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ORIGINAL RESEARCH

Serological investigation of five diseases; Influenza, Newcastle disease, Salmonella, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in native hens of Eghlid, Iran

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Abstract

Aim: The study was conducted to determine seroprevalence of the five diseases influenza, Newcastle, *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and salmonella, among around native hens of Eghlid in Iran, on spring 2011.

Materials and Methods: On the basis of native Hens distribution, this region divided into four parts of Eghlid, Doskord, Sedeh and Hasan-abad. Fifty unvaccinated native Hens randomly selected from each part. Blood samples were aseptically collected from the wing veins using 5-ml sterile syringe. Serum from hens was tested for detection and titration for Mycoplasma and Salmonella by the rapid slide agglutination method, and was tested for influenza and Newcastle by the Hemagglutination Inhibition Assay. The data was analyzed completely in randomized design with four treatments, 50 repetitions for each disease.

Results: 34 out of 200 samples (17%) were positive for influenza. There were significant differences between regions ($p < 0.01$). 38 out of 200 samples (19%) were positive for Newcastle. The maximum infectious rate obtained from Eghlid. There were significant differences between regions ($p < 0.05$). 170 out of 200 samples (85%) were positive for Mycoplasma gallisepticum. 4 from 200 samples (2%) were positive for Mycoplasma synoviae. The results do not show a significant difference for salmonella ($p < 0.05$).

Conclusion: Contamination of Influenza, Newcastle and *Mycoplasma gallisepticum* was high, and the highest contamination rate was related to *Mycoplasma gallisepticum*. It is usually recommended that preventive strategies, such as appropriate husbandry and hygiene, sanitary handling of chicks and eggs, routine health monitoring and vaccination of Native hens should be emphasized.

Keywords: influenza, mycoplasma, newcastle, salmonella, seroprevalence

Introduction

Agents of the disease, influenza, Newcastle disease, Salmonella, and Mycoplasma (two of two) are a virus and bacteria respectively. Those diseases are highly contagious, dangerous and almost zoonotic. Annual great economic losses to the poultry industry have been imposed, and may threaten human health. Therefore, knowledge of disease process and contamination rate in several regions is needed for control and prevention of disease.

Avian influenza is a contagious viral respiratory disease, which is caused by influenza virus A of the family *Orthomyxoviridae*. This disease has greater economic importance in the poultry industry. Avian influenza virus can infect various species of domestic and wild birds. Influenza disease in poultry occurs in two forms; highly pathogenic avian influenza (which "Plague Birds" was recognized and causes severe systemic disease with high mortality up to 100%) and the low virulence avian influenza (which created at least clinical signs in poultry, and egg production is slightly reduced) [1,2]. Signs of infection with influenza vary greatly depending on factors such as the strain of virus, the health, age and species of the host, other diseases such as coli-bacillus, Newcastle, Mycoplasma, and physicochemical factors such as dust and ammonia.

Newcastle is a highly contagious viral disease that infects the respiratory and nervous system. Agent of this disease is a member of the family *Paramyxoviridae*. That can grow and multiply in egg embryo. Chickens are highly susceptible to disease, but turkeys, ducks and other birds do not tend to develop severe signs. Newcastle disease virus in humans is mild, and can cause conjunctivitis. Wild birds are the source of contamination and can spread the virus. The virus may survive a part of its evolution in a wild bird's body. In chick mortality is very high, and sometimes reaches one hundred percent. In laying hens it decrease egg production. Newcastle disease can also cause softening of the shell eggs. Eggs produced by infected birds have carried Newcastle virus, so those are not worth to incubation.

Salmonella is one of the main microflora of the

gastrointestinal tract, and often is transmitted through food to humans, and almost annual one million of people infected and died because of salmonella [3]. The disease can be usually transmitted by a very wide variety of foods and wild animals such as rodents, pets, birds, reptiles and insects [3,4]. In developed and developing countries, infectiousness of salmonella is one of the most important and widespread of infectious food. But the rate of infection is varied among countries [3]. Foods such as meat, chicken and other poultry products are one of the most important source of ransmission of salmonella to humans. More than 50% of Salmonella infections can be transmitted to humans through poultry products.

