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DETECTION OF ANTIBODY TO NEWCASTLE DISEASE VIRUS IN HUMAN SERA IN PAKISTAN

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ABSTRACT

Ability of Newcastle disease virus (NDV) to elicit antibody response and clinical illness was assessed in poultry workers from 12 districts of Punjab, Khyber Pakhtoonkhwa and capital areas of Pakistan through a cross sectional sero-surveillance. Blood samples collected from 465 exposed and 25 unexposed individuals were tested for antibody titer against NDV through modified horse RBC haemagglutination inhibition (HI) test. Among different worker groups, the highest geometric mean titer (GMT) value of 149 was recorded for vaccinator's group followed by poultry attendants (86); lab technicians (65); veterinarians (61) and butchers (26). Among in different districts, the highest GMT value (160) was recorded for Haripur, Sialkot and Toba Tek Singh districts followed by Multan (139); Gujranwala (130); Faisalabad (113); Bahawalnagar (98); Lahore (80); Rawalpindi (75); Chakwal (53); Sheikhupura (53) and Islamabad (26). Occupation/group and district wise the highest seropositivity was recorded for vaccinator's group (85.71%) and for Toba Tek Singh district (82.14%) whereas that of the lowest was recorded for butcher's group (20.63%) and for Islamabad district (9.68%). No marked evidence of clinical illness was recorded in exposed individuals. It was concluded that continuous exposure to NDV initiated antibody response in humans without causing clinical illness.

Key words: NDV, HI, RDE, Poultry workers

INTRODUCTION

Newcastle disease virus (NDV) of paramyxoviridae family is non segmented single stranded RNA virus, cosmopolitan in distribution and causes acute infection of digestive, respiratory and nervous system in chickens of all ages. Regular Newcastle disease (ND) epidemics have previously been reported from Africa, Asia, Central America and parts of Southern America whereas sporadic cases have been reported from Europe (Alexander, 2003). Transmission of virus is through movement of live birds, infected occupational people, sharing of contaminated equipment and intake of contaminated feed and water (Alexander, 1988). Although birds are natural hosts of NDV, mild and self limiting human infections without systemic involvement have previously been reported in laboratory and field workers either due to accidental introduction and/or exposure with contaminated materials (Nolen, 2003). Both vaccinal and virulent strains of NDV can cause conjunctivitis, fever, headache, chills, pharyngitis and photophobia with general apathy in human beings (Chang, 1981; Alexander, 2003). Intranasal and intratracheal inoculation of African green monkey and Rhesus monkey with lentogenic and mesogenic strain of NDV gave rise to limited replication of virus in respiratory tract. However it did not exhibit any clinical sign with little or no virus shedding (Bukreyev *et al.,* 2005). In response to NDV, low levels of neutralizing antibodies are produced in humans (Alexander, 2003). According to previously conducted sero-surveys for detection of NDV antibodies in human beings, a seropositivity of 29% (Miller and Yates, 1971) and 20% (Charan *et al.,* 1981) was recorded for exposed groups whereas none of unexposed individual had detectable antibodies. Keeping in view the zoonotic importance of ND, present study was designed to understand the potential of NDV to initiate antibody response as well as clinical illness in human beings.

MATERIALS AND METHODS

Study area: From 61 districts of Punjab, Khyber pakhtoon khwa and federal area of Pakistan, a total of 12 districts including Bahawalnagar, Chakwal, Faisalabad, Gujranwala, Haripur, Islamabad, Lahore, Multan, Rawalpindi, Sheikhupura, Sialkot and Toba Tek Singh were randomly selected for the present study.

Sampling: From selected districts, coagulated blood samples and retrospective information about clinical illness were collected from 465 exposed and 25 unexposed individuals including poultry attendants (354); butchers / poultry retailers (63); vaccinators (7); lab technicians (18) veterinarians (23) and controls / unexposed individuals (25). Blood samples were collected from these selected (n= 490) individuals in

sterilized disposable syringes without anticoagulant, sera were separated and stored at -82^{0}C till further processing. The sera were analyzed at University Diagnostic Laboratory (UDL), University of Veterinary and Animal Sciences, Lahore, Pakistan

Hemagglutiantion Inhibition (HI) Test

Reference Antigen and Antiserum

NDV antigen: La Sota vaccine strain (Avinew, Merial, France)

NDV antiserum: Veterinary Laboratory Agency, UK

Protocol: The sera were treated with receptor destroying enzyme (RDE) with 1:3 ratio. The treated sera were kept in water bath at 37^{0}C for overnight to destroy the inhibitory substances in the serum. Prior to test the sera were treated in water bath at 57^{0}C for half an hour to inactivate the activity of RDE. Antibody titter to NDV was measured by modified horse RBC HI test. The titre was calculated by the reciprocal of the dilution which completely inhibit hemagglutination test (WHO, 2007). In our study HI titre of 1:160 or greater was considered positive which was also the standard used in other serological infection in human against other hemagglutinating H5N1 avian influenza virus (WHO, 2007).

