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DETECTION OF ANTIBODIES AGAINST AVIAN PNEUMOVIRUS IN BROILER BREEDER FLOCKS IN PAKISTAN

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ABSTRACT

A total of 282 serum samples were collected from 10 flocks, representing eight poultry breeding companies. These birds were reared in Punjab and North West Frontier Province of Pakistan. The antibodies against Turkey Rhinotracheitis virus/Swollen Head Syndrome (TRT/SHS) were detected by using a commercial kit based on Blocking Enzyme Immuno-Assay. Out of 282 samples tested for TRT/SHS antibodies, 53 were positive, 28 were in grey-zone and the remaining 201 samples were declared non-reactive. On the basis of this study it is evident that the poultry industry of Pakistan is at risk of avian pneumovirus (TRT/SHS) infection. It would be advisable to protect the valuable poultry flocks using TRT/SHS vaccine.

Key words: Turkey Rhinotracheitis virus, avian pneumovirus, Swollen Head Syndrome, antibody detection.

INTRODUCTION

Avian pneumovirus is the etiological agent of Turkey Rhinotracheitis (TRT) in turkey and Swollen Head Syndrome (SHS) in chicken (Wyeth *et al*., 1986; Seal, 2000). Turkey Rhinotracheitis, a characteristic respiratory disease of turkeys, was first observed in South Africa in 1978 (Buys *et al*., 1980) and subsequently, from various other parts of the world (McDougall and Cook, 1986; Wilding *et al*., 1986; Cook *et al*., 1988; Decanini *et al*., 1991). The important clinical signs are swelling of the peri and infraorbital sinuses, torticollis, opisthotonus and incoordination. Affected birds are reluctant to move and usually die as a result of their inability to feed. A decrease in egg production also occurs in most of affected flocks (Wyeth *et al*., 1987). The disease has a high morbidity and might also have a high mortality. *Escherichia coli* has been consistently isolated from skin lesions of the head (Morley and Thomson, 1984; O'Brien, 1985; Pattison *et al*., 1989). Therefore, *E. coli* is thought to be a secondary complicating factor (Alexander *et al*., 1986; Naylor and Jones, 1993).

Many researchers have developed an enzyme-linked immunosorbant assay (ELISA) to detect antibodies to TRT virus (Wyeth *et al*., 1986). Where the virus isolation has not been done as a causative agent, seroconversion of birds helps in diagnosing the disease (Grant *et al*., 1987). The present study was conducted as a pilot project to determine the seroconversion of broiler parent flocks following exposure to TRT/SHS infection in Pakistan by using an enzyme linked immunosorbant assay (ELISA).

MATERIALS AND METHODS

In the present study, a total of 282 samples were collected from 10 flocks (A to J), representing eight poultry breeding companies reared in Punjab and North Western Frontier Province of Pakistan. Blood samples were collected from these birds and divided into ten groups. Information regarding age of the birds, vaccination history and clinical history was collected. TRT/SHS antibodies were detected by using a commercial kit (Svanova Biotech, Rhone Merieux) based on Blocking Enzyme Immuno-Assay (Blocking-EIA).

TRT antigen coated plates were obtained. One positive control and one negative control serum (100 µl of each) were added on each microtitre plate. Fifty microlitres of PBS-Tween was added to each well. Then 50 µl of undiluted blood serum was inoculated to these wells. Plates were sealed and incubated at room temperature (18 to 25°C) for 30 minutes. After incubation, plates were washed three times with PBS-Tween buffer and 100 µl of horseradish peroxidase conjugated anti-TRT monoclonal antibodies were added to each well and mixed thoroughly. The plates were incubated at room temperature for 30 minutes and washed 3 times with PBS- Tween buffer. Then 100 µl

of substrate solution was delivered to each well and the plates were incubated for 10 minutes. The reaction was stopped by the addition of 50 µl of the stop solution to each well. The optical density of negative reference serum, positive reference serum and each sample was then measured at 450 nm on an ELISA reader. An empty microtitre plate was used as a blank. Results were interpreted on the basis of difference in values of optical density percentage (ODp) of different sera samples and positive reference sera (the positive reference sera should have an ODp less than 50). Following formula was applied to calculate the optical density percentage for each sample and for positive reference sera:

$$OD_P = \frac{\text{Sample OD}}{\text{Negative reference OD}} \times 100$$

Before testing sera for TRT antibodies it was essential to establish the ODs from negative sera. To ensure validity, the negative reference should have an optical density value (OD) between 0.9 and 1.4. A negative result was indicated by a colour change. As per literature provided by the manufacturer, samples with ODp less than 75 were categorized as positive, those with ODp between 75-85 were considered in grey-zone, i.e. they were neither fully positive nor fully negative. Samples having ODp more than 85 were recognized as negative.

RESULTS

Out of 282 samples tested for presence of TRT/SHS antibodies, 53(18.79%) samples were clearly positive and showed antibodies against TRT/SHS antigen. Twenty-eight samples (9.93%) were in grey-zone and remaining 201(71.28%) samples were serologically negative for TRT/SHS (Table 1).