Pullorum disease is almost exclusively a disease of young chickens and turkeys. Other birds such as quail, pheasants, ducks, peacocks and guinea fowl are also susceptible. The agent can be recovered from almost all organs, tissues and excreta. In older birds that have become carriers, *S. Pullorum* is most commonly recovered from the ova and oviduct; and it is recovered only occasionally from other organs and tissues, including the alimentary tract. In the acute phase of fowl typhoid the organism is also widely distributed, but in carrier birds, the organism is found most often in the liver, spleen and reproductive tract, and occasionally in the caeca. Clinical signs are typical of a septicaemic condition in poultry and include increasing mortality and poor quality in chicks hatched from infected eggs. Older birds show signs of anaemia, depression, labored breathing and diarrhoea causing adherence of excreta to the vent. The highest mortality occurs in birds of 2–3 weeks of age. In older birds disease may be mild or unapparent. In breeding flocks reduced egg production and hatchability may be the only signs, and trans-ovarian infection resulting in infection of the egg and hatched chicks or pullets are one of the most important transmission routes for the disease [5,6].

Avian mycoplasmosis can be caused by several species of *Mycoplasma* (class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae) including *Mycoplasma gallisepticum, M. synoviae, M. meleagridis* and *M. iowae*. *M. gallisepticum* is the most important pathogen in poultry. *Mycoplasma gallisepticum* is the most economically significant mycoplasmal pathogen of poultry. *M. gallisepticum* infections are also known as chronic respiratory disease (CRD) of chickens, infectious sinusitis of turkeys and house finch conjunctivitis [7,8].

M. gallisepticum infection vary from asymptomatic to severe, depending on the infecting strain and other factors. More severe infections are seen when the birds are infected concurrently with Newcastle disease virus, infectious bronchitis virus, *Escherichia coli* or pathogens other [8].

Infected chickens usually develop respiratory symptoms that may include rales, coughing, sneezing, nasal discharges and dyspnea. Turkeys typically experience more severe disease, often accompanied by swelling of the paranasal (infraorbital) sinus. Conjunctivitis with a frothy ocular exudate is common in turkeys and occurs occasionally in chickens. Production is lower in infected flocks, with decreased weight gain, feed efficiency and egg production. The symptoms of avian mycoplasmosis are typically slow to develop, and the course of the disease can be prolonged. However, acute respiratory disease sometimes occurs in young birds, particularly turkeys.

Mycoplasma synoviae disease is asymptomatic, mortality rate is less than ten percent. The disease has a long incubation period (generally between 11 to 21 days after the transmission). Transmission is also horizontal or vertical. Survival of *Mycoplasma synoviae* is low outside of the body. But transmission though stool is very important [3].

The aim of this study was to evaluate the prevalence of influenza, salmonella, New castle Disease virus (NDV), *Mycoplama gallisepticum* (Mg) and *Mycoplasma synoviae* (Ms) in native hens in region of Eghlid (a city in south of Iran).

Materials and Methods

Out study was conducted between March and June 2011. 200 apparently healthy native hens in the urban and rural of Eghlid (a city in south of Iran) were used in the study. With the distribution of native birds, Eghlid was divided into four regions (Eghlid, Hassanabad, Doskord and Sedeh). 50 unvaccinated hens were selected randomly from each region (total 200 hens). A total of 200 blood samples were collected from the wing vein of native hens (unvaccinated, mature, and healthy hens). However, in order to avoid confusion, before use, the tubes are numbered. To avoid corruption of samples, all samples were maintained in the refrigerator and transported to laboratory within 48hrs. If a delay in sample transportation was expected, samples were centrifuged and frozen at -20^{oc} before being submitted to the laboratory. All antigens were used from company Golbid, and imported from the Netherlands (Nobilis® Antigens, Intervet International Co Netherlands). Antigens of Mg, Ms, and Sp were maintained at refrigerator in 4^{oc} temperature. Before testing, in order to the balance temperature, antigens were maintained 30 minutes at room temperature. And before use, Antigens were stirred. Because of serological test, blood samples were centrifuged and the serum was separated from it.

The detection of antibodies against Mg, Ms, and *Salmonella pullorum* (Sp) were achieved by the rapid slide agglutination method. The first, antigen of Mg, Ms and salmonella and serum samples without dilution are mixed at room temperature (0.03 cc of serum 0.03 cc of antigen, proportion 1:1). Then, the plates were studied under a light source. If agglutination (clot) occurs, the sample was positive. Test was carried out for positive samples of Mg and Ms with a dilution one-eighth (1/8) and for Salmonella with a dilution one-

Table-1. Comparison average contamination percent of native hens to several diseases among native hens around Eghlid in Iran.

