SEROPREVALENCE OF DIFFERENT INFECTIOUS BRONCHITIS VIRUS STRAINS IN CHICKENS

Muhammad Akram Muneer, Khushi Muhammad, Khalid Munir and Khalid Naeem[1]
College of Veterinary Sciences, Lahore
[1]*Animal Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan*

ABSTRACT

A total of 2185 blood samples obtained from 110 poultry flocks were analyzed for antibodies to various strains of avian infectious bronchitis virus (IBV). These samples were obtained from commercial broiler, broiler-breeder, commercial layer and layer breeder flocks located in various geographical regions of Pakistan. The flock histories in terms of production performance, growth patterns and IB disease prevalence, vaccines and vaccination programs were recorded. Samples of blood from locally bred desi (native) birds and pheasants were also analyzed. These samples were primarily collected from flocks which had experienced low production, misshaped eggs and mortality in the near past. All the serum samples were analyzed using haemagglutination-inhibition (HI) test. This study indicated the presence of antibodies to various IBV types such as Arkansas, Massachusetts-41 (M-41), JMK, D-274 and D-1466. It was further observed that the breeder and commercial flocks which did not receive any killed or live IBV vaccination had significant titres of humoral HI antibodies indicating that various field IBV types were circulating in those flocks. The presence of IBV antibodies in unvaccinated flocks was higher in areas where poultry farms were located in clusters or where multiple age groups were housed.

INTRODUCTION

Avian infectious bronchitis (IB) continues to be an economically important disease of chicken despite the use of vaccines. First described in the United states in 1930, the IB virus (IBV) infections have been identified all over the world including Pakistan (Muneer *et al.*, 1987). IBV may cause pathology in respiratory (Hassanin *et al.*, 1995) and reproductive tracts and in the kidneys of affected birds (Kinde *et al.*, 1991). The IBV may be associated with mortality in young chicks, marked drop in egg production, laying of soft, misshaped and poor quality eggs for long periods and incomplete recovery to the rate of laying to pre-infection levels. In addition, various IBV types are the cause of poor weight gains and feed conversions in broilers. Early work by Jungherr *et al* (1956) suggested that more than one antigenic types of IBV existed. Since then, over 25 IBV stereotypes have been identified in various poultry producing countries of the world.

Live attenuated and killed IBV vaccines have been used prophylactically for controlling IBV infection in various types of chickens. Outbreaks of IB in vaccinated and unvaccinated flocks have been attributed to multiple types or strains of the IBV, emergence of new antigenically distinct strains (Gelb *et al*, 1991), and poor correlation of humoral neutralizing antibody titers (Wit *et al.*, 1997) to protection against reinfection with virulent IBV. Inspite of the fact that neutralizing antibodies persist for quite long periods, immunization of chickens with one antigenic type of IBV may induce partial or no protection against heterologous virus type. In addition, the mutagenic nature (Wang and Tsai, 1996) of IBV is considered as an important cause of antigenic variation and failure of vaccines and vaccination programs. It is necessary to isolate and characterize the IBV strains in any area where this disease is not adequately controlled. Monitoring of immune status of the vaccinated flocks for protection against homologous IBV and for the emergence of new serotypes or variant IBV viruses is also needed. The objective of the present study was to investigate seroprevalence of various IBV types in different areas of Pakistan and to suggest an IB control strategy in those areas.

MATERIALS AND METHODS

Collection and Preparation of Samples

Over a period of one year, a total of 2185 blood samples were collected from different groups consisting of vaccinated and unvaccinated birds (Table 2) with or without the history of ailment, similar to IBV. Serum samples collected were stored at -20°C till analyzed.

Haemagglutination-Inhibition (HI) Test

The HI tests were conducted in 96-well U-bottom microtitre plates according to the procedure described by Alexander and Chettle (1977). Briefly, in each of the

row A wells 1:2 dilution of each test serum was prepared using PBS. All the other rows had 25µl of PBS. Two fold dilutions of the unknown serum sample placed in row A-wells was prepared with well No. 10 in each column. To well No. 1 to 11, 8 HA of IBV in 25µl quantity were added and incubated for 30 minutes at room temperature (20-25°C). To each well, 50µl of 0.5% chicken RBCs were added. After one hr incubation at 20-25°C, the results were recorded. Known negative and positive IBV-antisera obtained from SPAFAS, USA were used as controls.

