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Serologic profile of avian leukosis virus subgroup-J, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in broiler grandparent flocks of Iran

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ABSTRACT

Avian Leukosis Virus Subgroup-J (ALV-J), *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are important pathogens in chickens that cause severe economical losses in poultry industry throughout the world. Seven broiler grandparent flocks of Iran (six broiler strains) were sampled randomly at the ages of 8-63 wk (100 samples from each flock) for antibody detection to ALV-J, MG, and MS by ELISA. One sample (0.9%) of flock F was antibody positive to MG. About the MS, there were 2 (2.1%), 2 (2.1%), 2 (2.1%), 8 (8.7%), 4 (4.3%), 9 (9.8%) and 2 (2.1%) positive samples from those collected from flocks A, B, C, D, E, F and G, respectively. However, in all flocks tested, the mean S/P ratio was < 0.5 and thus all flocks as recommended by the manufacturer were antibody negative to MG and MS. All plasma samples collected from flocks A, B and D were negative for ALV-J antibody. However, there were 2 (2.9%), 39 (51%), 5 (6.6%) positive samples from those collected from flocks C, E, and F, respectively. Only the flock E was antibody positive to ALV-J (according to the manufacturer's instructions), because in other flocks the mean S/P ratios were < 0.6. The findings of this study showed that one of the broiler grandparent flocks in Iran exposed to the ALV-J virus.

Keywords: Serology, ALV-J, MG, MS

INTRODUCTION

Avian Leukosis Virus Subgroup J (ALV-J) first was described by Payne in 1988(Payne *et al* 1991). These viruses which cause myeloid leukosis and nephromas in meat-type chickens are prevalent through the world and impose significant economic losses due to tumor mortality, sub-optimal productivity, condemnation and loss of pedigree birds (Fadly & Payne 2003, Venugopal 1999). *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) also are causes of major economic losses in the poultry industry throughout the world especially in concurrent infections with respiratory viral infections and Escherichia coli (Kleven & Ley 2003). Control of MG and MS are very dependent on serologic monitoring. In this study ELISA assay was used to measure antibody levels generated to possible ALV-J, MG and MS infections in seven

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flocks belonging to six different broiler grandparent strains present in Iran.

MATERIALS AND METHODS

Chickens. Seven flocks from six different strains of broiler grandparent in Iran were sampled and assigned flocks A (8 weeks of age), B (12 weeks of age), C (35 weeks of age), D (37 weeks of age), E (48 weeks of age), F (59 weeks of age), and G (63 weeks of age), respectively.

Plasma Preparation. One hundred blood samples were randomly taken from birds in each flock and immediately poured into tubes containing ethylenediamine tetra acetic acid (1 mg/ml) as anticoagulant, and submitted to laboratory (In the Faculty of Veterinary Medicine, Tehran University). In the laboratory, plasma samples separated by centrifugation (4000 rpm/min for 4 minutes), then stored at -20 °C until tested.

ALV-J antibody detection (ELISA). Commercial ELISA kit (IDEXX, USA) was used to detect antibody levels to ALV-J in 76 plasma samples from each flock. Briefly, the reagents and antigen coated plates were allowed to come to room temperature before the test. The test sample was diluted five hundred folds (1:500) with sample diluent prior to being assayed. 100 µl of undiluted negative control was dispensed into wells A1 and A2 and 100 µl positive control into wells A3 and A4. This was followed by 100 µl of diluted samples into appropriate wells. The plate was incubated for 30 minutes at room temperature. Each well was then washed 5 times with approximately 350 µl of diluted wash solution. The wash concentration was diluted 1:10 with distilled water. Goat anti-chicken conjugate (100 µl) was dispensed into each well. The plate was incubated at room temperature for 30 minutes, followed by washing each well as before. TMB substrate (100 µl) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally, 100 µl of stop solution was dispensed into each well to stop the reaction.

The absorbance values were measured and recorded at 650 nm using an automatic ELISA reader (Anthos 2020, Austria). The xCheck software (IDEXX) was used to generate the results.

MG and MS antibody detection (ELISA). The antibody to MG and MS detected in 92 plasma samples from each flock with commercial ELISA kits (IDEXX, USA). The procedure was followed according to the manufacturer's instructions as described for ALV-J Ab-ELISA.

RESULTS

All plasma samples collected from flocks A, B and D were negative for ALV-J ELISA antibody. However, there were 2 (2.6%), 39 (51%), 5 (6.6%) positive samples from those collected from flocks C, E, and F, respectively (Table 1).

Table 1. Serum antibody levels (by ELISA assay) to avian leukosis virus subgroup J (ALV-J) in broiler grandparent flocks of Iran.

Flock	Age (wks)	Positive/Total	S/P ratio[a](Mean ± SD[b])
A	8	0/76	0.014 ± 0.045
B	12	0/76	0.018 ± 0.047
C	35	2/76	0.048 ± 0.109
D	37	0/76	0.087 ± 0.097
E	48	39/76	0.756 ± 0.552
F	59	5/76	0.187 ± 0.549

[a] S/P: Sample to Positive. The Mean of S/P ratio > 0.6 for ALV-J was considered as positive.
[b] SD: Standard of deviation

Plasma samples collected from all flocks except flock F failed to show antibody to MG. One sample (0.9%), however, obtained from flock F was positive. The titer C.V(Coefficient of variation) of MG of flocks A, B, C, D, E, F and G were 102.1, 146.9, 175.8, 183.8, 232.6, 640.6 and 228.8 respectively (Table 2).There were a number of

positive samples in all flocks (A-G) for MS antibody. The corresponding numbers of positive samples for flocks A, B, C, D, E, F, and G were 2 (2.1%), 2 (2.1%), 2 (2.1%), 8 (8.7%), 4(4.3%), 9 (9.8%), and 2 (2.1%), respectively. The titer C.V. of MS of flocks A, B, C, D, E, F and G were 102.2, 111.9, 699.2, 162.7, 103.7, 114.3 and 117.4 respectively (Table 3).

