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Sero-prevalence of avian influenza among broiler-breeder flocks in Jordan

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Abstract

Thirty blood samples were collected randomly from each of the 38 breeder-broiler farms in Jordan. Serum samples were examined using indirect ELISA for specific antibodies to avian influenza virus. The overall true flock-level sero-prevalence of avian influenza was 71% (95% CI: 55,83). Positive flocks had 2–30 sero-positive chickens and half of flocks had >20 sero-positive birds. The number of sero-positive flocks varied in the studied localities with more sero-positives in farms located within the migratory route of migratory wild fowl. The examined broiler-breeder flocks had no clinical signs, or noticeable decrease in egg production; mortalities were within the normal range (0.1–1%). The number of positive sera/flock correlated with flock size. There were a no significant (Pearsons $r = 0.21$, $p = 0.21$) correlation between positive flocks and age. A non-pathogenic AI virus infects broiler-breeder farms in Jordan. Wild local and migrating birds might promote the further spread of this virus in Jordan and other countries.

Keywords: Avian influenza; Poultry; Viral diseases; Broiler-breeder; ELISA; Age influence; Jordan

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1. Introduction

Avian influenza (AI) is a respiratory disease of poultry caused by influenza virus A of the family Orthomyxoviridae. The disease is of an economic significance to the poultry industry worldwide (Easterday et al., 1997). For example, the total estimated cost (adjusted to 2001) of the highly pathogenic H5N2 virus outbreak in poultry during the years 1983–1984 in the northeastern USA was $588 millions (Swayne and Halvorson, 2003).

AI viruses cause natural infection in a variety of domestic and wild bird species throughout the world (Swayne and Halvorson, 2003). Influenza A virus infection in poultry occurs in two forms. The highly pathogenic AI (HPAI; previously known as ''fowl plague'') causes a severe systemic disease with mortality up to 100%, and the low-pathogenic AI (LPAI) usually causes minimal clinical signs other than a slight drop in egg production (Alexander, 2000).

HPAI derivatives emerged from LPAI H5 by mutation in a combination of in vitro and in vivo experimental systems (Brugh, 1988; Ohuchi et al., 1989; Brugh and Perdue, 1991; Perdue et al., 1996; Swayne et al., 1997). Wild waterfowl (especially ducks) serve as natural reservoirs and important sources of infection to domestic waterfowl and poultry (Easterday et al., 1997). Sporadic cases of influenza caused by the entire AI viruses were reported in humans (Swayne, 2000).

During 1998–2000, H9N2 viruses were reported in Middle Eastern countries and were responsible for widespread and serious disease in commercial chickens in Pakistan (Naeem et al., 1999, 2003), Iran (Nilli and Asasi, 2002, 2003), the United Arab Emirates (Manvell et al., 2000) and Saudi Arabia (Banks et al., 2000). Phylogenic analysis of H9N2 isolates from Pakistan, Iran and Saudi Arabia showed very close relationships – suggesting a common source (Banks et al., 2000). Numerous infections of poultry and other birds with the subtype H9 during the 1990s originated from separate introductions from feral birds (Banks et al., 2000).

Jordan is on the route of migratory wild birds and has local wild and feral birds. However, AI infection was never investigated in poultry in Jordan. We described the sero-prevalence of AI in all operating broiler-breeder flocks in central and southern Jordan during October to December 2001 and the association of the seropositivity with flock size and age.

2. Materials and methods

2.1. Birds and study area

The survey was conducted during October to December 2001 in the central and southern area of Jordan. A total population of 872,500 broiler-breeder chickens is distributed over the 38 operating broiler-breeder farms in the area (Anon, 2001). Each farm has 1–6 houses. The houses were built of brick and cement with metal-plate roofs and are of different sizes. Table 1 shows the quartiles for age, hen-day egg-production percent, flock size and total mortality percent of AI sero-positive and sero-negative broiler-breeder flocks. The farms

Table 1
Descriptions of avian influenza sero-positive and sero-negative broiler-breeder flocks during October to December 2001, in Jordan

Variable (flock-level)	Flock sero-status	Minimum	Quartiles			Maximum	p (rank sum)
			Q1	Q2	Q3		
Age (weeks)	+	28	36	45	49	55	0.20
	−	27	32	40	45	52	
Hen-day egg production (%)	+	22	65	75	78	85	0.06
	−	50	65	65	70	82	
Flock size ($\times 10^3$)	+	4.5	12	22	40	50	0.23
	−	8	11	15	23	34	
Total mortality (%)	+	0.1	0.3	0.5	0.7	1	0.97
	−	0.1	0.3	0.5	0.8	1	

followed an open-house system. The stocking density was 5 birds/m^2. The birds are housed in an intensive deep-litter system. Before birds were placed, the houses were cleaned, washed, disinfected and provided with new wood shavings. Most breeder flocks were imported from France (17), the UK (8), Holland (3) and Germany (2) and the rest were hatched locally. Feeding programs are according to manuals supplied to each flock by the grand parent companies.