RESULTS AND DISCUSSION

IBV Antibodies in Commercial Broilers

A total of 404 blood samples tested in this group were collected from 20 flocks of commercial broilers of various age groups varying between days 14 to 56. All those flocks had different history of disease incidence and vaccination programs. The major problems observed at those farms were poor body growth and poor feed conversion ratios, respiratory distress and mortality. Most of the farms had housed multiple age chickens.

Of the 20 broiler flocks, 13 were not vaccinated with any IBV vaccine, whereas 7 flocks had received live IBV H-120 vaccine during first week of their brooding.

The details of HI antibody titers against IBV among various groups are presented in Table-2. The results showed high levels of antibody titers in all the flocks, whether vaccinated or not. Birds in group A to C showed distinct clinical signs relating to IBV infection, which appears to confirm the occurrence of IBV infection. Furthermore, groups B and C also showed increased levels of antibodies against IBV variants, particularly antibody titres against D- 1466 variants attributes to the field exposure of this variant in the past. However, titres in groups A & D against variants were very low, which could be due to the shared antigens among IBV serotypes (Table-2). These findings confirm earlier reports of seroprevalance of M-41 and its isolation in Pakistan (Ahmad *et al.*, 1985 and Muneer *et al.*, 1987).

IBV infection in commercial layers

A total of 20 commercial layer flocks were investigated in various parts of the country. The HI results presented in Table-3 indicate that the problem was present in both vaccinated and unvaccinated layers. The flocks (A to B) had broad variation in its antibody titre range. Out of this 4 vaccinated flocks showed poor quality of eggs, such as soft egg shells, misshaped eggs & inferior inner contents. This when related with high serum titres against IB reflects field exposure with M-41 strain. In an earlier study a positive correlation between poor egg quality & increased antibody titres against IBV have been reported (Muneer *et al.*, 1986). Data in Table-3 also indicates high level of serum antibodies against two IBV variants, i.e., D-27 and D-1466 among IBV H-120 vaccinated and unvaccinated flocks indicating field exposure of birds to these variants.

The birds among some flocks in group B also showed high antibody titers against all the 3 IBV serotypes among various flocks. However, the level of titres were relatively higher against M-41 & D-274 indicating chances of field exposure against these two serotypes. Gelb *et al.* (1991) have also reported on the variant/serotypes of IBV isolated from the commercial layer and broiler chickens.

The overall findings of the serum analysis of commercial layers indicate that M-41 serotype of IBV is present in high quantity in vaccinated and unvaccinated flocks. On the other hand D-274 and D-1466 IBV antibody levels were high in 2 flocks among vaccinated birds whereas antibody titres against those viruses were also significantly high in 2 unvaccinated flocks. Role of IBV variant infections in both H-120 virus vaccinated and unvaccinated flocks needs to be determined and a correlation of serum antibody against IBV variants and egg production performance needs to be developed.

Status of IBV in layer breeder flocks

Only 4 breeder flocks in the districts of Sahiwal & Lahore were examined. All those flocks were vaccinated against IBV H-120 live and killed vaccines. The birds showed quite satisfactory rise in antibody titres against the IBV vaccines. Sero-analyses further indicated that the birds in 3 out of the 4 flocks had negligible antibody titers against D-274 and D-1466. The birds in flock 4 in group C (Table 3) had high titers against D-274 but very low titers against D-1466. As all these flocks were vaccinated against M-41 only, so the presence of low titers against the variants could either be a reflection of cross reactive antigens among the M-41 and variants or the titres earlier attained during any infection may have declined.

status of IBV in broiler-breeder flocks

A total of 1427 serum samples obtained from birds of different age-groups housed in areas of Abbotabad, Mansehra, Rawalpindi and Murree in the Northern Mountaneous Region and Gujranwala, Arifwala and Lahore in the central Punjab were evaluated. All those flocks had received IBV vaccination (both live H-120 and oil based) at different time intervals during rearing and production. Those flocks had mostly experienced respiratory disease and necropsy examination of dead and those live birds killed indicated effects on nasal sinuses, trachea, lungs, air sacs, liver and kidneys. The laying flocks had history of low egg production and laying of poor quality deformed and soft shell eggs

having watery albumen. Mortality in those affected laying flocks was quite negligible.

