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SHORT COMMUNICATION

Serological Survey of the Reticuloendotheliosis Virus Infection in China Native Chicken Flocks

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ABSTRACT

To investigate the reticuloendotheliosis virus (REV) infection status in China, 2531 sera samples from 26 farms of native chickens were collected and tested for antibodies against reticuloendotheliosis virus. Serum samples analysis revealed 32.16% samples positive for REV-antibody. All 26 kinds of China native chicken strains have REV infection. REV-antibody positive rates of different flocks ranged from 1.01 to 67.68%. This study suggests that REV infection is very common in China native chickens flocks and more emphasis be given on its prevention.

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INTRODUCTION

Reticuloendotheliosis virus is a member of the family Retroviridae, it differs from another avian retrovirus, avian leukosis viruses and capable of inducing neoplasms, runting, and immunosuppression in chickens. REV caused losses to chickens mainly due to vertical infection and early horizontal infection. Usually, adult chickens infected by REV only produced transient viremia and it is difficult to see typical tumor or clinical symptoms. However, if the chicken infected by REV in embryo as a vertical infection form, it often caused persistent viremia and immune suppression even for the lifetime in some individuals (Witter *et al*., 2003).

More and more attentions were given to contaminations with REV in some live poultry vaccines, such as Fowl pox virus (FPV) vaccine (Awad *et al.*, 2010). More importantly, some FPV vaccine and field strains contained the intact REV genome (Biswas *et al.*, 2011), indicating that REV has a very special transmission way.

For a long time, the economic losses caused by natural REV infections were not completely recognized. Early in the 1980's, REV infection was only occasionally reported in China, but recent studies indicated that REV infection is becoming more and more common in chicken farms (Zhang *et al.*, 2003; Jiang *et al.*, 2005). While there are no large-scale serological surveys in China for more than ten years, the REV infection status in chickens is not clear especially for the amount of China native chicken flocks. In this study, 2531 sera samples from 26 kinds of China native chickens were collected and tested to investigate the REV infection status in these native chickens.

MATERIALS AND METHODS

In this study, 26 kinds of China native chicken strains i.e., Taihe, Xianju, Gushi, Xiaoshan, Beijing, Langshan, White ear, Luyuan, Big bone, Chahua, New Langshan, Shiqi, Youxi, Chongren, Tibetan, Green-shell layer chicken and so on. All these kinds of chickens are protected as National Gene Pool for Native Poultry Breeds in China, according to the requirements of manufacturers it was marked with No. 01 to 26 instead of the chicken's strain as listed in Table 1. Each chicken was subjected to collect blood from the vein and the sera were installed in -40°C.

Antibody against REV: The sera were assayed for antibodies to REV by ELISA using the Reticuloendotheliosis Virus Antibody Test Kit (IDEXX Laboratory) according to the manufacturer's instructions. If the S/P>0.5, the sera was judged as positive to REV antibody. In order to ensure the accuracy of results, each sample was tested twice.

RESULTS

Among 2531 sera samples 814 (32.16%) were detected positive for REV-antibody. Although these 26 kinds of native chickens invested all have REV infection, their positive rates were quite different from each other as the lowest positive rate of 1.01% for Farms No. 02 while the highest positive rate 67.68% for farm No.07 was recorded. The S/P value for different sera samples was quite different even for the same chicken group, such as

the samples from Farms No. 26, the lowest S/P value was only 0.503, while the highest was up to 16.290. The same situation was also found in Farms No.03, 05, 06, 07, 09, 10, 24 and so on (Table 1). Among 814 sera samples for REV-antibody positive, level of S/P values varied from low, middle, higher and the highest in 38.33, 27.15, 30.47 and 4.05% sera samples, respectively (Table 2).

