ABSTRACT Ornithobacterium rhinotracheale (ORT) has been associated with avian respiratory disease. On coinfection with other pathogens, ORT can cause serious health problems in avian species, leading to financial losses. To monitor the serologic prevalence of ORT in chicken flocks in China, 1,280 sera were collected to determine ORT antibodies among 64 flocks from 15 provinces of China using a commercial ELISA kit. The overall seroprevalence of ORT among the birds tested was 44.06%. In younger chickens, the serum positive rate was lower than that in older chickens, and with increased age, the serum positive rates increased. Older chickens had not only higher positive rates but also higher antibody levels. These data indicated that ORT infections were common in China. Because an ORT vaccine is currently not available, good disease management and biosecurity measures are required for effective disease control.

Key words: Ornithobacterium rhinotracheale, ORT, seroprevalence

INTRODUCTION

Ornithobacterium rhinotracheale (ORT), a gram-negative rod-shaped bacterium, was first introduced into domesticated poultry from wild birds (Amornsin et al., 1997). The first observation of a new disease syndrome in turkeys was reported in the early 1990s in Germany (Hinz et al., 1994), and the causative bacterium was firstly named in 1994 (Vandamme et al., 1994). ORT infection is common in turkeys and chickens and can be transmitted horizontally and vertically. To date, 18 ORT serotypes (A–R) have been identified, but no direct relationship with virulence has been reported (Zahra et al., 2013; De la Rosa-Ramos et al., 2018; Montes et al., 2018). Analysis of the rpoB gene has been used to distinguish similar strains (Veiga et al., 2019). ORT can cause retarded growth, decreased egg production, and increased mortality and has been associated with avian respiratory disease. Viral infections caused by avian metapneumovirus, infectious bronchitis virus, and Newcastle disease virus and bacterial infections caused by Avibacterium paragallinarum, Bordetella avium, and Escherichia coli can cause severe clinical symptoms when coinfected with ORT (Ellakany et al., 2019). The severity of ORT depends on the strain pathogenicity and other factors such as age, coinfections, and management practices.

Antibiotics are often used to treat ORT. However, the sensitivity of ORT to antibiotics is variable depending on the strain and the environmental conditions of the chickens. The administration of amoxycillin (250 ppm) in water for 3 to 7 D or chlortetracycline (500 ppm) for 4 to 5 D is the current recommended treatment for ORT. The prevention of ORT is based on good hygiene and preventative therapy. Vaccination is not currently available in China. In other countries, some inactivated, attenuated, or subunit vaccines have been developed and evaluated (Lopes et al., 2002; Schuijffel et al., 2006; De Herdt et al., 2012). Clinical diagnosis of ORT is based on isolation of the organism. Cultures of ORT from the trachea of chickens grow slowly producing tiny colonies on blood agar, so this method is slow and lacks sensitivity. ORT can also now be detected by PCR or ELISA (Refai et al., 2005; Abdelwhab et al., 2013).

Despite the economic importance of this pathogen, few epidemiological studies on ORT have been reported in China. To monitor the immune status and serologic identification of ORT in large flocks, ELISA has proven...
efi cacious in the quantification of antibody levels to ORT in chicken serum. To investigate the current prevalence of ORT in healthy chicken flocks in China, 1,280 serum samples were collected randomly from varied ages and breeds of chickens in 2019 across 15 provinces of China and detected for ORT antibodies using the ELISA method.

MATERIALS AND METHODS

Sampling

A total of 1,280 sera were obtained to determine positivity to ORT infection among nonvaccinated chickens from 64 flocks of 15 provinces in China in 2019. The sera were stored at −20°C before use.

