Serological evidence for the presence of infectious avian hepatitis E virus among chicken flocks in Egypt

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ABSTRACT

This report on the prevalence of avian hepatitis E virus (avian HEV) among chicken flocks in Egypt. A total of 510 serum samples were collected from broiler breeder (n=191), layer breeder (n=235) and commercial broiler chickens (n=84) from different farm flocks (24 flocks) in Egypt. These samples were examined for the presence of avian HEV antibody using enzyme linked immuno-sorbent assay (ELISA). The results revealed that the occurrence of avian HEV was 15.7% (80/510) and 66.7% (16/24) of the examined flocks. HEV antibody prevalence increased as age dependent and most commonly occurred among broiler breeder flocks (10/24). In conclusion, the occurrence of avian HEV was confirmed for the first time among chicken flocks in Egypt.
Key words: Avian hepatitis E virus, ELISA, Sero-prevalence, Chicken, Egypt

INTRODUCTION

Hepatitis E virus (HEV), non-enveloped, positive sense single-stranded RNA virus with a genome of approximately 7.2kb in length, is classified in the family *Hepeviridae*, a genus *hepevirus* (Emerson and Purcell, 2003; Emerson et al., 2004). HEV has four recognized major genotypes 1- 4 in mammalian species (Okamoto, 2007; Meng, 2013). Genotypes 1 and 2 HEV isolates mainly obtained from human while 3 and 4 infected both human and other species (Cossaboom et al., 2011). It is well known that genotypes 3 and 4 are zoonotic. However, the identification of numerous HEV strains from animals and the confirmed ability of cross-species infection by these strains have significantly broadened the host range, diversity of HEV and raised public health concerns for zoonosis and food safety associated with genotypes 3 and 4 HEV infection (Meng et al., 2002; Meng, 2010; Cossaboom et al., 2011).

Avian HEV is associated with hepatitis-splenomegaly (HS) syndrome or big liver and spleen (BLS) disease in chickens (Payne et al., 1999). In both broiler breeder hens and egg laying hens, avian HEV is accompanied with increased mortality (at age of 30 – 72 weeks), drop in egg production, bloody fluid in the abdomen and an enlarged liver and spleen which cause significant economic loss in the poultry industry (Haqshenas et al., 2001; Morrow et al., 2008). Avian HEV has been detected in healthy chicken and chicken with HS syndrome in the USA (Haqshenas et al., 2001; Huang et al., 2002, Sun et al., 2004).

The sero-prevalence of avian HEV antibodies among 35 chicken flocks in Korea using an enzyme linked immuno-sorbent assay (ELISA) has been performed with positive rate of 57% out of the checked flocks (Kwon et al., 2012). It has been reported that cross-species infection of HEV could be occurred in zoo-like location and birds can be infected naturally with mammalian HEV (Zhang et al., 2008). Avian HEV has also been detected in UK, Hungary, Czech Republic, Ukraine, Germany, Spain, Poland, Russia, Taiwan, and China (Morrow *et al.*, 2008; Bilic *et al.*, 2009; Peralta et al., 2009;Marek, *et al.*, 2010, Sprygin et al., 2012; Hsu and Tsai, 2014).

HEV is a major cause of acute viral hepatitis in Egypt (Blackard et al., 2009; Eldin et al., 2010). The sero-prevalence of anti- HEV IgG in Egyptian pregnant women is high especially in rural areas (78.58%). With chronic HCV co-infection, a marked increase in anti-HEV IgG sero-

positivity has been reported (Gad et al., 2011). Children are susceptible to HEV infection in the infant stage and the occurrence of HEV antibodies is increased by 2 times more in the children over 4 years old (Aboulata et al., 2005). The serological prevalence of HEV among animals in Egypt had been recorded in 21.6%, 14%, 4.4% and 9.4% from examined cows, buffaloes, sheep and goat, respectively (El-Tras et al., 2013).

The status of avian HEV infection among chicken in Egypt is largely unknown as there is no report on detection of avian HEV up-to-date. So, this study was conducted to investigate the prevalence of avian HEV antibody among chicken flocks in Egypt.