Among the flocks examined at Abbottabad (Table 4), some had shown increased antibody levels against M-41, D-274 and D-1466 IBV strains. The overall GMT against IBV in these cases remained 40-90 against M-41, 35-113 against D-274 and 139-146 against D-1466. The remaining 19 flocks examined at Mansehra had high GMT antibody titres against M-41 ranging between 130-982 whereas low GMT titres (13-130) were recorded against D-274. Here only 2 flocks had titres more than 37. On the other hand GMT HI titres against D-1466 ranged between 2-146 and only the 2 flocks had high titres against this serotype. The data indicates that in most of the cases the high antibody titres against M-41 were primarily due to repeated vaccines whereas high titres against D-274 and D-1466 in a few cases could be attributed to infection of the flocks with these strains/variants from the field.

The samples received from Rawalpindi - Murree area belonged to seven flocks (Table 4). Out of it only 3 flocks showed good antibody titres against M-41 serotype (> 100). The other serotypes were tested in only 3 flocks, which had low titres against D-274 but high titres against D-1466. Here results indicate exposure of variant strains of IBV in these flocks at some stage of life. Unless proper biosecurity & flock sero-monitoring is continued there is a danger of spread of the variants at a greater scale. The four flocks examined at Gujranwala had high antibody titres against M-41 and low titres against D-274 (titre 24-161) and JMK (titre 6-20). However, in one flock higher titres against D-1466 were recorded. Samples from Arifwala also showed very low antibody titres (4-16) against the D-274, D-1466 (2-8) and JMK (4-8) but good titres against M-41 (24-130).

Among the flocks examined at Lahore, 23 showed low antibody titres against IBV D-274 strain (Table 4). However low titres in other flocks may be due to sharing of common antigenic determinants with other types of IBV used in different vaccines. Out of 25 flocks, 4 had significantly high range of HI antibody titres against D-1466 type (235-342) while other flocks had titres below 57, being non-significant.

As all the flocks were earlier vaccinated against M-41 serotype so interpretation of those results was not attempted.

IBV antibodies in desi chickens and pheasants

The samples collected from desi-chickens at 4 different areas were tested (Table 5). None of those birds were previously vaccinated against IBV. The seroanalysis of samples from Mansehra and Gujranwala detected no titres against any serotype of IBV. However, samples collected from Sialkot and Jehlum showed significant rise in antibody titres against M-41 & D-274. The presence of antibody titres against IBV is a reflection of field exposure.

As regards pheasant serology against IBV, high HI antibody titers against M-41 IBV were detectable but non-significant seroconversion against D-274, D-1466 and ArKansas virus were detectable (Table-6). It is apparent from the results that those birds had field exposures to M-41 serotype. Findings in various flocks indicate that high antibody to IBV strains of M-41, D-274 and D-1466 was present in vaccinated and unvaccinated flocks and some of these flocks also had the history of low egg production, poor egg quality and decreased hatchability despite the use of live and killed IBV vaccines. This indicates that some other variant strains of IBV may be prevalent in the poultry flocks of Pakistan. However, this study did not include their testing. It is therefore, necessary to establish strong flock seromonitoring program at each farm both for determining the efficacy of vaccines and also for revision of the existing vaccination programs. Since there are over 25 known serotypes of IBV and new variants are continuously emerging, there is a need to launch nation wide studies to undertake serotyping and virus isolations for selecting, those vaccine strains which may be more useful than the serotypes presently used as vaccines. Isolation and characterization of new IBV types for effective broad spectrum IBV vaccine is needed to control the growth and egg production losses to farmers (Muneer *et al.*,1986).

Table 1: Area wise distribution of flocks investigated for IBV antibodies

Type of birds	Location	Flocks examined	Blood samples investigated
Commercial Broiler	Faisalabad, Lahore, Gujranwala, Kasur	20	404
Commercial Layers	Lahore, Gujranwals, Sahiwal, Sialkot, Kasur	20	188
Layers breeder	Sahiwal, Lahore	4	68
Broiler breeders	Lahore,Mansehra,Arifwala, Abbottabad, Gujranwala, Ralwalpindi, Murree	60	1427
Desi hen	Gujranwala, Jehlum, Sialkot, Mansehra	4	72
Pheasant	Mansehra	2	26
Total		110	2185

Table 2: Flock history and IBV antibody titres in commercial broilers

Group/flocks	Serum samples	Clinical history related to IB	IBV Vaccination status	GMT Range of IBV antibody titres against		
				M-41	D-274	D-1466
A(6)	105	Mortality & Poor growth	Nil	511-755	10	10 (105)
B(5)	134	Mortality & Poor growth	Yes [a](H-120)	17-578	24-61	36(123)
C(7)	125	Respiratory problems	Nil	41-688	5-61	6(123)
D(2)	40	No Clinical history	Yes(H-120)	128-642	10	16

A IBV H/120 vaccine at day 2-5 of age.

