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SHORT COMMUNICATION

Seroprevalence of Hepatitis E Virus in Four Species of Parrots in China

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ABSTRACT

In this study, antibodies against avian Hepatitis E Virus (avian HEV) were detected in 6.43% of examined serum samples (n=311) from budgerigars (*Melopsittacus undulatus*), lovebirds (*Agapornis* sp.), cockatiel (*Nymphicus hollandicus*) and Alexandrine parakeets (*Psittacula eupatria*) by indirect ELISA. Seroprevalence of avian HEV infection in different parrot species varied from 0 to 7.92%. Statistical analysis of the origins of parrots demonstrated that parrots from Weifang city had a higher avian HEV seropositivity (7.84%) compared with parrots from Beijing city (5.06%). The seroprevalence in parrots of different age groups varied from 4 to 7.62%. The avian HEV seroprevalence in parrots examined in spring and summer was 7.19 and 5.81%, respectively. This is the first report of avian HEV seroprevalence in four species of parrots in China, which will provide base-line data for the control of HEV infection in parrots in China.

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INTRODUCTION

Hepatitis E virus (HEV), a non-enveloped virus that belongs to the genus *Hepevirus*, is a zoonotic pathogen spreading in many developing countries (Pérez-Gracia et al., 2013). The pathogen is usually transmitted by contaminated water due to poor sanitation (Johargy *et al.*, 2013). Several animals were reported as reservoirs of HEV, including birds (Zhang *et al.*, 2008; Peralta *et al.*, 2009).

Avian HEV has been detected in chickens that have hepatitis-splenomegaly (HS) syndrome in the USA (Haqshenas *et al.*, 2001). The virus is genetically related to human and swine HEV, and the identity of the two HEV strains is about 50-60% (Huang *et al.*, 2004). However, non-human primates can be infected only by swine HEV (Meng *et al.*, 1998), but cannot be infected by avian HEV (Huang *et al.*, 2004). Avian HEV was reported to spread readily between chicken flocks and can lead to significant economical losses to the poultry industry (Peralta *et al.*, 2009).

Avian HEV infection in chickens has been reported all over the world (Zhu *et al.*, 2004; Peralta *et al.*, 2009), but only one report of avian HEV infection in the parrot *Psittacus erithacus* (Zhang *et al.*, 2008). The objective of the present investigation was to examine the seroprevalence of avian HEV infection in four species of parrots, namely, budgerigars (*Melopsittacus undulatus*), lovebirds (*Agapornis* sp.), cockatiel (*Nymphicus hollandicus*) and Alexandrine parakeets (*Psittacula eupatria*) from Beijing and Weifang cities, for the first time, which would provide "base-line" information for assessing the effectiveness of strategies for controlling avian HEV infection in parrots.

MATERIALS AND METHODS

Study site: The survey was carried out in Beijing and Weifang cities, North China. Beijing City is located between east longitudes of 115°25' to 117°30' and north latitudes of 39°26' to 41°03' and is the capital of China with an average altitude of 43.5 meters. It has a typical temperate and monsoonal climate, with an annual precipitation of 626 mm and an average annual temperature of 12.6°C. Weifang City is situated in the middle of Shandong Peninsula (35°41′ 37°26′ N, 118°10′

120°01′ E). The average altitude of Weifang city is 19.3 meters. This city has a north temperate and monsoonal climate, the average annual temperature is 14.0°C, and the annual precipitation is 615.3 mm. The above two cities are the main origins of parrots in China.

Collection and preparation of serum samples: A total of 311 (158 from Beijing and 153 from Weifang) blood samples were randomly selected from 100,000 parrots (Win Episcope 2.0) via wing vein between March and June 2013. The serum samples were separated by centrifugation at 1000 × *g* for 10 min and were stored at -20°C until for antibody detection. Data regarding species, age, geographic origin and gender of parrots were obtained from owners of the parrots and local veterinary practitioners.

Serological examination: All serum samples were tested for circulating antibodies (CAbs) against avian HEV by indirect ELISA using a commercially available kit (Nuoyuan Co, Ltd, Shanghai, China) according to the manufacturer's instructions. The positive and negative controls were supplied in the kit and contained in each test.

Statistical analysis: Statistical analysis of the seroprevalence of avian HEV infection in parrots from different locations, ages, genders and species were tested by Chi-square in SAS (Statistical Analysis System, Version 9.0) (Ahad *et al.*, 2013). Results were considered statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

In the present survey, CAbs against avian HEV were detected in 20 (6.43%) of the 311 examined parrot serum samples by ELISA (Table 1). Seroprevalence of avian HEV infection in different parrot species varied from 0 to 7.92% (Table 1). Male parrots had a higher HEV seroprevalence (7.36%) than females (5.41%), although the difference was not statistically significant. Parrots from Weifang city had a higher avian HEV seropositivity (7.84%) compared with that from Beijing city (5.06%), but the difference was not statistically significant (Table 1). The HEV seroprevalence in parrots of different age groups varied from 4 to 7.62%, and parrots examined in spring had a slightly higher (7.19%) HEV seroprevalence compared to parrots collected in summer (5.81%), although these differences were not statistically significant (Table 1).