MATERIALS AND METHODS

Sample collection

Serum samples were collected from 24 chicken flocks; broiler breeder, layer breeder and commercial broiler, of different age ranged from 45 days – 80 weeks (Table 1) from various geographical regions in Egypt. The samples were transported in ice-cooled insulated box to the laboratory and stored at -80°C until use. The data included; type of flock, age, strain of chickens, flock capacity, rearing system, egg production, mortality rate, other specific clinical signs (if any) and farm location were recorded.

Table 1. The collecting data of the examined chicken flocks for avian HEV antibodies in Egypt

Type of Flock	Sample number	Age/weeks	Strain of birds	Flock size	Rearing system
Layer breeder	**34**	**79**	**White Hy-Line**	**22000**[b]	**Cages**
	53	**52**	**Balady mix**[a]	**13000**	**Open litter**
	15	35	Lohman	8800	Cages
	16	**55**	**Lohman**	**15000**	**Cages**
	26	**53**	**Bovans**	**6500**	**Closed litter**
	26	50	Bovans	6500	Cages
	8	**36**	**Hy-Line**	**25000**	**Cages**
	7	**34**	**Hy-Line**	**25000**	**Cages**
	23	70	Lohman	15000	Semi closed litter
	17	50	Lohman	15000	Semi closed litter
	10	40	Ross	40000	Closed litter
Broiler reeder	**13**	**58**	**Hubbard classic**	**3500**	**Closed litter**
	12	**58**	**Hubbard classic**	**3500**	**Closed litter**
	12	**39**	**Hubbard classic**	**3500**	**Closed litter**
	13	**39**	**Hubbard classic**	**3500**	**Closed litter**
	12	**47**	**Lohman**	**10000**	**Closed litter**
	10	**80**	**Balady mix**	**1200**	**Open litter**
	40	**43**	**Hubbard classic**	**10000**	**Closed litter**
	18	**43**	**Hubbard classic**	**40000**	**Closed litter**
	12	**47**	**Ross**	**40000**	**Closed litter**
	25	13	Ross	40000	Closed litter
	14	34	Rose	15000	Closed litter
	10	**40**	**Lohman**	**10000**	**Closed litter**
Commercial broiler	84	6.5	Avian	30000	Closed litter

[a] Fayome and Behaire breeds
[b] Avian HEV positive flock shown in bold

ELISA to detect avian HEV antibodies in the chicken sera

Chicken serum samples were tested by BLS ELISA kit (BioChek, UK, Ltd) for the presence of avian HEV antibodies according to manufacturer's instructions. Briefly, chicken serum samples were diluted to 1:500 and added to microtitre plates have been coated with inactivated avian *hepevirus* antigen. The anti-chicken IgG labelled with alkaline phosphatase will bind HEV antibodies and antigen complex. A yellow color was developed after adding p-Nitrophenyl Phosphate substrate. The optical density (OD) values were measured at 405nm using ELISA reader (inifinte-F50, Tecan, Austria) and Magellan version 7.0 software. The sample to negative ratio (S/N) and virus titer was calculated according to kit instructions. The sample had an S/N value of 0.2 or greater considered positive for avian HEV antibody while that had an S/N value of 0.199 or lower was negative.

RESULTS

The positive serum samples were recovered from 16/24 (66.7%) of the examined flocks. The broiler breeder flocks were highly susceptible to infection (10/24) than layer breeder flocks. There is no positive samples could be detected among broilers (Table 1).

The prevalence of avian HEV among chicken flocks

Eighty out of 510 serum samples were contained avian HEV antibodies and the peaks of antibody detection were from 40 to 55 weeks old and 80 weeks old (Fig. 1). Also, the distribution of avian HEV positive samples were 62/80 in broiler breeder and 18/80 in layer breeder

