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Serological surveillance for FMDV antibodies serotypes O and A in cattle in Tongliao, Inner Mongolia, China*

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Summary

Foot-and-mouth disease (FMD) is an acute, febrile and highly contagious disease caused by foot-and-mouth disease virus (FMDV), mainly affecting cloven-hoofed animals. FMDV antibody detection can provide a timely understanding of the immune level after vaccination. In this study, 22,902 cattle blood samples from free-range households and large-scale farms were collected from eight districts in Tongliao city, Inner Mongolia Autonomous Region, China, from January to December 2020. Serum antibodies against serotypes O and A FMDV were detected by liquid-phase blocking ELISA. The results showed that the positive rates of serotypes O and A FMDV antibodies in Tongliao were 91.21% and 90.18%, respectively. The positive rates of serotypes O and A FMDV antibodies in 8 regions ranged from 85.80% to 98.41% and 84.48% to 96.77%, respectively. The positive rates of serotypes O antibodies in cattle from households and farms were 82.15% to 97.41%, and 90.51% to 99.32%, respectively. The positive rates of serotypes A FMDV antibodies in cattle from households and farms ranged from 83.47% to 96.77% and 84.98% to 97.30%, respectively. The positive rates of serotype O FMDV antibody in spring, summer, autumn and winter were 92.68%, 94.40%, 88.84% and 84.32%, respectively, and those of serotype A FMDV antibody were 93.50%, 92.19%, 87.26% and 88.86%, respectively. The results indicated that the immunization level of FMD was high in Tongliao city, and all positive rates exceeded the requirement that the qualified rate of immune antibody should be kept above 70% all year long according to the National Compulsory Immunization Plan of Animal Epidemic Diseases in 2020. However, vaccination and antibody detection of cattle raised by households in autumn and winter should be strengthened. The results of this study can provide a basis for authorities to formulate FMD prevention and control plans.

Keywords: antibody detection, liquid-phase blocking ELISA, cattle, serotypes O and A foot-and-mouth disease, FMD virus

Foot-and-mouth disease (FMD) is one of the most contagious animal diseases characterized by fever, loss of appetite, depression, lameness and the appearance of vesicles (fluid-filled blisters) that most commonly occur on the feet and in, or around, the mouth (1, 3, 15). The causative agent FMDV affects cloven-hoofed animals both domestically and wild species (2, 4, 13). Animal exposure could result in 100% morbidity (12). FMD is caused by an *Aphthovirus* of the family *Picornaviridae*, and seven strains (A, O, C, SAT1, SAT2, SAT3, and Asia1) are endemic in different countries worldwide. There is great antigenic diversity between the serotypes, and often there is also no cross-protection between them (7, 8, 11). Each strain requires a specific vaccine to provide immunity to a vaccinated animal. To date, over 160 countries and regions have reported FMD (20). The disease is estimated to circulate in 77% of the global livestock population in Africa,

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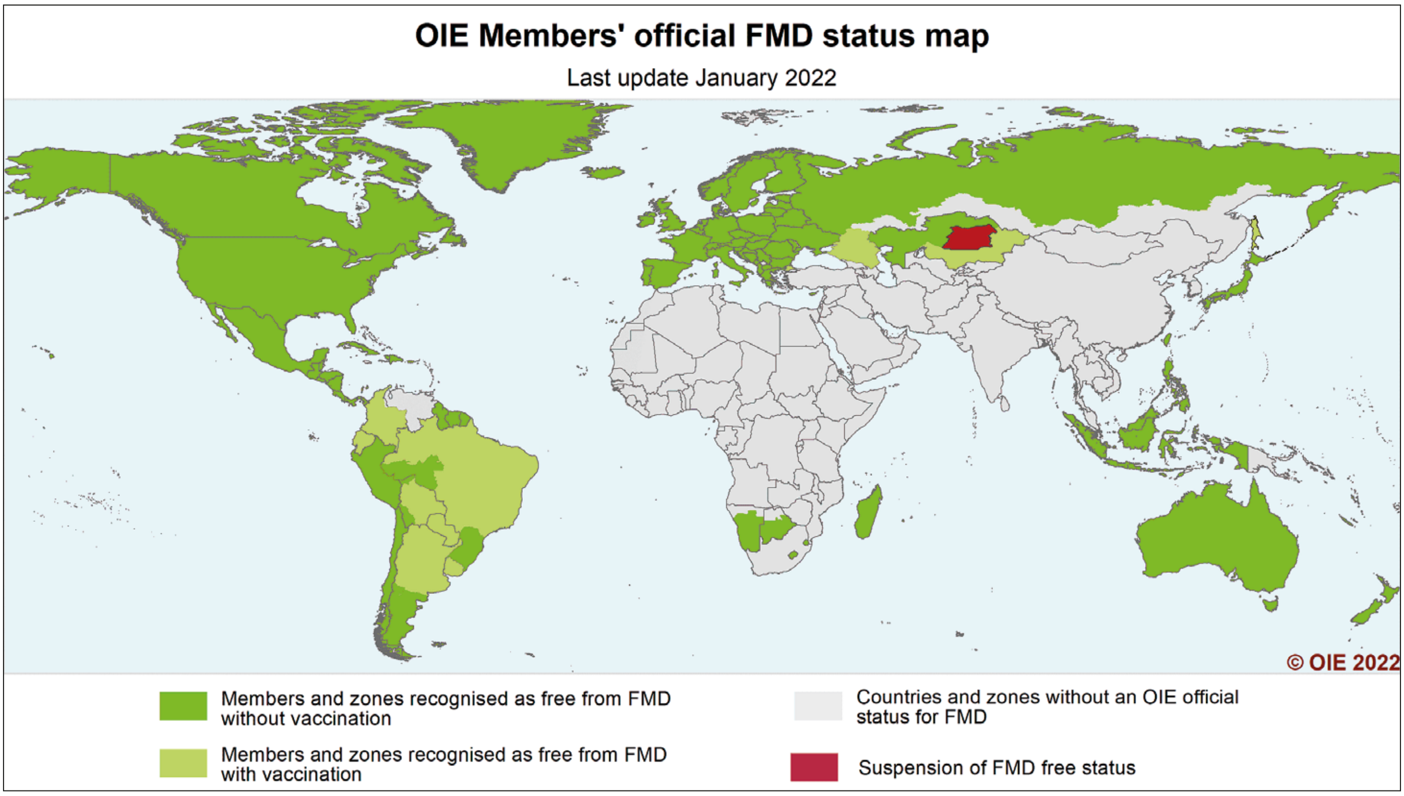

