the most important of these challenges. Influenza A viruses are classified by their surface glycoproteins, haemagglutinin and neuraminidase. Up to now, 18 haemagglutinin and 11

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neuraminidase subtypes of influenza viruses have been identified [2]. On the basis of pathogenicity, avian influenza is categorized into two categories, highly pathogenic avian influenza and low pathogenic avian influenza. The avian influenza virus A(H9N2) is considered a low pathogenic avian influenza and is endemic in Pakistan [3]. The highly pathogenic avian influenza subtypes include A(H5) and A(H7) but these have not been reported in Pakistan since their last outbreak in 2008.

Highly pathogenic influenza can be deadly to humans with many deaths reported in recent years around the globe [4]. Its indications are illness like seasonal influenza, conjunctivitis, respiratory distress with short breaths leading to acute respiratory disorders and failure of the respiratory system. The symptoms of low pathogenic avian influenza in humans are conjunctivitis, influenza-like illness, cough, fever, sore throat, muscle aches, and pneumonia. As low pathogenic avian influenza is not fatal, most of the time it goes unnoticed. However, its widespread circulation in human populations has been reported from different parts of the world. This widespread circulation and high rate of mutation may cause active transmission of avian influenza virus among humans because of regular reassortment of the virus [5]. Therefore, it could have the potential to cause a future global influenza pandemic [6]. As this disease is highly prevalent in Pakistan [7], there is a need for effective disease surveillance in poultry as well as in poultry professionals working directly or indirectly within the industry.

Currently very few data are available on the seroprevalence of avian influenza in human populations in Pakistan. In view of these gaps and the importance of the pathogen, we determined the seroprevalence of avian influenza A(H9) in poultry professionals and assessed the preventive practices they used against the influenza virus.

Materials and methods

Study design

A descriptive cross-sectional study was conducted from December 2016 to May 2017 in Rawalpindi, Pakistan.

Sampling strategy

Professionals working in Rawalpindi district were our target population. Five different categories of poultry professional were included in this study: poultry-farm workers, field veterinarians, staff working in diagnostic laboratories, butchers and vaccinators. The number of poultry professionals working in the target area was estimated to be 125,000 based on information from poultry associations and government organizations. The sample size for simple random sampling was calculated using *Epi Calc*^{TM} 7, with an expected prevalence of A(H9) of 46% as reported by Ahad et al. [3] at 95% confidence level. The sample size initially calculated was 381; this was increased to 419 to account for 10% potential non-response.

Sample selection

Poultry professionals working directly with poultry for atleast 6 months in the geographical boundaries of Rawalpindi were included in the study. Lists of the five categories of poultry professional were taken from the district administration and poultry associations. List of Poultry Disease Diagnostic Laboratory workers, Private practitioners was obtained and they were selected using balloting technique. Accurate list of poultry farms and shops was not available; therefore, these premises were randomly approached. It was ensured that the selected farms are not located in close proximity. In the selected proximities every Poultry farm worker, butcher or vaccinator fulfilling our inclusion criteria was included in the study.

Sample collection

All participants were interviewed about their sociodemographic background, contact with poultry and their practices when in contact with poultry. They were also asked if they would provide a blood sample. Blood samples were collected after informed written or verbal consent from the study participants. The samples were immediately transported to the Virology Laboratory of Disease Diagnostic Section, Poultry Research Institute, Rawalpindi, Punjab. In addition, demographic information and information on preventive practices was collected on a structured questionnaire adapted from a previous study with necessary modifications.

Laboratory procedures

The serum was separated from the blood samples and processed for detection of antibodies against avian influenza A(H9N2) using the haemagglutination inhibition test. Reference antiserum of A(H9N2) (A/Turk/Wisc/1/66) was procured from the Veterinary Laboratory Agency in the United Kingdom. A solution of 0.5% chicken red blood cells was used for this purpose. The haemagglutination inhibition test was performed using the method described by Kazufumi et al. [8]. Briefly, sera were treated with receptor destroying enzyme by diluting one part of serum with three parts of enzyme and incubating at 37 °C overnight. Sera were further diluted with phosphate buffer saline to a final dilution of 1:10. Two-fold serial dilution of serum in 25 μL phosphate buffer saline was done in a 96-well microtitration plate: 25 μL of 4 haemagglutinin unit virus were added up to well 10. Then 50 μL of the red blood cell solution were added. Wells 11 and 12 were kept as positive control of the virus and red blood cells, respectively. The plate was incubated 4 °C and the result was noted after 60 min. The titre was taken as the reciprocal of the dilution level of the wells. The HI titre of 1:160 or above was taken as cut off value and considered positive for AIV H9 infection.