Table 3: IBV antibody titres of commercial layers and layer breeder flocks

Flock/ group	Species	Samples/Flock	IBV Vaccination	GMT IBV antibody titres against		
				M-41	D-274	D-1466
A	Layers	77/4	H-120 live	47-736	7-235	6-437
B	Layers	111/16	NIL	99-589	7-256	8-148
C *	Layer breeder	68/4	H-120 live	89-467	6-92	5-21

A None of the flocks indicated history of egg production or egg quality drops.

Table 4: IBV-HI Antibody titres in broiler breeder flocks

Group	Sampling area	No. of Samples/Flock	Clinical history related to IB	IB Vaccination status	Range of GMT IBV antibody titre			
					M-41	D-274	D-1466	JMK
A	Abbottabad & Mansehra	487/22	Low egg production	+	40-982	13-130	2-343	-
B	Rawalpindi & Murree	230/7	Respiratory signs + low egg production	+	65-260	35-57	80-106	-
C	Gujranwala	94/4	Respiratory signs + low egg production	+	172-406	24-112	18-45	6-20
D	Lahore	577/25	Low egg production	+	45-724	02-161	04-342	-
E	Arifwala	39/2	Poor egg quality	+	24-130	4-16	2-8	4-8

All the flock were vaccinated 2-3 times with IBV H-120 live vaccine upto 15th week and an oil-based shot at 18-20 week of age; M41: Masachusetts strain; D=2747 and D 1466 = Dutch variants of IBV JMK+ Jamican Strain

Table 5: IBV serum HI antibody titres of desi (native) chickens and pheasants of various locations

Flock Group	Sampling area	No. of Samples	IB Antibody Titres to			
			M-41	D-274	D-1466	ARK
A*	Sialkot	16	128-1024	64-1024	NT	NT[a]
B*	Jehlum	34	0-1024	0-256	NT	NT
C*	Mansehra	11	4	0	4	NT
D*	Gujranwala	11	4	4	4	NT
E**	Mansehra	26	16-512	0-64	2-8	2-16

* = Desi Chickens; ** = Pheasants; NT= Not tested

REFERENCES

Ahmed, J., M. Ashfaque, M.D. Ahmad, 1985. Replication and cytogenicity of IBV in Poultry. Pakistan Vet. J., 5: 107-109.

Alexander, D.J., and N.J. Chettle, 1977. Procedures for the hemagglutination and the hemagglutination-inhibition tests for avian infectiious bronchitis virus. Avian Path., 6: 9-17.

Gelb, J. Jr., J.B. Wolf and C.A. Moran, 1991. Variant serotypes of infectious bronchitis virus isolated from commercial layer and broiler chickens. Avian Dis., 35(1): 82-87.

Hassanin, H.H., A. Ibrahim, A. El-Attar and M.Z. Eldimerdash, 1995. Seroepidemiological studies on some respiratory viruses in broilers. Assiut Vet. Med. J., 33(65): 217-22.

Jungherr, E.L., T.W. Chomiak, and R.E. Luginbuhl, 1956. Immunologic differences in strains of infections bronchitis virus. Proceeding of 6th Annual Meeting of US Livestock Sanitary assoc., Chicago, USA.

Kinde, H., B.M. Doft, A.E. Castro, A.A. Bickford, J. Gelb-Jr. and B. Reynolds, 1991. Viral pathogenesis of a nephrotopic infectious bronchitis virus isolated from commercial pullets. Avian Dis., 35(2): 415-21.

Muneer, M.A., D.A. Halverson, J.A. Newman, and C.N. Coon, 1986. Effects of infectious bronchitis virus on laying chickens. Avian Dis., 30: 644-647.

Muneer, M.A, J.A. Newman, S.M. goyal and M. Ajmal, 1987. Antibodies to avian infectious bronchitis in Pakistani Chickens. Poult. Sci., 66: 765-767.

Wang, C.H. and C.T. Tsai, 1996. Genetic grouping for the isolates of avian infectious bronchitis virus in Taiwan. Archives of virology, 141(9): 1677-1688.

Wit, J.J., D.R. de-Makkes, B.Kouwenhoven and J.H.M. Verheyden, 1997. Sensitivity & specificity of serological tests for detection of infectious bronchitis virus induced antibodies on broilers. Avian Path., 26: 105-118.