Seroprevalence of avian metapneumovirus infection in broiler and broiler breeder chickens in Iran

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ABSTRACT: Avian metapneumovirus causes an acute highly contagious upper respiratory tract infection primarily of turkeys and chickens. 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 and broiler breeder flocks in Kermanshah province, west of Iran. All the flocks had not been vaccinated against avian metapneumovirus. The province were divided into four geographic areas; southwest, southeast, northwest, and northeast. Flocks in each area, and 14–15 birds in each flock, were randomly sampled. The blood samples were taken regardless of the presence of any signs of respiratory or any other clinical disease in the flocks. A total of 435 blood samples were collected from 30 commercial chicken flocks (24 broiler flocks, aged between six and eight weeks, and six broiler breeder flocks, aged between 56 and 72 weeks). The presence of antibodies against avian metapneumovirus in each serum sample was tested twice by enzyme-linked immunosorbent assay using a commercial kit which was able to determine antibodies against A, B and C subtypes of avian metapneumovirus. Out of 347 serum samples obtained from broiler chickens, 167 (48.1%) were positive to avian metapneumovirus antibodies, which represented 20 (83.3%) of 24 examined broiler flocks. Out of 88 samples obtained from broiler breeder chickens, 82 (93.2%) were positive to avian metapneumovirus antibodies, which belonged to six (100%) of examined broiler breeder flocks. Detection of anti-avian metapneumovirus antibodies among broiler breeder (100%) was higher than broiler (83.3%) flocks. A higher rate of seropositivity (83.3% of samples and 100% of broiler flocks) was observed in northwest. The results of this study may indicate the possible involvement of avian metapneumovirus in the respiratory disease we are seeing in chickens in Iran. Its prevalence has to be investigated in other parts of Iran. 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 making autogenous vaccines.

Keywords: avian metapneumovirus; seroprevalence; avian pneumovirus; turkey rhinotracheitis; chicken

Avian metapneumovirus (aMPV), previously referred to as avian pneumovirus (APV) and avian rhinotracheitis (ART) virus, causes an acute, highly contagious upper respiratory tract infection of turkeys and chickens (Pedersen and Gough, 2009). It was first reported in South Africa in the late 1970s (Buys and Du Preez, 1980), initially in turkeys and later in chickens (Buys et al., 1989). The infection can cause significant economic losses in turkey and chicken flocks, particularly when exacerbated by secondary pathogens (Pedersen and Gough, 2009).

The etiological agent is an enveloped virus with an unsegmented single-stranded negative-sense RNA virus with a helical symmetry (Gough, 2003). The virus exhibits some characteristics of a pneumovirus, but differs from mammalian pneumoviruses at the molecular level and has recently been classified as the type strain of a new genus, *Metapneumovirus,* in the family *Paramyxoviridae* (Pedersen et al., 2000). The avian metapneumovirus has been classified into four subtypes: A, B, C and D based on nucleotide sequence analysis. Using monoclonal antibodies limited cross reactivity between subtypes has been observed in enzyme-linked immunosorbent assay (ELISA) and neutralization test (Collins et al., 1993; Cook et al., 1993; Toquin et al., 2000).

Serological evidence suggests aMPV is widespread throughout the world and of considerable economic importance, particularly in turkeys. Oceania and Canada are the only regions that have not reported aMPV (Cook, 2000; Gough, 2003). There is serological and molecular evidence that aMPV occurs in a variety of other avian species, including pheasants, guinea fowl, ostriches, passerines and various waterfowl (Shin et al., 2002; Gough, 2003; Bennett et al., 2004; Lee et al., 2007), but there is no evidence of disease (Pedersen and Gough, 2009).

In turkeys, the virus causes a disease known as turkey rhinotracheitis (TRT). Infection with aMPV can occur from a very young age in turkeys and is characterized by snicking, rales, sneezing, nasal discharge, foaming conjunctivitis, swelling of the infraorbital sinuses and submandibular edema (Pringle, 1998). Secondary adventitious agents can dramatically exacerbate the clinical signs. In an uncomplicated infection, recovery is rapid and the birds appear normal in approximately 14 days. When husbandry is poor or secondary bacterial infection occurs, airsacculitis, pericarditis, pneumonia, and perihepatitis may prolong the disease and there may be an increase in morbidity and mortality (Cook et al., 1991; Mekkes and De Wit, 1999; Marien et al., 2005).