Statistical analysis: The data were analyzed by non parametric Kruskal-Wallis one way analysis of variance (ANOVA) method using statistical software (SAS, 2004).

RESULTS AND DISCUSSION

In this study we evaluated human sera to ND who are directly exposed to poultry industry in Pakistan specially in Punjab province. We found the highest GMT 149 was observed in vaccinator groups and the lowest GMT 26 observed in butchers (graph-1). Similarly the highest seropositivity 85.71% was found in the vaccinators group and the lowest seropositiviy 20.63 % was found in the poultry retailer / butcher group. Vaccinators frequently move from one poultry farm to another poultry farm for vaccination of poultry. They are more exposed than other groups of professional people. This might be one of the reasons having high titre of antibody to ND in vaccinator groups in compare to other group. In case of butcher they sacrificed only broilers. It means butchers have been exposed when broiler reach at the age 40 days and above. In case of broilers NDV vaccine is generally administered minimum two weeks before of vaccination (Nabi *et al*., 2012) so that virus shedding is low. This might be reason the butchers had the lowest GMT. Within the butcher group the lowest percentage (6.35%) was observed in the people who had titre of 1:20. Among the professional groups the highest percentage (52.38%) of butcher group had no detectable antibody titre to ND. In all groups of people (4% - 92%) found that they don't have detectable antibody titre except in the vaccinator group. In the vaccinator group every body had titre at least 1:80 or above. Among the poultry attendants highest number of people 106 (29.94%) had titre of 1:160 and 97 (27%-40%) number of people had the tire of 1:80. Among the veterinarians the highest number 6 (26.09%) people had titre of 1:80. Same number of people 5 (21.74%) had the titre of 1:160 and 1: 20 respectively. It showed that titre of control group had significant difference with veterinarian ($p<0.01$) and lab technician group ($p<0.05$) while the titre with the butcher, poultry attendant and vaccinator was insignificant (Table-1).

Antibody titre to NDV in human population belonging to 12 different districts of Pakistan were evaluated. It showed that the highest GMT 160 was observed in Haripur district from Khyber Pakhtoon Khwa province, Sialkot and Toba Tek Singh district of Punjab province which is also considered as infected titre in our study (graph-2). All the three districts had the same GMT. The lowest GMT 26 was observed in Islamabad, capital of Pakistan. In Multan district 139 GMT was recorded which is closed to infected titre. In all the places the poultry worker had no detectable antibody titre except Chakwal and Islamabad districts. In Chakwal and Islamabad 28.26% and 38.71% respectively poultry workers had no detectable antibody titre. In seven districts out of twelve districts all the poultry workers had more than 1:20 antibody titre. The name of the places are Haripu, Sialkot, Toba Tek Sing, Multan, Gujranwala, Faisalabad and Bahawalnagar districts. In terms of seroinfectiviy highest seropositivity 82.14% was found in Toba Tek Singh districts and lowest 9.68% was found in Islamabad district. Out of 12 districts in 6 districts more than 50% poultry workers were seropositive. In the category of titre 1:320 highest 42.86% was observed in Sialkot districts and in three places like Faisalabad, Rawalpindi and Islamabad no poultry worker had that above mentioned titre. It showed that titre of all districts had significant difference ($P<0.01$) when compared with Toba Tek Singh district. (Table - 2).