In flock "A" serum samples of 28 birds were obtained at the age of 50 weeks. Five birds showed positive reaction when tested for TRT/SHS antibodies, three birds showed grey-zone reaction and 20 were negative for TRT/SHS. In flock B, serum samples of 28 birds at the age of 34 weeks were taken. Three birds were positive for TRT/SHS antibodies, one was in grey-zone and 24 were negative. Flock C also comprised of 28 birds in age group of 34 weeks. In this flock four birds were positive, five were considered in grey-zone and 19 were negative. In flock D, comprising of 28 birds at the age of 34 weeks, eight birds showed positive reaction, two were in grey-zone and 18 were negative. In flock E having same number of birds at the age of 38 weeks, five showed positive results, three were in grey-zone and 20 were non reactive. In flock F comprising 28 birds at the age of 44 weeks, five birds were positive, two were considered as in grey-zone and 21 were negative. Flock G was also comprised of 28 birds at the age of 34 weeks, out of these, eight birds were positive, three were in grey-zone and 17 were negative. Flock H with 28 birds at the age 44 weeks, showed five positive, 19 negative and four in grey-zone. Flock I comprising of 29 birds at the age of 50 weeks, out of which six birds showed positive reaction for TRT/SHS, two were in grey-zone and 21 were negative. Flock J comprising of 29 birds in age group of 50 weeks, showed four birds as positive, three in grey-zone and 22 as negative.

DISCUSSION

This study was conducted by using an ELISA kit, which is commercially available. In Poland, Minta *et al*. (1995) also used ELISA to detect seroprevalence to avian pneumovirus in sera collected from 39 broiler breeder flocks aged 12-96 weeks, 56.4% of broiler breeder flocks were positive. Similarly, Lu *et al*. (1994)

Table 1: Presence of antibodies against TRT/SHS in broiler breeder birds reared at different breeder farms in Pakistan

S.No.	Flock	No. of birds	Age (weeks)	Positive (<75 ODp)	Grey-zone (75-85 ODp)	Negative (>85 ODp)
1	A	28	50	5	3	20
2	B	28	34	3	1	24
3	C	28	34	4	5	19
4	D	28	34	8	2	18
5	E	28	38	5	3	20
6	F	28	44	5	2	21
7	G	28	34	8	3	17
8	H	28	44	5	4	19
9	I	29	50	6	2	21
10	J	29	50	4	3	22
Total	8	282	--	53	28	201

ODp = Optical Density Percentage

conducted a serological survey to detect TRT/SHS antibodies in broiler breeder farms in Taiwan and found 398 birds positive for serum antibodies.

In this study, birds showed no clinical signs of TRT but titre of antibodies against TRT/SHS according to ELISA results were clearly positive in 18.79% samples and 71.28% showed strong negative but 9.93% samples gave doubtfull results i.e. in grey-zone. Pollan *et al*. (1992) also used similar ELISA test to examine 16 flocks in which 14 had birds with respiratory signs and 2 without any clinical sings. According to their results, clinically healthy and newly infected flocks had no antibodies against TRT; within 5 days low titres appeared and maximum levels were attained after 10 to 13 days. Grant *et al*. (1987) also recommended this test for the serodiagnosis and is being used to evaluate large number of samples.

Like the study under discussion, Maharaj *et al*. (1994) also conducted an ELISA to determine the antibody status of TRT in two adult broiler breeder flocks just before the onset of clinical sings, but failed to find any positive result, while samples taken 14 and 21 days after infection showed a significant rise in antibodies against TRT virus. Bell and Alexander (1990) also conducted an ELISA test to detect antibodies against TRT virus in 639 serum samples collected from 38 flocks. In contrast to our findings, they found all samples negative for TRT antibodies. Similar to our study, Mona *et al*. (1997) recorded high seroprevalence in 14 flocks with ELISA-positive birds using two commercial kits i.e. Pathasure (British strain of TRT) and Svanova (French strain of TRT).

The present study was an initiative to record the serological presence of TRT virus in local area. The results revealed that 18.79% birds were serologically positive for TRT/SHS. Tanaka *et al*. (1996) also conducted a serological survey to detect Turkey Rhinotracheitis virus infection in chicken in Japan and concluded that the TRT virus started to infect chicken in Japan before 1988 and was widespread thereafter. Although the number of strongly positive sera detected in this study is very small but it is sufficient to indicate that the birds are at risk to TRT infection. Moreover, the sera found in grey-zone might have had an infection of TRT in the past. The antibodies level thus produced might have increased and ELISA antibodies titers dropped to such an extent that it could not completely block the reaction with conjugated antibodies. So the results of those samples, which were in grey-zone, are doubtful. They might have had exposure with TRT virus.

On the basis of these results it is concluded that avian pneumovirus is present in Pakistan. As the vaccination against TRT/SHS infection is not routinely done in our region, commercial breeder farmers and broiler farmers are advised to vaccinate their birds with TRT/SHS vaccine to avoid infection and losses. It is also concluded that more work is required to isolate and characterizer TRT virus, and to see role of other factors e.g. *E. coli* infection in the causation of this disease.

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