Clinical signs of infection in chickens are less characteristic than those in turkeys. Severe respiratory distress may occur in broiler chickens particularly when exacerbated by secondary pathogens such as infectious bronchitis virus, mycoplasmas, and *Escherichia coli* (O'Brien 1985; Pattison et al., 1989).

In chickens, there is strong evidence to suggest aMPV is one of the etiological agents of swollen head syndrome (SHS). SHS is reported in both broilers and broiler breeders, and in breeders the evidence for a primary role of aMPV as one of the etiological agents of SHS is stronger. However, aMPV is not the only agent associated with SHS (Cook, 2000). The syndrome is characterized by respiratory disease, apathy, swelling of infraborbital sinuses and unilateral or bilateral periorbital and facial swelling, extending over the head. These signs are frequently followed by cerebral disorientation, torticollis and opisthotonos. Although mortality does not usually exceed 1–2%, morbidity may reach 10%, and egg production is frequently affected (Morley and Thomson, 1984; O'Brien, 1985, Picault et al., 1987; Pattison et al., 1989; Gough et al., 1994; Tanaka et al., 1995).

In broilers, aMPV may not be a primary pathogen, but rather be involved with other agents in SHS or in another respiratory disease complex. The benefit derived from using aMPV vaccines in broilers provides strong circumstantial evidence for the importance of aMPV in disease in broilers (Cook, 2000).

Clinical signs are not pathognomonic for a diagnosis of aMPV. A diagnosis may be made by either serology, polymerase chain reaction (PCR) or virus isolation (Stuart, 1989; Gough, 2003). Isolation of virus is rarely successful from birds showing severe chronic signs as the extreme clinical signs are usually due to secondary infectious agents. Furthermore, for reasons that are unclear, virus isolation from chickens may be more difficult than from turkeys (Naylor and Jones, 1993). Current serological tests include ELISA, virus neutralization, and immunofluorescence (Stuart, 1989; Gough, 2003).

Serology is the most common method of diagnosis of aMPV infections, particularly in unvaccinated flocks, because of difficulties in isolating and identifying aMPV. The most commonly employed method is the ELISA. A number of commercial and in-house ELISA kits are available that are suitable for testing both turkey and chicken serum. These kits claim to have a broad spectrum of sensitivity and specificity for all subtypes of aMPV and can be used for testing sera from a variety of avian species.

In Iran, the chicken industry is the most developed industry in the animal sector. This industry is composed of broilers, broiler breeders, and layers. The Iranian poultry industry which, with nearly 1.2 million tonnes of output, is the largest in the Middle East is ranked fourth in Asia and 17th in the world (Shariatmadari, 2000). There are serious respiratory diseases in chickens in Iran causing catastrophic economic losses to the chicken industry. Although, serological evidence of aMPV is now available from many countries (Pedersen and Gough, 2009), it has not been reported from Iran. It is believed that aMPV is a part of several multifactorial respiratory diseases in western parts of Iran. The purpose of this study was to determine the prevalence of aMPV antibodies in broiler and broiler breeder flocks in this area.

MATERIAL AND METHODS

In a one year period, from April 2009 until February 2010, a total of 435 blood samples were

collected from 30 commercial chicken flocks (24 broiler flocks, aged between six and eight weeks, and six broiler breeder flocks, aged between 56 and 72 weeks) located in Kermanshah province, west of Iran. All the flocks had not been vaccinated against aMPV, but they had been vaccinated against main respiratory disease, including infectious bronchitis, Newcastle disease, and avian influenza (H9N2). The province were divided into four geographic areas; southwest (SW), southeast (SE), northwest (NW), and northeast (NE). Flocks in each area, and 14–15 birds in each flock, were randomly sampled. The blood samples were taken regardless of the presence of any signs of respiratory or any other clinical disease in the flocks.

The presence of antibodies against aMPV in each serum sample was tested twice by ELISA using a commercial kit (Flock Chek® Avian Pneumovirus, IDEXX, Leibefeld-Bern, Switzerland), which was able to determine antibodies against A, B and C subtypes of aMPV. The test procedure and analysis of results were performed as recommended by manufacturer. Positive and negative control antisera were provided in the kit and used in each run. Absorbance was read at a wave length of 650 nm on an ELx 800® ELISA reader (Bio-Tek, Winoski, VT, USA). The relative level of antibody in the unknown was determined by calculating the sample to positive (S/P) ratio. Serum samples with S/P ratios of greater than 0.2 (titers greater than 396) in repeated tests were considered positive to exposure to aMPV.