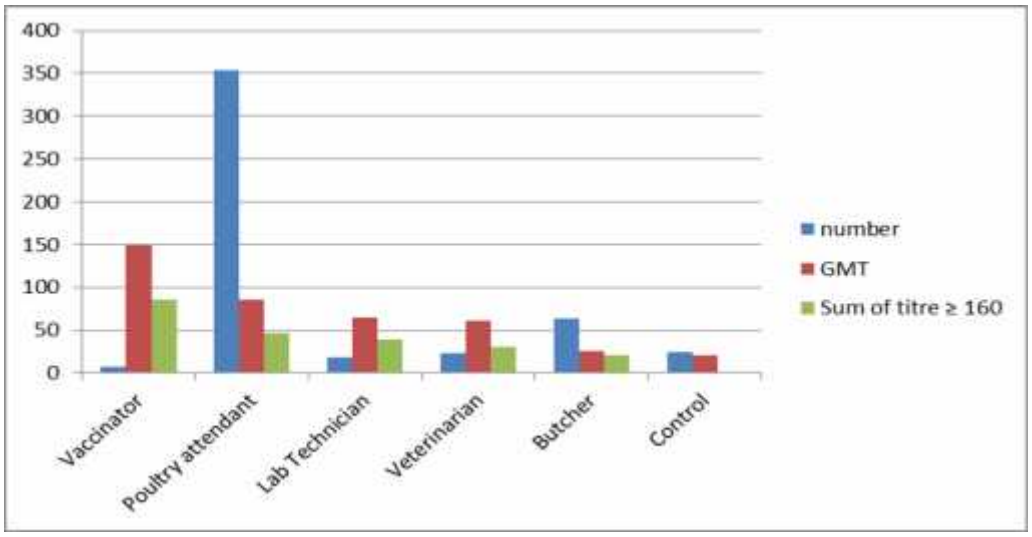
In one study total 213 human sera were evaluated for the detection of NDV antibodies by HI and enzyme linked immuno sorbent assay (ELISA). Out of 213, 104 samples were taken from the people who were working in farm and veterinary vaccine institute. Rest 109 sera samples were taken from general population whose exposure to poultry was limited. It showed that 39% of sera sample from high risk people were positive by both HI and ELISA and 20% sera sample from high risk population were negative by both HI and ELISA test (Charan et al., 1981). In our study almost same percentage of lab technician were seropositive for ND based on HI test. In the control group 93.5% population

were sero negative by both HI and ELISA (Charan et al., 1981). This result has concord with our findings. Among the high risk groups of people 61.54 % had below titre of 8 (Charan et al., 1981). In our study 52.38% of butcher had no detectable antibody titre to ND. A similar study was undertaken to evaluate antibody to avian viruses in human population. Antibody to three different avian viruses like Infectious bursal disease virus (IBDV), NDV and infectious bronchitis virus (IBV) were measured in two groups of population. One group of people was exposed to poultry industry and another group of people exposure to poultry industry was limited. Antibody was measured by indirect sandwich ELISA method. In case of IBDV it showed there was no significant difference of antibody titre between the two groups. However in case of NDV where sera were diluted from 1/40 to 1/640 and it showed the mean titre was double in the group who has been exposed to poultry industry in compare to other group who has not been exposed to poultry industry (Pedersden et al., 1990). In our study we also observed the same result in the control group. We evaluated sera of 25 people as control group whom are not exposed to poultry industry. None of the individual was found seropositive against ND. 92% of the people of control group did not contain any detectable antibody titre to NDV. Only 2% people of control group had titre of 1:20. A similar type of study was taken in USA to evaluate antibodies to ND in two groups of people. In group I people had close contact with poultry and in group II had limited association with poultry. In group I 29% people had antibody to ND where as in group II none of individual had antibody to ND. Antibody was measured by HI test using guinea pig RBC. Within group I, 2.58 % people had antibody titre ranging from 1:160 to 1:320 (Miller and Yates, 1971). But in our study 46.61 % poultry attendant had the titre of 1:160 and above. This might be due not taking proper biosafety measure during handling of the birds. When sera of same individual were measured by neutralization test it showed that in group I and II 17% and 7% people respectively were positive for ND. It showed there was no correlation between HI antibody titre and neutralizing antibody titre (Miller and Yates, 1971). In UK there were two outbreaks of conjunctivitis in people who were working in a broiler factory. The people were engaged in eviscerating of carcass and dressing of broiler. Altogether 16 isolates of NDV were isolated from 37 samples of lacrimal fluid during the two outbreaks (Trott and Pilsworth, 1965). During analysis of human sera for evaluating titre against ND one scientist himself was affected with bilateral conjunctivitis for 3-4 days. The virus was isolated from eye washing. Two years later same individual was affected with ND related conjunctivitis but the strain was different (Miller and Yates, 1971). During our study personal clinical history was also taken during take blood sample from the poultry worker. People specially lab technician personnel they had some episodes of conjunctivits during their routine work. Although no attempt was made to isolate NDV from the lacrimal fluid of clinical affected lab technician people. By district wise we found highest 82.14% seropositivity in Toba Tek Singh district. In that district about 14.28% of the people were lab technician. The lab technician who did postmortem examination of dead or clinically sick birds for investigation of any poultry disease. They also routinely performed serological test like HI, ELISA for diagnosis of different poultry diseases including NDV.