Table 1: Detection of REV antibody in sera from 26 native chicken flocks

Farms No.	Total birds	Chicks Positive for REV-antibody		S/P value for REV positive serum	
		No.	%	Minimum	Maximum
01	100	3	3.00	0.667	1.475
02	99	1	1.01	0.517	0.517
03	99	52	52.53	0.513	8.170
04	99	25	25.26	0.505	4.983
05	101	39	38.61	0.510	8.148
06	100	39	39.00	0.508	11.145
07	99	67	67.68	0.504	11.92
08	100	60	60.00	0.503	6.769
09	101	64	63.37	5.508	9.990
10	98	45	45.92	0.503	12.273
11	100	36	36.00	0.510	4.393
12	99	5	5.05	0.641	1.812
13	98	3	3.06	1.218	8.053
14	100	9	9.00	0.581	1.282
15	42	12	28.57	0.511	6.902
16	98	4	4.08	2.708	8.542
17	100	11	11.00	0.545	6.892
18	97	4	4.12	3.057	8.068
19	99	23	23.23	0.523	7.665
20	100	56	56.00	1.783	8.028
21	100	62	62.00	0.601	8.135
22	101	49	48.51	0.607	9.717
23	101	24	23.76	0.538	9.649
24	96	49	51.04	0.837	10.707
25	100	18	18.00	0.795	9.161
26	104	54	51.92	0.534	16.290
Total	2531	814	32.16		

Note: All flocks investigated were more than 150 days old and every farm was vaccinated with MDV live vaccine and FPV live vaccine.

Table 2: Analysis of different S/P value for REV-antibody positive serum

Level of S/P values	S/P value	Number	%
Low	0.500-0.999	312	38.33
Middle	1.000-4.999	221	27.15
Higher	5.000-9.999	248	30.47
Highest	>15.000	33	4.05
	Total	814	100

DISCUSSION

In recent years, more and more studies have found that REV genome components can be integrated into other viral genome, such as the REV gene fragment can be integrated into the genome of FPV (Biswas *et al.*, 2011). Our previous study has proved the co-infection of Marek's disease virus (MDV) and REV in Marek's tumor samples and some recombinant field MDV strains with partial REV genome were identified (Zhang and Cui, 2005). The phenomena of natural genetic recombination between REV and MDV or FPV warned that the co-infection and recombination of REV with other viruses would speed up evolution of some viruses and make the detection to REV using the molecular biological methods becoming more and more difficult. For there is no commercial vaccine to control REV, it is reasonable to reflect the REV infection status with serological method.

China has 48 strains of native chicken. Although they are the important protection subject of the government, however, these native chickens also face a lot of viral infectious disease threats especially for immuno-suppressive virus. In this study, serum samples from 26 kinds of China native chickens were collected and tested the antibody against REV and we found all these native chickens have REV infection. REV-antibody positive rate of different flocks ranged from 1.01 to 67.68%. The results indicated it is very necessary to take some strong measures to prevent and control REV infection in china native chickens. We also found the S/P value in the ELISA test for different sera samples was quite different even for the same chicken group, such as the samples from farms No.26, the highest was up to as high as 16.290 while the lowest S/P value was only 0.503. This indicate different individuals infected by REV will show different response and this may be related to REV epidemic situation in different regions or susceptibility to REV of different native chickens.

Evidence of REV-antibody can be accomplished by maternal antibodies (Wu *et al.*, 2009), but it's impossible for these flocks with so high maternal antibody level for all of them were more than 150 days old, more likely the high antibody titer in these chickens is caused by wild virus infection. Of course, there may be some chickens using FPV live vaccine and MDV vaccine with REV contamination for all these chickens have been vaccinated with FPV live vaccine and MDV live vaccine. As the vaccines used in these native chickens have no storage and can't be verified one by one, this speculation cannot be identified. While the REV was isolated and identified from a SPF chicken farm in China recently (Wang *et al.*, 2012) and the REV contamination in live poultry vaccine may be a very important channel for the REV infection.

Although these chickens investigated have such a high REV positive rate, no significant tumor or death found in these flocks. As reported early REV infection in chickens could not only cause growth retardation but also severely suppress immune responses to vaccinations against Avian influenza virus (AIV) and Newcastle disease virus (NDV) (Sun *et al.*, 2006). So the REV infection in these chickens may cause great losses by depressing immunity induced by Avian influenza and Newcastle disease vaccine. Of course, another possible reason is there is certain REV resistant strains in China native chickens, but this need further research and validation.

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