Data analysis

Rates and frequencies were calculated using *Epi Info* 7 while Chi square test of independence was performed using SPSS software.

Results

A total of 419 people were interviewed, of whom 406 (96.9%) were male. The mean age of the participants was 36.4 (SD 10.86) years. Among these professionals, 261 (62%) were poultry-farm workers, 24 (6%) were vaccinators, 67 (16%) butchers, 44 (11%) field veterinarians and 23 (5%) laboratory staff. Of the 419 participants, 100 (24%) had no formal education whereas 51 (12%) were professional veterinarians. Most of the participants had primary (39%) and secondary school (25%) level education. Of the 419 people interviewed, 332 (79%) agreed to a blood test whereas the rest 87 (21%) did not allow blood sample collection hence were not included in serological study. Among the professionals (n = 332) screened for sero-conversion against A(H9) 235 (71%) were poultry-farm workers, 18 (5%) were vaccinators, 21 (6%) butchers, 39 (12%) field veterinarians and 19 (6%) laboratory staff. Among them 167 were positive for A(H9) antibodies, the seroprevalence among various groups ranged from 38.5% to 100% (P < 0.001) with an overall seroprevalence of 50.3%. Laboratory staff had the highest seroprevalence (19/19, 100%) and veterinarians had the lowest (15/39, 38.5%). Vaccinators, butchers and farm workers

Table 1
Exposure of different poultry professionals with risk factors and sero-positivity against Influenza A(H9N2) based on these individual risk factors.

Exposure categories		Professional categories					Serological result			
		Butchers	Farm workers	Field vets	Lab staff	Vaccinators	Positive	Negative	Chi square	P-value
Contact with live birds	Yes	20	223	39	14	18	159	155	0.261	0.609
	No	1	12	0	5	0	8	10		
Contact with dead birds	Yes	6	235	39	14	0	142	152	4.118	0.042
	No	15	0	0	5	18	25	13		
Contact with offals/tissue	Yes	19	0	39	19	0	43	34	1.232	0.267
	No	2	235	0	0	18	124	131		
Contact with blood	Yes	10	0	18	17	0	29	16	4.165	0.041
	No	11	235	21	2	18	138	149		
Contact with virus/antigen	Yes	0	0	3	10	0	12	1	9.55	0.002
	No	21	235	36	9	18	155	164		

Table 2
Use of different personal protection equipments/practices by poultry professionals and sero-positivity against Influenza A(H9N2).

Personal protection equipments/practices used		Professional categories					Serological result			
		Butchers	Farm workers	Field vets	Lab staff	Vaccinators	Positive	Negative	Chi square	P-value
Outer Garments	Always	2	121	17	13	1	66	88		
	Sometimes	16	64	22	6	15	71	52	6.521	0.038
	Never	3	50	0	0	2	30	25		
Gloves	Always	4	82	15	7	0	27	81		
	Sometimes	7	74	19	12	2	64	50	44.745	0.001
	Never	10	79	5	0	16	76	34		
Face mask	Always	7	119	20	11	5	44	118		
	Sometimes	7	76	16	8	10	75	42	77.988	0.001
	Never	7	40	3	0	3	48	5		
Shoe covers	Always	1	116	2	4	8	41	90		
	Sometimes	3	41	17	7	9	46	31	31.691	0.001
	Never	17	78	20	8	1	80	44		
Hand wash with water	Always	12	168	35	19	11	104	141		
	Sometimes	9	45	4	0	5	44	19	23.664	0.001
	Never	0	22	0	0	2	19	5		
Hand wash with water & sanitizer	Always	5	99	17	7	3	43	88		
	Sometimes	6	69	16	12	9	62	50	30.497	0.001
	Never	10	67	6	0	6	62	27		

Table 3
Antibody titre to Influenza A(H9N2) in different professionals of the Rawalpindi region.

Professional categories	No. of samples	Antibody titre						
		<1:20	1:20	1:40	1:80	1:160	1:320	1:640
Butcher	21	3	1	4	2	6	5	–
Field veterinarian	39	–	9	9	6	12	3	–
Laboratory staff	19	–	–	–	–	12	5	2
Poultry-farm worker	235	29	47	17	35	71	25	11
Vaccinator	18	1	–	1	1	9	3	3

Values are number of the participants in the professional group.

had a seroprevalence of 83.3% (15/18), 52.4% (11/21) and 45.5% (107/235) respectively.

