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Detection of antibody to avian viruses in human populations

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SUMMARY

The ability of three avian viruses to elicit antibody response in humans was surveyed for the purpose of identifying zoonotic diseases. Antibody levels in people associated with poultry were compared to those in people having limited poultry association. Antibody levels to three avian viruses: infectious bursal disease virus, a birnavirus; Newcastle disease virus, a paramyxovirus; and avian infectious bronchitis virus, a coronavirus were determined by enzyme-linked immunosorbent assays (ELISA). Differences between the two study groups were evident: people having a known association with poultry showed significantly higher levels of antibodies to Newcastle disease and avian infectious bronchitis virus. Antibodies detected may be due to virus exposure rather than zoonoses.

INTRODUCTION

Zoonotic disease can often be determined by studies in which antibodies against an infectious agent in a human population that has had a close association with animals is compared to the antibody level of a general population with little or no animal association. Such a study was undertaken in which the serum antibody levels of a population of chicken-associated individuals working in the Northeastern United States were compared to the serum antibody levels of a population from the New England states without known poultry exposure. The sera were collected between 1983 and 1985 and tested using ELISA testing, which offers a more sensitive method of antibody detection than any of the previously used serological tests (1, 2).

The postulate under which this study was done was that some poultry-associated persons might have atypically high antibody levels against viruses to which they had been exposed and that zoonotic infections would thereby be indicated.

The viruses under study were bursal disease virus (IBDV), Newcastle disease virus (NDV), and avian infectious bronchitis virus (A-IBV). Of these, only NDV has been considered capable of multiplying in humans and producing a zoonotic disease (3, 4).

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Infectious bursal disease virus

IBDV is one of a family of viruses which are causative agents of disease in chickens, fish, shellfish, and insects. There are no known human or primate birnaviruses. Chickens are the primary host for IBDV, which causes acute disease of chicks and results in destruction of their bursa, an antibody forming organ.

In vitro, IBDV replicates in both avian and mammalian cell lines. These include cells from several species of monkeys (5). The ability of IBDV to propagate in primate cells suggests that IBDV or a related virus might be capable of replication in humans. To date, there have been no reports of infection of humans with this avian virus nor studies of human sera for antibodies to it or a related birnavirus.

Newcastle disease virus

Newcastle disease virus causes an acute infection of all ages of chickens. The virus can infect the digestive tract, respiratory tract or nervous system. NDV is often found in the internal organs of poultry, due to either infection or vaccination with a live strain of the virus (3). Commercial chicken generally receive regular vaccinations against Newcastle disease with both inactivated and live-virus vaccines.

Reports of human infection with NDV have most often been associated with direct contact with either infected birds or concentrated virus. Although more than one hundred descriptions of human reaction to NDV have been noted in the literature, NDV is not considered to be a serious public health hazard (3, 4, 6, 7). NDV has been described as producing a subclinical as well as local and systemic responses in humans. Local reaction is commonly limited to a transient conjunctivitis not affecting the cornea; chills, malaise, headache, and fever have been attributed to systemic involvement. Human response to NDV usually results in production of low levels of neutralizing antibodies. Reactions to subsequent re-exposure to the virus (which have been reported) are similar to reactions to primary exposure; the presence of antibody does not prevent a second ocular inflammation (4). Multiplication of NDV in human hosts is considered to be limited and spontaneous transmission under natural conditions does not occur.

Avian infectious bronchitis virus

A-IBV is an acute, highly contagious respiratory disease of chickens. Poultry species are the only ones known to be naturally infected with this avian coronavirus. Most commercial chicken flocks are vaccinated with live attenuated strains of A-IBV.

This virus appears to have no public health significance. Clinical infection of humans with A-IBV has not been reported although subclinical infection with the virus has been suggested when two populations differing in exposure to poultry were studied for the presence of antibody to A-IBV. Antibodies were found only among individuals who had worked closely with poultry (8).

