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Serological Survey of Avian Metapneumovirus Infection in Broiler Breeder Chicken Farms in Tamil Nadu

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Abstract Avian metapneumovirus (aMPV) is an important poultry pathogen causing an acute highly contagious upper respiratory tract infection in chickens leading to swollen head syndrome. The disease can cause significant economic losses in turkey and chicken flocks, particularly when exacerbated by secondary pathogens. The purpose of this study was to determine the prevalence of avian metapneumovirus antibodies in broiler breeder flocks in Tamil Nadu, India. Twenty numbers of broiler breeder farms located in Tirupur district of Tamil Nadu were selected randomly and blood samples were collected. A total of 485 blood samples were collected from 20 broiler breeder chicken flocks (aged between 4 and 72 weeks). The serum samples were tested for the presence of antibodies against avian metapneumovirus by using a commercial enzyme-linked immunosorbent assay kit (IDEXX APV Ab test, Liebefeld-Bern, Switzerland) which was able to determine antibodies against A, B and C subtypes of avian metapneumovirus. Out of 485 serum samples, 165 (34.02%) were positive to avian metapneumovirus antibodies, which represented 14 of 20 (70%) examined broiler breeder flocks. All the chickens had not been vaccinated against avian metapneumovirus in India and these results indicate that commercial poultry birds are exposed to this important poultry pathogen. This is the first report of serologic evidence of AMPV in India. Its prevalence has to be investigated in other parts of India. Future work may and should include the use of molecular methods and isolation of the virus. Isolation of avian metapneumovirus will allow the possibility of controlling the disease.
Keywords *ELISA; Seroprevalence; Swollen Head Syndrome*

1. Introduction

Avian metapneumovirus (aMPV) is recognized as an important pathogen in many commercially poultry producing countries. It belongs to the family *Paramyxoviridae*, subfamily *Pneumovirinae*, genera *Metapneumovirus*, and has an enveloped unsegmented single-stranded negative sense RNA

[1]. aMPV causes Turkey Rhinotracheitis (TRT) in turkeys and Swollen Head Syndrome (SHS) in chickens. aMPV can cause damage to the upper respiratory tract (trachea), such as, lack of cilia movement and/or cilia loss—damage that may lead to respiratory clinical signs such as nasal discharge, coughing, sneezing and more complicated respiratory problems. This stress on the cilia and upper respiratory tract can facilitate the multiplication of *E. coli* and other bacterial infections such as *Mycoplasmas, Pasteurella, Bordetella sp* and *Ornithobacterium rhinotracheale*, etc. that lead to a respiratory syndrome called swollen head syndrome [2]. aMPV plays a role in the multiplication of infectious bronchitis virus (IBV) in the upper respiratory tract. aMPV can also affect the reproductive tract, impacting egg formation in turkey breeders, broiler breeders and egg-type chickens, resulting in an increase in the percentage of egg abnormalities and a drop in egg production [3, 4]. aMPV was first detected in turkeys in South Africa in the late 1970s [5]. Later it was reported in chickens in South Africa [6]. By 1993, Alexander described TRT in Israel, France and Great Britain. Serological evidence of aMPV is now available from many countries of the world [7, 3]. To our knowledge, there is no published information on aMPV in India. ELISA is the most common serological way of diagnosing aMPV infection in chicken and turkeys [3]. The present study was conducted to find out the status of aMPV infection in broiler breeder chickens in India by ELISA test.

2. Materials and Methods

2.1. Seroprevalence Survey

This serological survey was conducted as part of post graduate research by the department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary Animal Sciences University, India, to elucidate the incidence of aMPV in chickens.

2.2. Chicken Flocks and Serum Samples

A total of 485 blood samples were collected randomly from 20 broiler breeder chicken flocks aged between 4 and 72 weeks. Chicken serum was extracted by centrifugation at 1,500 × g for 10 min at 4°C and kept at −20°C before use.

2.3. ELISA

Chicken serum samples were tested individually using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of antibody against avian metapneumovirus (Avian Pneumovirus Antibody Test Kit, IDEXX Laboratories, Liebefeld-Bern, Switzerland). Positive and negative control sera were included for each test. Absorbance (ABS) was measured and recorded using an ELISA reader (Bio Rad, Japan) at 650 nm. Based on the instruction manual of the ELISA kits, serum samples with Sample to Positive (S/P) ratios of less than or equal to 0.20 should be considered negative. S/P ratios greater than 0.20 (titres larger than 396) should be considered positive and indicate exposure to aMPV.

$$S/P = \frac{\text{Sample ABS} - \text{Negative Control ABS}}{\text{Positive Control ABS} - \text{Negative Control ABS}}$$

3. Results

The results of present study showed 14 flocks (70%) were infected by aMPV infection. Totally 165 blood sera samples (34.02%) out of 485 blood sera samples collected from 20 flocks, have different

level of specific antibody titer against aMPV infection. Mean titer of positive samples were 2553±150.62 (Mean±SE) and negative samples mean were 99.40±7.75 (Mean±SE).

4. Discussion

The present study was an initiative to record the serological presence of aMPV in India. This was conducted by using an ELISA kit, which is commercially available. The results revealed that 34.02% of the tested birds were serologically positive for SHS. A higher rate of seropositivity for aMPV in chickens observed in India is in agreement with previous reports from other part of the world [8, 9]. The aMPV started to infect chicken in Japan before 1988 and was widespread thereafter [10].

In Poland, Minta *et al.* [11] 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. In this study, birds showed no clinical signs of SHS at time of blood collection, but titre of antibodies against SHS according to ELISA results were clearly positive in 34.02% samples. This is in agreement with previous reports from other parts of the world. aMPV has been isolated from chicken flocks without clinical signs [12] and chicken flocks free of clinical signs may have antibodies for aMPV [13, 14, 15]. In broilers, aMPV has been associated with clinical signs of swollen head syndrome. Layers infected with aMPV in the early phase of lay do not reach peak production, whereas layers in their late phase of lay suffer a drop in egg production [16, 3]. A positive detection of antibodies confirmed that the birds were exposure to the aMPV, but a negative result does not rule out the exposure. The aMPV infected chickens may not necessarily produce humoral antibodies, or antibodies may be at very low levels at the time of the sampling [17].

Chickens are not vaccinated for aMPV in India and the seropositivity for aMPV indicates that commercial chickens in India are exposed to this important poultry pathogen. aMPV may cause serious economic losses in turkeys and chickens especially in the presence of concurrent bacterial and viral infections.

On the basis of these results it is concluded that avian metapneumovirus is present in India. It is also concluded that more work is required to isolate and characterize AMP virus. Results of such a study would be strategic for institution of preventive measures against aMPV infection.

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