Serology

Sera were tested for antibodies to ORT using a commercial ELISA test kit according to the manufacturer’s instructions (IDEXX, Westbrook, ME). This ELISA test kit detects the serological response to ORT serotypes A–M. Briefly, serum samples was diluted ten-fold from an initial dilution of 1:50 in phosphate-buffered saline (final dilution was 1:500), then 100 μL of each diluted sample was added to the wells and incubated at 18°C to 26°C for 30 min. After the incubation, the plate was washed with 350 μL of wash buffer (3–5 repeats). A conjugate reagent was added to each well and incubated at 18°C to 26°C for 30 min. Then, the plate was washed with 350 μL of wash buffer (3–5 repeats). Then, 100 μL of tetramethylbenzidine substrate buffer was added to the wells and incubated at 18°C to 26°C for 15 min. The reaction was quenched with 100 μL of stop solution. The absorbance of the sera was measured at 650 nm. Sera with S/P values above the cutoff level of 0.40 (titer = 844) were considered positive.

RESULTS AND DISCUSSION

The data from this study revealed ORT seropositive chickens from all provinces in China, and the overall seroprevalence of the chickens was 44.06% (Table 1). Among the provinces, Henan had the highest positive rate of 98.33%. The data were obtained from 3 different flocks, and the age of these chickens were 22, 34, and 66 wk, respectively. Jiangsu had the largest sample size but the lowest positive rate, which may be related to the fact that most samples were from younger chickens. Guangdong and Yunnan had the same positive rate, but their chickens were of different ages. The Guangdong samples were from chickens aged 3 and 7 wk, whereas the Yunnan samples were from chickens aged 16 and 38 wk. Tianjin (12 w), Guangxi (13 w), and Hunan (24 w) had only one sample set each, but the difference

Table 1. Seroprevalence of Ornithobacterium rhinotracheale infection in China.

| Province | Flocks tested (n) | Birds tested (n) | Positive (n) | Positive rate (%) |
|----------|-------------------|-----------------|--------------|------------------|
| Beijing  | 8                 | 160             | 33           | 20.63            |
| Guangdong| 2                 | 40              | 23           | 57.50            |
| Guangxi  | 1                 | 20              | 13           | 65.00            |
| Hebei    | 10                | 200             | 104          | 52.00            |
| Heilongjiang | 3         | 60              | 31           | 21.67            |
| Henan    | 3                 | 60              | 59           | 98.33            |
| Hunan    | 1                 | 20              | 9            | 45.00            |
| Jiangsu  | 11                | 220             | 36           | 16.36            |
| Liaoning | 6                 | 120             | 58           | 48.33            |
| Inner Mongolia | 2    | 40              | 20           | 50.00            |
| Shaanxi  | 3                 | 60              | 38           | 63.33            |
| Shandong | 9                 | 180             | 104          | 57.78            |
| Shanxi   | 2                 | 40              | 27           | 67.50            |
| Tianjin  | 1                 | 20              | 4            | 20.00            |
| Yunnan   | 2                 | 40              | 23           | 57.50            |
| Total    | 64                | 1,280           | 564          | 44.06            |
in positive rate was obvious. In addition to the differences in age, the variations in the seropositive rates may also be affected by the management practices on different chicken farms.

The rates of ORT detection in chickens of different ages are shown in Figure 1. In the early stages of chicken growth, the positive rates were low or even zero. With increased age, the positive rates also increased. After 49 wk of age, the seroprevalence of ORT among the chickens was 90% or above. At 18 wk of age, the positive rate was zero, possibly because these chickens were from a flock in Hebei, which had good management practices. Chickens younger than 18 wk mainly tested negative, whereas those older than 18 wk mainly tested positive. The ORT antibody levels of each chicken are shown in Figure 2. Older chickens had not only higher positive rates but also higher antibody levels; in fact, the levels were higher than the threshold value. The highest antibody titer recorded was 39,954, which was 47 times higher than the cutoff level.

In conclusion, ORT infection is currently common among chickens in China. With increased age, the seroprevalence also increased, and older chickens had higher antibody levels. With the lack of an ORT vaccine, good management practices and biosecurity measures are required for effective disease control.

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