MATERIALS AND METHODS

Populations Two groups provided human sera for the study. Group I consisted of 59 individuals closely associated with poultry, including employees of poultry

dressing plants and diagnostic laboratory personnel working with avian viruses. Group II consisted of an equal number of individuals whose contact with poultry was presumed to be limited, including university students and city-dwelling women 18–25 years of age. The two groups investigated did not have equivalent age or sex distributions. Group I was 71% male; Group II only 15% male. Group I individuals were older: 58% of Group I individuals were between the ages of 30–75 years with an average age of 36 years, whilst 14% of Group II individuals were between the ages of 30–75 years with an average age of 25. The average duration of association with poultry for Group I was known to be 11·3 years.

Viruses All viruses used were produced in avian cells. Infectious bursal disease virus originated from a commercial live virus vaccine (Bursa Vac-M, Sterwin Lab.). Newcastle disease virus (B1-Hitchner-Blacksburg lentogenic type) was obtained from the NDV repository at the Department of Veterinary Science of the University of Wisconsin. Infectious Bronchitis virus originated from the Massachusetts strain of avian IBV.

ELISA procedure The indirect sandwich ELISA method for assay of antibody was used as has been described elsewhere (1, 2, 9). The ELISA assay was performed in polystyrene Micro-ELISA plates with 96 flat-bottomed wells (Dynatech Laboratories, South Windham, ME, USA). The following procedure was adopted as a general guideline: each antigen was brought to an appropriate dilution in carbonate-bicarbonate buffer (pH 9·6) and 45 μl was added to sensitize alternate horizontal rows in a Micro-ELISA plate. Adjacent wells were sensitized with the same dilution of control antigen. The plates were covered and incubated for 18–24 h at 4 °C. Following incubation, the plates were washed three times with phosphate buffered saline (pH 7·4) to which Tween 20 was added up to 0·08% (PBS-Tween). After washing, dilutions of a test serum were added. The test sera were serially diluted two-fold in PBS-Tween. Forty-five μl of each test serum was transferred to a well with the relevant positive antigen and to a well sensitized with negative antigen. Plates were again sealed and incubated for 1 h at 37 °C. Following incubation, three washings similar to the previous one were performed to remove unreacted serum components. An anti-human peroxidase-linked immunoglobulin antibody [goat antihuman immunoglobulin G (IgG), heavy and light chains, labelled with horseradish peroxidase (Cappel Laboratories, West Chester, PA, USA)] was then added and allowed to react with human antibody. The presence of captured peroxidase was calibrated colorometrically. IgG antibodies were detected. All sera were individually tested in duplicate at nine serial dilutions to 1 in 5120.

Two reagent controls were included in each plate: a substrate control where antigen-coated wells were incubated with substrate only (PBS-Tween was substituted for serum and conjugate) and a conjugate control where antigen coated wells were incubated with conjugate and substrate only (PBS-Tween was substituted for serum). The optical density of each well was measured by a photometer (Micro-ELISA Minireader, Dynatech Laboratories, South Windham, ME, USA) at an absorption wavelength of 492 nm. The substrate control acted as a blank for calibration of the photometer. The conjugate control values were averaged for each plate and that value was subtracted from every test well. Subtraction of corresponding control antigen readings completed the calculation of final absorbance values. Finally, mean values were calculated for each dilution

Table 1. *The P/N ratios of the net optical density readings produced by serum antibody to three avian viruses in two human populations as measured by ELISA. The mean values and standard error of the mean are given. The dilution of 1 part to 640 represents the end point dilution of both NDV and A-IBV. No end point could be determined for IBDV*

Human population groups*	Virus†	Reciprocal of serum dilution				
		40	80	160	320	640
I	IBDV	1·00±0·12‡	1·03±0·18	0·95±0·11	1·05±0·11	0·93±0·11
II		1·00±0·14	1·00±0·13	1·00±0·13	1·00±0·12	1·00±0·12
I	NDV	2·00±0·26	2·83±0·41	2·41±0·39	2·67±0·48	2·34±0·39
II		1·00±0·14	1·00±0·15	1·00±0·13	1·00±0·22	1·00±0·17
I	A-IBV	1·80±0·21	2·35±0·26	2·45±0·34	2·14±0·37	1·15±0·33
II		1·00±0·12	1·00±0·11	1·00±0·13	1·00±0·37	1·00±0·09

* Group I, individuals having had a close association with poultry; Group II. Individuals having had a limited association with poultry.