Retrospective information recorded at the time of sampling revealed that two of the eighteen laboratory technicians had an episode of eye infection/conjunctivitis during their routine work. None of the remaining 463 exposed individuals exhibited clinical illness. It was concluded that continuous exposure of human beings to NDV initiated antibody response without causing clinical illness.

Table-1. Antibody titre to NDV in different occupational people in Pakistan

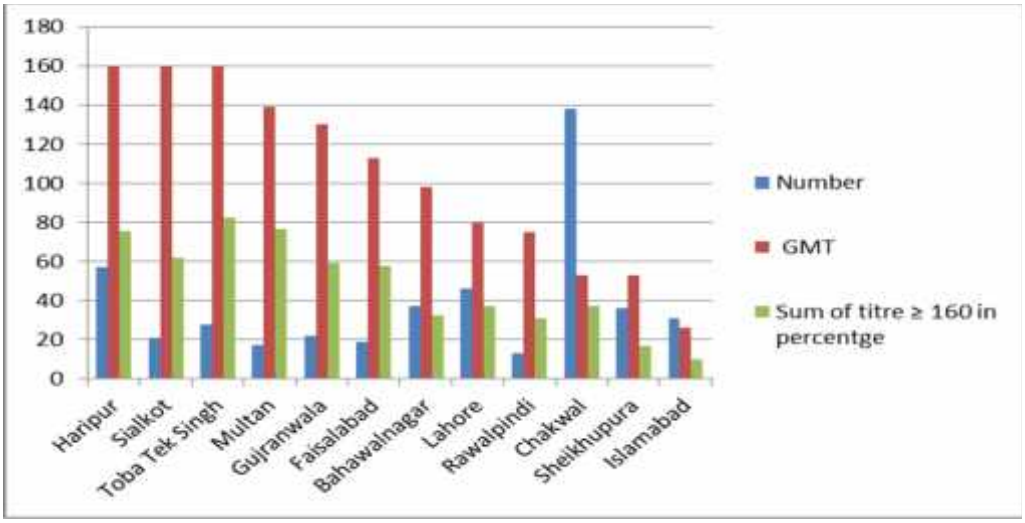
Occupation	number	GMT	<1:20	1:20	1:40	1:80	1:160	1:320	Sum of titre ≥ 160
Vaccinator	7	149				1(14.29)	6(85.71)		85.71
Poultry attendant	354	86	31(8.76)	26(7.34)	35(9.89)	97(27.40)	106(29.94)	59(16.67)	46.61
Lab Technician	18	65	2(11.11)	3(16.67)	3(16.67)	3(16.67)	4(22.22)	3(16.67)	38.89
Veterinarian	23	61	1(4.35)	5(21.74)	4(17.39)	6(26.09)	5(21.74)	2(8.70)	30.43
Butcher	63	26	33(52.38)	4(6.35)	5(7.94)	8(12.70)	13(20.63)		20.63
Control	25	21	23(92.0)	2(8.0)					0.00
Total	490								



Graph1: Antibody tittre to NDV in different occupational people in Pakistan

Table -2. Antibody titre to NDV in population in different districts of Pakistan

Study Site	Number	GMT	<1:20	1:20	1:40	1:80	1:160	1:320	Sum of titre ≥ 160
Haripur	57	160			2(3.51)	12(21.05)	27(47.37)	16(28.07)	75.44
Sialkot	21	160			1(4.76)	7(33.33)	4(19.05)	9(42.86)	61.90
Toba Tek Singh	28	160			1(3.57)	4(14.29)	18(64.29)	5(17.86)	82.14
Multan	17	139				4(23.53)	12(70.59)	1(5.88)	76.47
Gujranwala	22	130			2(9.09)	7(31.82)	9(40.91)	4(18.18)	59.09
Faisalabad	19	113			1(5.26)	7(36.84)	11(57.89)		57.89
Bahawalnagar	37	98			3(8.11)	22(59.46)	9(24.32)	3(8.11)	32.43
Lahore	46	80		9(19.57)	9(19.57)	11(23.91)	8(17.39)	9(19.57)	36.96
Rawalpindi	13	75		2(15.38)	1(7.69)	6(46.15)	4(30.77)		30.77
Chakwal	138	53	39(28.26)	24(17.39)	10(7.25)	14(10.14)	19(13.77)	32(23.19)	36.96
Sheikhupura	36	53		8(22.22)	16(44.44)	6(16.67)	3(8.33)	3(8.33)	16.67
Islamabad	31	26	12(38.71)	7(22.58)	2(6.45)	7(22.58)	3(9.68)		9.68
Total	465								



Grpah-2; Antibody titre to NDV in population different districts of Punjab, Pakistan

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