In terms of exposure to the different potential sources of A(H9N2) infection, most of the participants had direct contact with live birds 95% (314/332) and 294 (89%) had contact with dead birds. Only 13 (4%) of the participants reported handling the live virus or antigen. Contact with blood and animal tissue was reported by 45 (14%) and 77 (23%) participants respectively. Contact with virus/antigen was the most significant exposure as 92% (12/13) of the exposure positive participants showed sero-positivity as compared to 47% (155/319) in exposure negative group (P < 0.002). The details about exposure of different poultry professionals to different risk factors and their impact on seroprevalence is given in Table 1.

Practices regarding the use of different personal protection equipments and their impact on seroprevalence was also recorded. Hand wash with water after each task was the most frequently adopted preventive practice with 74% (245/332) of the participants reporting its consistent use while gloves were the

least used 33% (108/332) PPE. Consistent use of soap/sanitizer, outer garments/gowns, face masks and shoe covers was reported by 39.5% (131/332), 46% (154/332), 49% (162/332), and 39.5% (131/332) respectively. Use of face mask was significantly protective (P < 0.001) against Influenza A(H9N2) as only 27% (44/162) participants who reported its consistent use were sero-positive as compared to 64% (75/117) and 91% (48/53) sero-positivity among occasional users and those who never used mask at all, respectively. Other PPEs had the similar effect, the details of PPE usage practices by different groups of poultry professionals and sero-positivity against Influenza A(H9N2) is given in Table 2. Table 3 represents the antibody titres to A(H9N2) in the different professionals in the poultry business.

Discussions

Avian influenza is a contagious viral disease, distributed worldwide, which can not only infect all age groups of humans and the poultry population but is a serious threat to the people working

directly or indirectly with poultry [9]. In this study, we assessed the seroprevalence of A(H9N2) virus in professionals working with poultry and their practices to prevent infection. A high seroprevalence of 50.3% against influenza A(H9N2) observed during this study was not unexpected as Influenza A(H9N2) has been prevalent in poultry of this region since 1998 [10]. These results were also consistent with the findings of similar studies in Pakistan [11] and neighbouring country Iran where a similar seroprevalence 23–87% was reported [12]. However another study from china reported much lower (2.9%–11.1%) seroprevalence of A(H9N2) among various groups of poultry professionals [13]. This may be explained by the different poultry production and marketing systems in these two countries as well as the knowledge and PPE practices of the two study populations. Yu et al. reported a much higher compliance of PPE practices in their study population where 88.9% respondents said they wore specific work clothing, 84.1% workers regularly used disinfectant, 54.1% wore gloves, and 45.9% wore face masks.

The extent of direct contact with poultry or poultry products may vary based on type of poultry farming and live bird markets in the respective country. Personnel who always used facemasks had a lower seroprevalence of 29.6% (48/162) compared with 90.6% (48/53) in those who never used them. These results are in line with the findings reported in a study in Punjab [11]. A study conducted in Nepal on knowledge and practices of poultry workers on Influenza showed similar results, which reported 30% of the poultry professionals used gloves while 27% used face masks [14].

Serological evidence of human infection with A(H9N2) viruses has also been reported from neighboring countries of this region including India, China and Egypt [15–18]. In these studies, the seroprevalence ranged between 1.2% and 17% in various categories of poultry professionals. But in our study the seroprevalence of antibodies against A(H9N2) virus ranged from 38.5% to 100%. The high seroprevalence might be attributed to an increased avian-to-human transmission capacity of the avian influenza virus in Pakistan through mutations and reassortments [19]. Similarly, the high seroprevalence in the vaccinators might be due to close contact with live birds during vaccination as well as poor compliance to PPE usage. Overall the higher sero-prevalence may be due to the fact that vaccination against Influenza A(H9N2) is regularly used in poultry populations in Pakistan that may result in un-detected persistence of the pathogen facilitating its silent spread to other populations [20].

Our study had certain limitations, as this was a cross sectional study conducted over a period of six months only, therefore it may overlook seasonal trends.

Conclusion

The results demonstrated A(H9) seropositivity in poultry professionals which means they were exposed to A(H9) infection. Poultry professionals with direct exposure to live antigen or infected birds or who did not use personal protection equipment had the highest A(H9) seropositivity.

Funding

No funding sources.

Competing interests

None declared.

Ethical approval

Ethical approval was obtained from the Institutional Review Board, National Institute of Health, Islamabad, Pakistan.

Author contributions

MFT conceived the idea, designed the study, and collected and processed samples. MAA supervised the research. TG designed the study and analysed the data. SD, MMHB and QA collected and processed samples. MAS and MAK reviewed the manuscript. MAR analysed the data. MTN conducted interviews.

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