Disease	Areas				
	Eghlid	Doskord	Sedeh	Hasanbad	Total means ± SE
Influenza	8±0.27[b]	10±0.3[b]	28±0.45[a]	22±0.42[a]	19±0.39
Newcastle	26±0.44[a]	20±0.4[b]	12±0.34[c]	18±0.37[b]	17±0.37
Salmonella	0	0	0	0	0
Mg	88±0.33[a]	80±0.4[a]	90±0.3[a]	82±0.39[a]	85±0.35
Ms	0[b]	0[b]	2±0.123[ab]	4±0.2[a]	2±0.12

a,b,ab ; Do not differ significantly at P<0.05

sixteenth (1/16) (positive serum by phosphate buffer 0.5M and PH=7.2 was diluted). Therefore, diluted samples were positive, the test was positive, otherwise it was negative. Of course, if in the first experiment was negative, the test was negative and was not required to dilute. Antibodies against avian influenza and Newcastle virus in the serum were evaluated using the Hemagglutination Inhibition Assay (HI). The HI assay was performed using 96 'U'-well microtiter plates, 1% v/v red blood cells and according to present standards [7,9,10]. Due to unvaccinated hens, samples were considered positive if titers were greater than 2($\geq$2).

The resulting data using software mini tab 15, in a completely randomized design for each sample with four treatments (regions) and 50 repetitions of each treatment or region, were analyzed. The average infectious rate of each disease was compared with Duncan at 5% level.

Results

The results showed that 34 out of 200 samples (17%) were positive for titers of influenza. Contamination rate from Eghlid, Doskord, Sedeh and Hassan-abad were 8, 10, 28 and 22 percent respectively. The highest contamination rate was in the Sedeh. There was no significant difference between Sedeh and Hassan-abad contamination rate ($p < 0.05$), but concerning the other regions, there were significant differences among those regions. The contamination rate between Eghlid and Doskord were not significant ($p < 0.05$) (Table 1).

The results of the research showed that 38 out of 200 samples (17%) were positive for titer of Newcastle. Contamination rate from Eghlid, Doskord, Sedeh and Hasan-abad were 26, 20, 12, and 18% respectively. The highest contamination rate was related to the Eghlid. The different among the regions was significant ($p < 0.05$). Difference between Eghlid and Sedeh contamination rate were not significant ($p < 0.01$) (Table -1).

In this study any sample was not positive for titer of *Salmonella pollurum*.

The results showed that 170 out of 200 samples (85%) were positive for titers of *Mycoplasma gallisepticum*. Contamination rates from Eghlid, Doskord, Sedeh and Hasan-abad were 88, 80, 90, and 82% respectively. The highest contamination rate was related to the Sedeh. There were no significant differences among different regions ($p<0.1$), and the contamination rate was almost the same in four regions (Table-1).

The results showed that 4 out of 200 samples (4%) were positive for titers of *Mycoplasma synoviae*. Contamination rates from Eghlid, Doskord, Sedeh and Hasan-abad were 0, 0, 2, and 6% respectively. The highest contamination rate was related to the Hasan-abad (Table -1).

Discussion

In the present study, Avian Influenza Virus (AIV) antibody titer was found in all the areas and infectious lever in four areas weren't uniform. Migrant birds are one of carriers and agent incidence of AIV. Therefore, existence of more migrant birds and various other species of animals (such as ducks, goose and so on…) in the above mentioned areas is one of the causes of different infectious lever. The absence of clinical signs of influenza in native hens, in spite of high antibody titer in some birds, could be due to persistent and acquired resistance of birds to influenza virus in the environment.

Al-Natour (2005) reported that the sero-prevalence of AIV was 71% among broiler-breeder flocks in Jordan [2]. In another study conducted by Nooruddin (2006) in Bangladesh, an overall 9.82% sero-prevalence of AIV was recorded [11]. Dergham (2009) reported that sero-prevalence of AIV-H_9 was 54.2 and 78.3% in broiler and laying hen farms in Jordan, respectively [12].

In a serological investigation, Dadras (2006) reported that the sero-prevalence of AIV was 53.56% among the farms around Shiraz city in Iran [13]. In a study conducted by Hadipour (2010) in Iran, overall HI titer and sero-prevalence against H_9N_2 were recorded 6.52 and 72.98%, respectively [14].