† IBDV, Infectious bursal disease virus; NDV, Newcastle disease virus; A-IBV, Avian infectious bronchitis virus.

‡ The positive to negative antibody titers (P/N) are the mean ELISA absorbance readings of 59 serum samples of test Group I divided by the mean absorbance of the corresponding serum dilution of the control Group II. The standard error of the mean values are also expressed in relation to the Group II mean values.

of both populations and subjected to statistical analysis. All test samples giving an absorbance value above a threshold level were considered as positive. This threshold level was predetermined by testing a number of individuals presumed to have limited poultry association (Group II). The threshold value was equal to the upper limit of the Group II values plus the addition of 0·2 optical density (OD) units. The 0·2 OD units constitute a difference detectable by the unaided human eye.

The OD readings have been expressed as the positive to negative (P/N) ratios of Group I with the corresponding Group II sera. P/N is the mean OD of replicate wells of the exposed Group I samples divided by the mean OD of the corresponding serum dilution of the Group II samples. The P/N ratio of Group II is 1·00, the mean value divided by itself.

RESULTS

The P/N ratios of the mean ELISA titers of Group I are given in Table 1. The mean of Group I anti-IBDV values was very similar to the mean of Group II values at all dilutions. No end point could be established for detection of antibody, which suggests no differences between the populations. In contrast, in the test for NDV antibodies at serum dilutions of 1/40 through to the end point dilution at 1/640, Group I had mean ELISA readings more than twice as high as Group II at each dilution. In the test for A-IBV antibody, the end point dilution was also found at 1/640. At serum dilutions of 1/80, 1/160 and 1/320, the Group I population had readings more than twice as high at each dilution as did the Group II population. An analysis of variance for significance of mean OD difference between the two populations in regard to their antibody levels was done by means

Table 2. *Analysis of variance of the mean antibody titers at a dilution of 1/160. Variance between the two populations is shown by the Student t test. The results indicate that the null hypothesis is valid for antibody response to IBDV, but not for IBV or NDV**

Human population groups†	Virus‡	Mean	Probability of no difference in serum antibody response
I	IBDV	1·05 ± 0·11	0·50 > *P* > 0·10
II		1·00 ± 0·13	
I	NDV	2·40 ± 0·30	*P* = < 0·001
II		1·00 ± 0·13	
I	A-IBV	2·45 ± 0·34	*P* = < 0·001
II		1·00 ± 0·13	

* The null hypothesis was evaluated using the t distribution. It was also valid at 1/40 and 1/80 dilutions. The mean represents 59 human serum samples at a dilution of 1/160. Mean values were determined by ELISA and are expressed as P/N ratios of the Group II readings. The standard error of the mean are also expressed in relation to Group II values.

† Group I, Individuals having a close association with poultry; Group II, Individuals having limited association with poultry.

‡ IBDV, Infectious bursal disease virus; NDV, Newcastle disease virus; A-IBV, Avian infectious bronchitis virus.

of the Student t test (Table 2). Data are presented in Table 2 for the 1/160 dilution. Similar results were obtained at the 1/40 and 1/80 dilutions. The test showed that the two populations are significantly different in their antibody levels to NDV and A-IBV at a level of confidence of greater than 95%. No difference was shown between the means of the two populations regarding their antibody titers to IBDV.

DISCUSSIONS

ELISA, a sensitive method of detecting antibody, was the method chosen to screen the sera. Optimal conditions for performing ELISA for the detection of human antibodies to the virus were established in this laboratory. The tests were developed and the parameters calibrated for linearity by using previously determined high and low reacting human sera for antiserum to each virus (9). The ELISA technique was reproducible and sensitive for the detection of all IgG antibodies to both the viruses used and to related strains of those viruses.