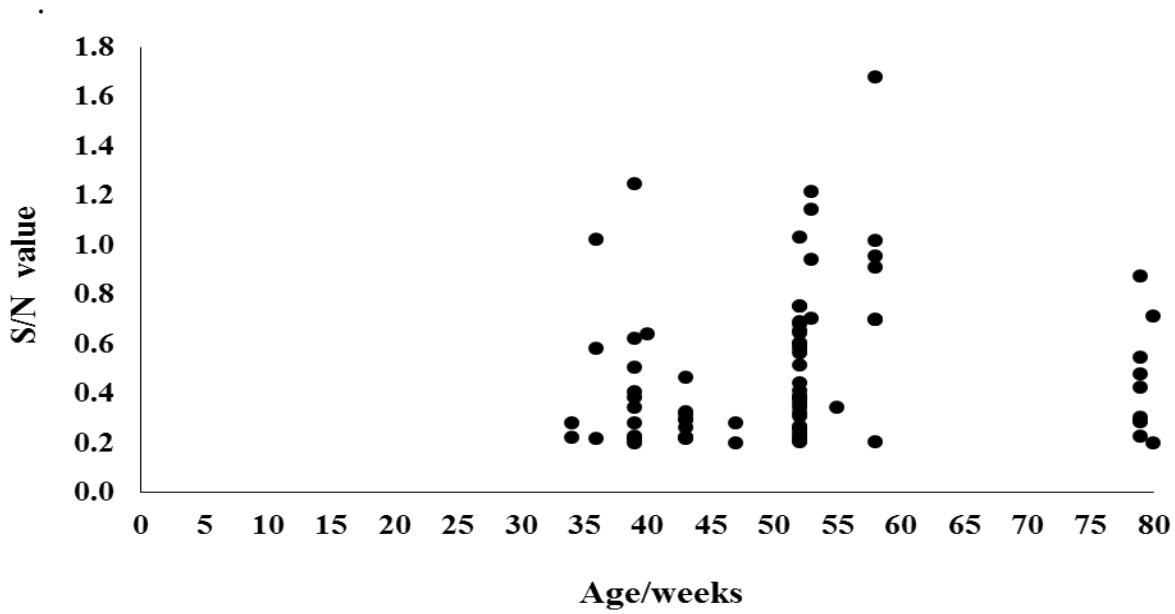

Fig. 1. The levels of avian HEV antibody in the positive sera from chicken of different ages. Samples with S/N ratio 0.2 or greater were considered positive

The correlation among the avian HEV, age, flock type and rearing system

The obtained results indicated that the antibody detection level was increased with age as shown in in Fig 1. The high S/N values (over 1) were obtained from elder chickens mainly at 50 weeks old with the highest S/N value was at 60 weeks old (Fig 1). The broiler breeder had the high avian HEV positive samples than layer breeders (Fig. 2). The majority of positive samples were collected from chicken flocks reared on litter which the most common rearing system for chicken flocks. Also, positive samples were obtained from chicken flocks have been reared in cages (Table 1) which mainly used for layer breeder.