The results showed that Newcastle antibody titer was found in all areas and prevalence rate of Newcastle in four areas wasn't uniform. Highest contamination rate was in the eghlid. Particles suspended in the air, water and the contaminated food are the most important agent of transmission of Newcastle virus. Prevalence rate was high, where there were maintained pigeon, because contamination can easily expand in the area. Lack of vaccination and incorrect vaccination could increase prevalence of Newcastle disease. Habibi (2007) reported that the sero-prevalence of Newcastle virus was 11.8% among native hens of Kazeron city in Iran [15]. Ghaem-meghami (2007), reported that the seroprevalence of NDV was 2.7, 19.5 and 0.55% among hens, goose and ducks in Iran,

respectively [16]. In another study conducted by Omidi (2010) in Amol city in Iran, an overall 15.8% sero-prevalence of NDV was recorded.

The results showed that salmonella antibody titer was not found in any area, that with results by Habbi (2007) had different (3.07%). In an investigation serology, Dadras (2006), reported that the sero-prevalence of salmonella was 34.4% among the farms around Shiraz city in Iran [13]. Fardi-pour (2007) reported that the sero-prevalence of salmonella was 36.7% among farms around Isfahan city in Iran. Contaminated Eggs or foods are one of the main transmission agents of the disease, that in these areas, foods aren't brought from other regions. Besides, housing of cow and poultry are very low, and foods aren't almost entered to these areas [4].

The results showed, that contamination rate by *Mycoplasma gallisepticum* is very high in all the areas. Our results are higher than of the similar studies. Dadras (2006) reported that the sero-prevalence of Mg was 67% among the farms around Shiraz city in Iran [13]. Haghighi-Khoshkhoo (2011) reported the prevalence of Mg and Ms were 10 and 45% respectively [17]. Seifi (2011) reported that sero-prevalence of Mg during years 2003- 2008 of 24.1% to 0% progressively declined, and the prevalence was the highest (18.5%) in winter and the lowest (6.8%) in summer [18].

M. gallisepticum is transmitted during close contact between birds as well as on fomites. Aerosol spread occurs over short distances and can be responsible for transmission within a flock. *M.* gallisepticum is also transmitted vertically in eggs. Shedding in the egg can vary; egg transmission is more frequent in birds infected during laying than in birds infected before they mature. Infected birds carry *M.* gallisepticum for life, and can remain asymptomatic until they are stressed. Turkey is the main carrier of this disease. Thus, the high infectious rate is due to rearing other poultry such as turkeys close to the native birds, feeding native birds on human food waste (which includes commercial shell eggs), and lack of disinfection and proper sanitation. A similar study conducted in Bangladesh showed that the rate of infection varies from 45.1 to 66.5% [19]. In a study conducted in Algeria showed that the rate of infection by Mg for chicken and laying hens was 84.81, 81.15 respectively. And the prevalence was higher (91.13%) in the winter season and lower (73.91%) in summer season [20].

The results show that contamination by *Mycoplasma synoviae* is very low in all regions. Our results are lower than other studies. Due to the lack of source of contamination (absence of infected birds) and the cleaning of the area was low contamination. Dadras (2006) reported that the sero-prevalence of Ms was 100% among the farms around Shiraz city in Iran [13]. Suzuki (2009) reported that sero-prevalence of Ms was 53% by Elisa test with the high rate of Ms infection [21]. Kapetanov (2010) reported that the overall sero-prevalence of the Mg and Ms of the flocks in Serbia were 9.01 and 47.49% in 2000 and was 11.59 and 22.17% in 2009 by SPA and Elisa tests respectively and concluded that sero-prevalence of MS was decreased ,versus the Mg increased [22].

Conclusion

Contamination of Influenza, Newcastle and *Mycoplasma gallisepticum* was high, and the highest contamination rate was related to *Mycoplasma gallisepticum*. It is usually recommended that preventive strategies, such as appropriate husbandry and hygiene, sanitary handling of chicks and eggs, routine health monitoring, and vaccination of Native hens should be emphasized.

Author's contributions

All authors contributed equally. All authors read and approved the final manuscript.

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Competing interests

Authors declares that they have no competing interest.