Parrot is one of the popular pet birds among Chinese families and zoos because of the exclusively ornamental use. Many families in Beijing and Weifang cities breed parrots for commercial purposes, and produce approximately 70% of the parrots sold in China every year. Previous study showed that birds are susceptible to HEV (Zhang *et al.*, 2008), and is considered an important factor influencing the economic income of the poultry industry.

In the present study, we investigated the seroprevalence of avian HEV in four species of parrots in Beijing and Weifang Cities. The overall avian HEV seroprevalence in parrots was 6.43% (95% CI 3.71-9.16), which was lower than that in chickens (35.9%) in Shandong province (Zhao *et al.*, 2013), but higher than that in chickens in Liuzhou (1.49%), and Hebei (0%) (Zhu *et al.*, 2004). The differences in HEV seropositivity may be resulted from differences in living environments, immune capacities, serologic tests used, feeding conditions, resistance to the pathogen, as well as differences in animal husbandry practices and animal welfares.

The HEV seroprevalence in juveniles (≤5 months, 7.62%, 95% CI 2.54-12.69) is similar to that in adults (13-18 months, 7.55%, 95% CI 2.52-12.58), but higher than that in sub-adults (6-12 months, 4%, 95% CI 0.16-7.84). But among age groups, the difference in HEV seroprevalence was not significant statistically (P=0.48) (Table 1). The findings of our study is different from that of previous studies which showed that the seroprevalence of avian HEV in older chickens (>18 weeks) was higher than in youngers (<18 weeks) (Peralta *et al.*, 2009).

In China, most parrots are bred in the semi-free range system, which increases the opportunity of exposure to food or water contaminated with HEV when they gather together. There was no statistical difference in HEV seroprevalence between male parrots (7.36%, 95% CI 3.35-11.37) and the females (5.41%, 95% CI 1.76-9.05) (Table 1), probably because they live in the same environment.

In north China, the climates are high temperature and rainy in summer but dry and strong wind in spring, which

Table 1: Seroprevalence of avian hepatitis E virus infection in pet parrots in Beijing and Weifang cities detected by indirect Enzyme Linked Immunosorbent Assay (ELISA)

Variable	Category	No. of serum samples	No. of positive samples	Prevalence (%) (95% CI)	P-value
Region	Beijing	158	8	5.06 (1.65-8.48)	0.32
	Weifang	153	12	7.84 (3.58-12.10)	
Sex	Male	163	12	7.36 (3.35-11.37)	0.48
	Female	148	8	5.41 (1.76-9.05)	
Species	Budgerigar (*Melopsittacus undulatus*)	202	16	7.92 (4.20-11.65)	0.26
	Alexandrine Parakeet (*Psittacula eupatria*)	61	4	6.56 (0.35-12.77)	
	Lovebirds (*Agapornis* sp.)	26	0	0 (-)	
	Cockatiel (*Nymphicus hollandicus*)	22	0	0 (-)	
Age	Juveniles (≤5 months)	105	8	7.62 (2.54-12.69)	0.48
	Sub-adults (6-12 months)	100	4	4 (0.16-7.84)	
	Adults (13-18 months)	106	8	7.55 (2.52-12.58)	
Season	Spring	139	10	7.19 (2.90-11.49)	0.62
	Summer	172	10	5.81 (2.32-9.31)	
Total		311	20	6.43 (3.71-9.16)	

is suitable for the spreading of the pathogen. The seroprevalence of HEV in budgerigar (7.92%, 95% CI 4.20-11.65) is similar to that in Alexandrine Parakeet (6.56%, 95% CI 0.35-12.77), but higher than that in lovebirds (0%) and cockatiel (0%), and the difference was not statistically significant among different parrot species (Table 1). No HEV seropositivity was detected for lovebirds and cockatiel parrots, possibly due to the small number of sampled parrots. Further studies should increase the number of sampled parrots.

Conclusion: the present investigation revealed that 6.43% of the examined parrots of four species in China were seropositive with avian HEV infection. This is the first report of the seroprevalence of HEV in budgerigars, Alexandrine parakeets, lovebirds and cockatiel in China, which provided the base-line data for taking integrated strategies and measures for the effective prevention and control of HEV infection in birds in China.

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