The birds are usually kept for a production life of 1–2 years, then sold for slaughter. The breeds were Hubbard (19), Ross (8), Lohman (7), Cobb (2), and Shaver (2). Breeder flocks in Jordan adopt health programs that include bacterial- and viral-disease vaccines except AI.

The studied area is on the route of many migratory waterfowl (except for the Karak farms). More than 374 wild bird species are recorded in Jordan of which about 220 are migrants or winter visitors, and as many as 150 are known to breed at least once/year (Anderws, 1995). Waterfowl aggregate around surface-water bodies. Local wild birds (such as ducks, sparrows, pigeons, doves and crows) frequently are observed in houses and/or farms.

All studied localities except Karak have 1–4 water dams. Poultry farming density is low (<300 birds/km^2) in Karak, medium (>300–1000 birds/km^2) in Jerah and Balqa and high (>1000 birds/km^2) in Zarqa, Qutrana and Madaba (Anon, 2001).

2.2. Sampling

All the operating broiler-breeder farms ($n = 38$) in the central and southern parts of Jordan were contacted through the appropriate veterinarians and all owners agreed to participate in this study. Each farm was visited once, the houses were numbered in each farm, then a number was drawn to be sampled. Then, as three parallel imaginary lines were drawn along the length of the house (left, right and center), 10 birds were picked along one walk covering the length of the house using each of the three lines. A total of 30 birds/farm were picked up and blood samples were collected by veno-puncture of the wing vein. Sera were separated and stored at −20 °C until used. Thirty serum samples per flock were tested for anti-AI virus antibodies.

2.3. ELISA

An indirect ELISA test kit, recommended by Swayne et al. (1998) to detect type-A group-specific RNP and M antibodies was used according to the manufacturer's (Kirkegaard and Perry Laboratories {KLP}, Gaithersburg, Maryland 20879, USA) instructions. The plates have been coated with inactivated AI virus, subtype H9N2. An ELx800 ELISA reader (BIO-TEK Instruments, Inc. Winooski, VT, USA) was used. Optical-density values were transformed into titres using the ProFlock software (KLP, USA). This test's sensitivity and specificity are 99 and 98%, respectively.

2.4. Data collection

At the time of sampling, information was collected with the help of the appropriate veterinarian. These included; the farm location, flock source, bird breed, flock size, flock age, vaccination history for avian influenza, percent hen-day egg production, clinical signs (including: respiratory symptoms accompanied with cyanosis in the comb and wattles and percent of total mortality from the start of the cycle up to the sampling day).

2.5. Statistical methods

Descriptive statistics of flock characteristics and 95% confidence intervals were calculated for the sero-prevalence after correcting for the sensitivity and specificity (Rogan and Gladen, 1978). Mann-Whitney and Pearson's correlation tests were also used.

Table 2
Proportions of farms with 0–30 avian influenza sero-positive chickens during October to December 2001 in Jordan ($n = 38$ farms)

No. of sero-positive chickens out of 30 chicken examined per farm	No. of farms examined	% of farms
0	11	29
1 or 2	4	11
3 or 4	0	0
5 or 6	0	0
7 or 8	1	3
9 or 10	0	0
11 or 12	1	3
13 or 14	0	0
15 or 16	1	3
17 or 18	1	3
19 or 20	2	5
21 or 22	0	0
23 or 24	3	8
25 or 26	6	16
27 or 28	4	11
29 or 30	4	11

3. Results

The overall true flock-level sero-prevalence of AI was 71% (95% CI: 55.83). Positive flocks had 2–30 sero-positive chickens (Table 2).

The number of positive sera/flock correlated significantly (Pearsons $r = 0.42$, $p = 0.01$) with flock size. There was no significant (Pearsons $r = 0.21$, $p = 0.20$) correlation between positive flocks and age. The examined breeder-broiler flocks had no clinical signs or noticeable decrease in egg production; mortalities were within the normal range (0.1–1%). There was no significant difference between sero-positive and sero-negative flocks' age, hen-day egg production percent, flock size or total mortality percent (Table 1).

4. Discussion

This is the first report of sero-prevalence of AI in commercial Jordanian broiler-breeder chicken flocks. The sero-positive birds had no history of clinical signs – suggesting the existence of a LPAI virus. More than half of the examined birds in each of the flocks were seropositive (median-18, range of 2–30 birds) (Table 2).

Among the six areas of commercial broiler-breeder farming examined, the only one that was negative (Karak, three flocks sero-negative) was the one not located in the migratory fowl routes. The Karak farms are 30 km from other poultry farms and Karak has a low poultry-farm density (100 birds/km^2) and has no surface water.

The indirect ELISA is an accurate test amenable to semi-automation and the rapid survey of large number of samples. However, the test results should be interpreted on a flock and not on individual-bird basis (Swayne et al., 1998).

Most of broiler-breeder flocks we examined in Jordan had evidence of prior exposure to AI. Because cumulative flock mortalities were nevertheless low, we infer that exposure was to LPAI. To date, all AI isolates from Jordan were identified to be H9N2 (Al-Natour, unpublished observation). All infected flocks were near migratory waterfowl routes and large bodies of surface water.

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