References

1. Alexander D.J. (2000) A review of avian influenza in different bird species. *Vet. Microb*. 74:3-13.
2. Al-Natour MQ., Abo-Shehaha MN. (2005) Sero-prevalence of avian influenza among broiler-breeder flocks in Jordan. Preventive. *Vet. Med*. 70:45-50.
3. Betancor L., Pereira M., Martinez A., Giossa G., Fookes M., Flores K., Barrios P., Repiso V., Vignoli R., Cordeiro N., Algorta G., Thomson N. and Maskell D. (2010) Prevalence of Salmonella enterica in Poultry and Eggs in Uruguay during an Epidemic Due to Salmonella enterica Serovar Enteritidis. *J. Clin. Microb*. 48(7). pp. 2413-2423.
4. Fardi-pour A. (2007) Recognition of serotypes salmonella among farms broiler chicks of Isfahan city in Iran. The 5st meeting veterinarians of clinical science, Iran. pp: 59-64.
5. Fauquet C., Fauquet M. and Mayo M.A. (2005) Virus Taxonomy: VIII Report of the International Committee on Taxonomy of Viruses. Academic Press.
6. Ley DH., et al. (2008) Mycoplasma gallisepticum infection. *In*: Disease of poultry, Fadly A.M., Gilson J.R., Mc Dougald L.R., Nolan L.K., Swayne D.E. (eds), 12th edition, Iowa State University Press, Ames, Iowa, pp: 807-834.
7. World Organisation for Animal Health, (2008) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OIE, Paris.
8. Vahedi N. (2007) Recognition of serotypes Mycoplasma among farms broiler chicks around Amol city in Iran. The 5th International Meeting of clinical science veterinarians in Iran. pp: 76-82.
9. Alexander DJ, Allan Wh. and Wilding GP., (1982) Standard technique for hemagglutination inhibition test for antibodies to avian infectious bronchitis virus. Veterinary record113:64.
10. Butcher G.D. (2009) Factors to consider in serologic testing for Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS)," in Electronic Data Information Source (EDIS), University of Florida, VM 126, http://edis.ifas.ufl.edu/vm093.

11. Noorruddin GM, MT. Hossain and M Mohammad, (2006) Sero-epidemiology of avian influenza virus in native chicken in Bangladesh. *Int. J.Poult. Sci.* 5:1029-1033.
12. Dergham A., et al. (2009) Avian influenza virus-H_9 surtype in poultry flocks in Jorda. *Prev.Vet. Med.* 84; 77-81.
13. Dadras H. (2006) Investigation of five diseases among native hens around Parishan Lake in Iran. The 4st international congress of clinical science veterinarians in Iran. pp: 90.
14. Hadipour MM. (2010) Sero-prevalence survey of H_9N_2-AIV in Backyard chicken around the Caspian Sea in Iran. Rev. Bras. Cienc. Avic. V: 12(1): pp:53-55.
15. Habibi GH. (2007) Serological investigation of five diseases among native hens around Lake Parishan in Iran. The 5th meeting veterinarians of clinical science, Iran. pp: 90.
16. Ghaemmeghi S., et al. (2007) Serological investigation of NDV disease among hens, duck,and goose in Iran. *J. Res. Vet.* (univ. Tehran).62(5): 297-300.
17. Haghighi-Khoshkhoo P., et al. (2011) Seroprevalence of Mycoplasma gallisepticum and Mycoplasma synoviae infection in the commercial layer flocks of the Centernorth of Iran. *African J. Micro. Res*. 5(18): 2834-2837.
18. Seifi S. and Sherzad M.R. (2012) Risk factors and sero-prevalence of Mycoplasma allisepticum nfection in broiler breeder farms in Mazandaran province, north of Iran. *Revue Méd. Vét.*, 163(5): 215-218.
19. Hossain KM., Hossain Md. and Yamato I. (2010) Sero-prevalence of Mycoplasma gallisepticum infection in chicken in Rajshahi and surrounding Districts of Bangladesh. *Int. J. Boil*. 2:74-80.
20. Heleili N., Mamche B. and Chelihi A. (2011) Incidence of avian Myciplasmosis in the region of batna, eastern Algeria. *Vet. World* 4(3).101-105.
21. Suzuki K., Origlia J., Alvarez F., Faccioli M., Silva M., Caballero J., Nunrez L. and Castro L. (2009) Relative risk estimation for Mycoplasma synov*iae* in backyard chickens in Paraguay. *Int. J. Poult. Sci.*, 8: 842-847.
22. Kapetanov M., Orlic D., Potkonjak D. and Velhner M. (2010) Mycoplasma in poultry flocks in the year 2009 compare to the year 2000 and significance of the control measures in Serbia. Lucrari scientific Med. Vet. XLIII: 249-253.

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