The lack of age and gender-matched controls and of positive and negative reference sera are considered by the investigators to be drawbacks of the study. All other things being equal, opportunities for exposure to any of the agents could be assumed to be likely to increase with increasing age of the individual, though differences in age are not likely to account for all of the observed differences in antibody level. Gender does not affect the general ability to produce antibodies but it is certainly related to occupational exposure. Most of the people who work with chickens are male. The observation that young females had the same mean reading for antibody to IBDV as did the older male test subjects strengthens

confidence in the validity of the controls. Inherent in a study of this nature is the difficulty of determining what could approximate a 'negative' human serum; that is, one from a mature person known never to have been exposed to any of the agents under study. Young adult sera were used, in part, because IgG levels have been found to peak around age 20 years and slowly decline thereafter (7). These Group II individuals were presumed to have limited direct contact with poultry. In actual fact, no information was available for these individuals regarding their degree of poultry association. If misclassification of exposure status of any of the Group II individuals did influence the results, the effect would be to reduce the differences between the groups and thus underestimate the true differences.

Whilst morphologically similar, human coronaviruses isolated in human respiratory infections are serologically distinct from A-IBV and so would be unlikely to react in this test system. Heterogeneous antibody to human coronavirus could, however, give some low level of cross-reactivity with A-IBV (8). NDV also has a low level of antigenic cross-reactivity with human mumps virus, a fact that may have contributed to the positive reactions found in some of the serological tests used including the ELISA (10). These facts would make it likely that Group I might have slightly higher levels of antibody able to react to NDV and IBV because of the greater number of years which Group I have had to encounter related viruses and possibly develop cross-reacting antibodies. For this reason, only differences shown to be greater than 400% between the levels of the two groups have been considered.

Human antibodies to the two avian viruses most widely used as live vaccines in poultry were detected. IBDV is not widely used as a vaccine; generally only chickens from areas where infectious bursal disease outbreaks have occurred are vaccinated. These birds are immunized as chicks with a non- or weakly-pathogenic live strain of IBDV. Mature birds are refractory to natural infection with IBDV, so chickens entering slaughterhouses would be unlikely to have bursal disease virus in their systems (11). In handling chickens, there would be much less opportunity for contact with IBDV than with the other two viruses; thus the likelihood of a Group I antigenic response due either to exposure or infection would be lower.

The results of this study corroborate the findings of previous investigators that human populations in close contact with poultry have greater incidence of antibodies to NDV than those with limited associations (3, 6, 7). It also corroborates an earlier report of A-IBV antibody in humans (8).

The multiplication of the avian virus in human tissues might be inferred from the presence of antibody to avian virus. However, repeated exposure to the virus, as could occur during the routine handling of virus or infected chickens, might also result in antibody response without viral replication having occurred in the subject. The testing system did not distinguish between the two possible cases. The strongest indication of infection from these data are the high levels of antibodies detected in Group I in comparison to Group II. An individual demonstrating a four-fold rise in level of specific serum antibodies is generally considered to be mounting an immune response to infection (3). This study shows Group I mean antibody levels against NDV and A-IBV exceed the Group II readings by 8- to 16-fold.

At the time of an individual's initial encounter with a virus, it is possible to distinguish between an allergic type of response and response to virus infection by monitoring rises in the classes of specific antibodies produced. Viral multiplication in the host would be likely to elicit IgM antibodies followed by IgG. An allergic type response without virus multiplication would initially elicit IgE antibodies followed by IgG. This study did not deal with classes of specific antibodies and it cannot be determined by the testing format used whether the IgG antibody levels found retrospectively were produced due to viral exposure or viral replication in the test population.

Seroconversion from antibody-negative to antibody-positive status has been shown to be related to ocular reaction to NDV (3, 4, 6). The ocular symptoms produced in response to NDV exposure are more characteristic of infection than allergy, as are the 'flu-like' systemic responses, which numerous members of Group I have described as accompanying their exposure to NDV (personal communications).

It was not determined if the same individuals had elevated antibodies to several avian viruses. Correlation of individuals having high anti-NDV titers with individuals having high anti-A-IBV titers would have suggested more strongly whether the antibody levels were due to a common exposure to both viruses, as might have been encountered during the course of chicken handling and flock vaccination procedures. Chicken vaccination is often done with divalent vaccine containing both NDV and A-IBV. The observation that the antibody levels against NDV and A-IBV were similar both at the dilution at which they could be detected and the relative amount of antibody at each dilution suggests a certain commonality of exposure.

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