RESULTS

The results revealed that 48.1% broiler and 93.2% broiler breeder chickens were serologically positive for aMPV (Table 1). Out of 347 serum samples obtained from broiler chickens, 167 (48.1%) were positive to aMPV antibodies, which represented 20 (83.3%) of 24 examined broiler flocks. A higher rate of seropositivity (83.3% of samples and 100% of broiler flocks) was observed in NW.

Out of 88 samples obtained from broiler breeder chickens, 82 (93.2%) were positive to aMPV antibodies, which belonged to six (100%) of examined broiler breeder flocks. Detection of anti-APV antibodies among broiler (83.3%) was lower than among broiler breeder (100%) flocks.

DISCUSSION

This is the first report on the seroprevalence of aMPV antibodies in broiler and broiler breeder chickens in western parts of Iran. The results showed that 48.1% broiler and 93.2% broiler breeder chickens were serologically positive for aMPV (Table 1). Out of 347 serum samples obtained from broiler chickens, 167 (48.1%) were positive to aMPV antibodies, which represented 20 (83.3%) of 24 examined broiler flocks. Gharaibeh and Algharaibeh (2007) reported that out of 38 chicken flocks in Jordan tested by ELISA, 18 locks (47.4%) were found to have positive antibody titer for aMPV, the positive flocks comprised 21.7,

Table 1. Serological prevalence of avian metapneumovirus antibodies in broiler and broiler breeder chickens in western parts of Iran

Breeding type	Geographic area	Age (weeks)	Number of flocks	Number of samples	Positive samples		Positive flocks	
					n	%	*n*	%
Broiler	SW	6–8	5	75	13	17.3	4	80
Broiler	SE	6–8	8	116	44	37.9	6	75
Broiler	NW	6–8	6	84	70	83.3	6	100
Broiler	NE	6–8	5	72	40	55.6	4	80
Total			24	347	167	48.1	20	83.3
Broiler breeder	NW	56–62	4	60	58	96.7	4	100
Broiler breeder	NW	72	2	28	24	85.7	2	100
Total			6	88	82	93.2	6	100

SW = southwest, SE = southeast, NW = northwest, NE = northeast

75, and 100% of broilers, layers, and broiler breeders, respectively. Goyal et al. (2003) reported that the average seroprevalence rate of aMPV in Minnesota turkeys was 36.3%.

A higher rate of seropositivity (83.3% of samples and 100% of broiler flocks) was observed in NW; this area had the highest concentration of broiler flocks. Goyal et al. (2003) reported a higher rate of seropositivity to aMPV in counties with the highest concentration of turkeys. Out of 88 samples obtained from broiler breeder chickens, 82 (93.2%) were positive to aMPV antibodies, which belonged to six (100%) of examined broiler breeder flocks. In Poland, Minta et al. (1995) 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. Gharaibeh and Algharaibeh (2007) reported that 100% of broiler breeder flocks in Jordan tested by ELISA were found to have positive antibodies to aMPV.

Detection of anti-APV antibodies among broiler (83.3%) was lower than among broiler breeder (100%) flocks. This may be due to the short life span of broiler flocks. The higher prevalence of aMPV in broiler breeder chickens (93.2%) compared with broiler chickens (48.1%) also supports the previous point that the long life span of breeders allows them to develop a stronger immune response detectable by ELISA.

Because all studied flocks were more than four weeks of age and none was vaccinated against aMPV, results of this survey data suggest field exposure of these flocks to aMPV and exclude the possibility that the detected antibodies were due to maternal antibodies or vaccination. This confirms the endemic nature of the disease in Iran. All of the examined broiler flocks were more than four weeks of age. This is the period when most of the respiratory problems begin in broiler flocks in Iran. This may indicate the possible involvement of aMPV in the respiratory disease we are seeing in chickens in Iran. As aMPV can initiate or exacerbate poultry respiratory diseases, its prevalence has to be investigated in other parts of Iran. Future work may and should include the use of molecular methods and isolation of the virus. Isolation of aMPV will allow the possibility of making autogenous vaccines.

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