Table 2: Serum antibody levels (ELISA assay) to *Mycoplasma gallisepticum* (MG) in broiler grandparent flocks of Iran.

Flock	Age (wks)	Positive/Total	S/P ratio[a](Mean±SD[b])	Titer C.V.[c]
A	8	0/92	0.031 ± 0.030	102.1
B	12	0/92	0.025 ± 0.036	146.9
C	35	0/92	0.036 ± 0.062	175.8
D	37	0/92	0.024 ± 0.042	183.8
E	48	0/92	0.020 ± 0.045	232.6
F	59	1/92	0.110 ± 0.628	640.6
G	63	0/92	0.011 ± 0.025	228.8

[a] S/P: Sample to Positive. The Mean of S/P ratio > 0.5 for ALV-J was considered as positive.
[b] SD: Standard deviation
[c] CV: Coefficient of Variation

Table 3: Serum antibody levels (ELISA assay) to *Mycoplasma synoviae* (MS) in broiler grandparent flocks of Iran.

Flock	Age (wks)	Positive/Total	S/Pratio[a](Mean±SD[b])	Titer C.V.[c]
A	8	2/92	0.103 ± 0.101	102.2
B	12	2/92	0.139 ± 0.142	111.9
C	35	2/92	0.099 ± 0.637	699.2
D	37	8/92	0.136 ± 0.209	162.7
E	48	4/92	0.177 ± 0.168	103.7
F	59	9/92	0.242 ± 0.254	114.3
G	63	2/92	0.133 ± 0.146	117.4

[a] S/P: Sample to Positive. The Mean of S/P ratio > 0.5 for ALV-J was considered as positive.
[b] SD: Standard deviation
[c] CV: Coefficient of Variation

DISCUSSION

ALV-J antibody test kit has been developed as a flock screening tool for monitoring horizontal transmission of the virus and as recommended by the manufacturer, the mean S/P ratio greater than 0.6 is considered positive. In this study, only the flock E was positive for ALV-J antibody (the mean S/P ratio was 0.756). ALV-J seroconversion varies across the lines and may depend on endogenous leukosis virus expression and slow the rising of serological titres (Smith *et al* 1990). Therefore, the kit is not recommended for testing meat-type chickens less than 12-14 weeks of age, then, the results associated with flocks A and B may be inaccurate.

Importation of contaminated great grandparent and grandparent meat breeder lines has however enabled ALV-J to enter many countries causing significant economic losses in meat-type chickens (Fadly & Smith 1999, Hair-Bejo *et al* 2004., Hwang & Wang 2002., Wang & Juan 2002). Occurrence of ALV-J in commercial egg-type chickens has been recently reported too (Xu *et al* 2004).

The range of antigenic variation and the efficiency of horizontal transmission and ability to cause disease or infection in all meat-type chicken lines are characteristic of the ALV-J which makes the control of infection very difficult (Venugopal 1999, Bacon *et al* 2000). Avian Leukosis viruses are widespread among chickens; its antigen or antibody detection, or its isolation has limited value. However, the result of antibody detection as virus isolation or antigen detection is useful to prepare appropriate programs for reducing and eradication of avian leukosis viruses (Fadly 2000). The ELISA antibodies are more valuable in monitoring the flocks for ALV-J infection because they seem to be more cross reactive among variant viruses than the more specific neutralizing antibodies (Venugopal 1999). In addition, The ALV-J Ab-ELISA is a useful tool for identifying flocks that should be further investigated by other diagnostic methods. A positive result on the ALV-J antibody test kit indicates exposure to the ALV-J virus but not the actively shedding of the virus. Hence, determination of ALV-J flock status should include testing for the virus. In spite of low

sensitivity of PCR in natural infection (Zavala *et al* 2002), these techniques together with serological and histological methods are useful in diagnosis and investigation of ALV-J. The findings of this study showed that the broiler grandparent flocks in Iran are not free from ALV-J suggesting that the broiler breeders and chickens are at high risk of ALV-J infection.

MG and MS Ab-ELISA kits have been developed for flock screening and as recommended by the manufacturer the mean S/P ratio greater than 0.5 is considered positive. In all flocks tested, in spite of a number of positive samples(the titer C.V was great), the mean S/P ratio was less than 0.5 and thus all flocks were negative for MG and MS antibody (Tables 2 and 3). Serological assays for MG and MS are useful for flock monitoring and can determine the status and the time of the infection (Kleven & Ley 2003). Serologic testing for MG and MS infection have limited specificity and sensitivity because of the presence of antigenic variation and the cross reactions that may occur between MG and MS strains (Ansari *et al* 1983, Opitz *et al* 1983, Kleven & Ley 2003). More recently other techniques especially molecular techniques have developed (Liu *et al* 2001).

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