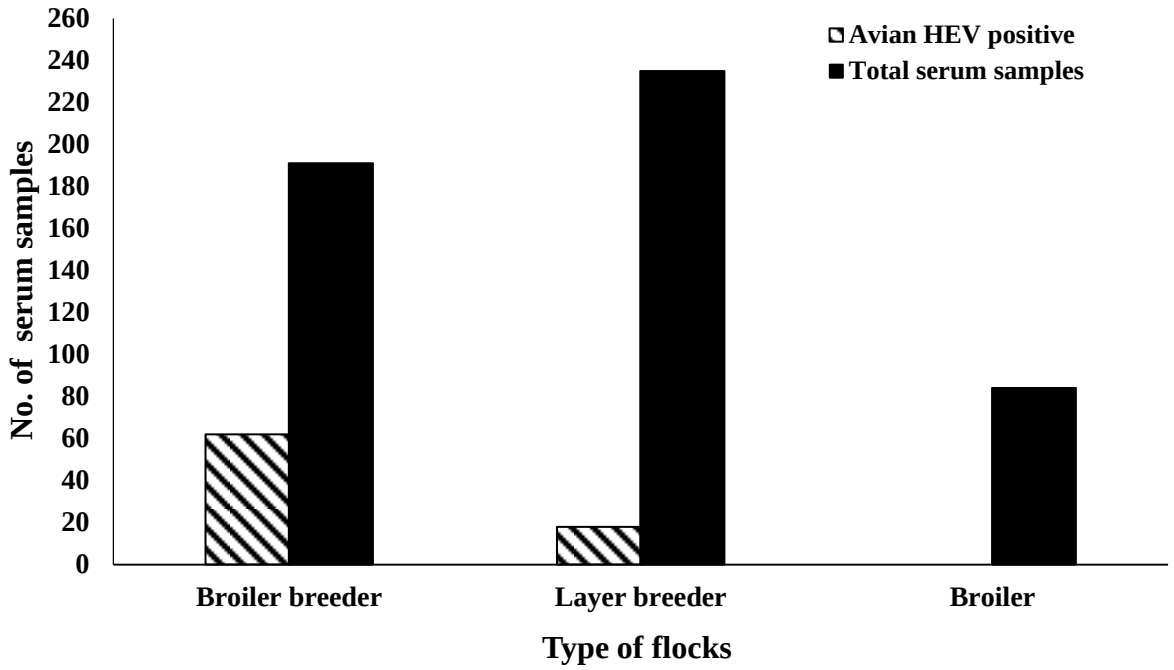

Fig. 2. The relationship between flocks types (broiler breeder, layer breeder and commercial broiler) and the prevalence of avian HEV antibodies in the examined serum samples.

DISCUSSION

Although the previous studies had reported that a high prevalence of HEV virus occurred among human and animals in Egypt (Blackard et al., 2009; Eldin et al., 2010; Gad et al., 2011; El-Tras et al., 2013). Up to date, there is no evidence for the detection of the HEV among poultry in Egypt.

This study reports on the prevalence of avian HEV among commercial chicken flocks in Egypt using ELISA. The results revealed that 80 out of 510 serum samples obtained from 24 clinically health flocks in spatially separated locations in Egypt were avian HEV positive. This arises the public health concerns due to the possibility that the chicken may act as a reservoir for the HEV, zoonotic viruses, and transmit it to human and animals (Meng et al., 2002; Zhang et al., 2008; El-Tras et al., 2013). However, further investigations and genetic characterization of avian HEV infected chicken are essential to confirm this possibility.

The avian HEV positive samples were age dependent which increased in birds over 34 weeks of age and reached its peak (30/80) at 52 weeks of age (Fig 1), this manner is similar to that observed in human and swine HEV (Huang et al., 2002). The majority of serum samples have an S/N ratio lower than 0.8 (Fig 1), which can correlated to the detection of avian HEV in clinically normal birds. The effect of virus is dose dependent, birds infected with a high dose of the virus usually progress to a clinical hepatitis with significant lesions, whereas birds received a low dose succumb to a subclinical infection without clinical lesions (Agunos et al., 2006).

In this study almost all examined chicken flocks were apparently health with no case history or characteristic post mortem problems except for slight decrease in egg production in some flocks. Avian HEV were detected by Huang et al., (2002), Sun et al., (2004), Peralta et al., (2009) and Sprygin et al., (2012) in apparently healthy birds. Billam et al., 2009 and Marek et al., 2010 revealed that no distinguished biological or genetic differences among avian hepatitis E viruses isolated from birds showing BLS or HS clinical symptoms and that obtained from clinically health ones has been

detected. Only one-fourth of the avian HEV infected chickens showed gross lesions which indicated that other co-factors exhibit the clinical features in avian HEV infected chicken (Billam et al., 2005).

The prevalence of avian HEV antibody in the samples obtained from broiler breeder (62/191) was higher than that obtained from layer breeder (18/235) as shown in Fig 2. Also, there is no antibody could be detected in the serum samples from commercial broiler (Fig 2). This attributed to the age susceptibility where the natural infection of chicken with avian HEV occurs 12 weeks of age and the fecal-oral transmission of the virus infection enhanced the viral spread among chickens reared in litter system (Huang et al., 2002; Sun et al., 2004; Billam et al., 2005).

CONCLUSION

Avian HEV infection of chicken flocks in Egypt was demonstrated for the first time by detection of avian HEV antibody. The avian HEV infection is highly prevalent in broiler breeder.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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