Fig. 1. Epidemic distribution of foot-and-mouth disease worldwide (from OIE website)

the Middle East and Asia, as well as in a limited area of South America (Fig. 1). Environment transmission plays a key role in the disease spreading process (20). Although FMD does not result in high mortality in most outbreaks, the effects of this disease have been far-reaching, not only on animal health and rural economies but also on the prospects of the livestock industry and international trade (14, 16). The annual global impact of FMD in endemic regions amounts to between 6.5 and 21 billion USD resulting from visible production losses and vaccination costs, while outbreaks in FMD-free countries and zones cause additional losses exceeding 1.5 billion USD per year (9, 10). In view of the seriousness of the disease, the World Organisation for Animal Health (OIE) listed FMD as the first of a list of infectious diseases in animals.

Foot-and-mouth disease is classified as a class I animal disease and a class I infectious disease of imported animals in China. FMD outbreaks have been reported in China for many years. The earliest written outbreak record in mainland China was in 1893 in Yunnan Province and the Tibetan Plateau, and the first reports of FMDV isolates were in 1958 in the Xinjiang Uygur Autonomous Region and Yunnan Province (20). Since June 2011, no positive samples of Asian type I FMD have been found in China (21). At present, serotypes O and A FMD are prevalent in mainland China. Applicable control measures for FMD involve animal movement restrictions, a vaccination program, animal quarantine, environmental sanitary controls, outbreak investigations, serological surveillance, and slaughtering of sick animals (18). To contribute to the long-term goal to control and eradicate FMD in China, the Chinese government has adopted a series of control measures, including compulsory routine vaccination against the disease and a surveillance programme. Compulsory routine vaccination is one of the most effective and influential methods for the prevention and control of FMD. It is also the most important means for the prevention and control of FMD in China. Compulsory routine vaccination against FMD in China involves vaccination of all pigs, cattle, sheep, camels and deer against serotype O FMD. All dairy cattle and breeding bulls must be vaccinated against serotype A, and all cattle and sheep, camels and deer in border areas from Inner Mongolia, Yunnan, Tibet, Xinjiang and the Xinjiang Production and Construction Corps must be vaccinated against serotype A. The immunization effect is directly related to the prevention and control effect of FMD. When a compulsory vaccination programme is completed, a surveillance programme is required to be implemented. The surveillance is designed to assess vaccine coverage and its effectiveness. Serological surveillance is an important means to measure the immune effect. Serum antibody level monitoring of immunized animals can provide a timely understanding of the occurrence and prevalence of FMD, effectively carry out scientific prevention and control and risk assessment and is also an important basis for formulating FMD prevention and control programs.

Inner Mongolia has traditional advantages in regional resources, cattle raising experience and cattle product reputation. The cattle industry has always been its characteristic industry. Tongliao, located in eastern

Inner Mongolia, is one of the main cattle-raising areas. The number of cattle, breeding cows and fattening cattle ranks first in Inner Mongolia and even the whole country. Tongliao has a developed beef cattle industry, excellent geographical location, convenient transportation routes, and active cattle trading, greatly increasing the chance of FMD transmission and the serious situation of FMD prevention and control.

FMD is sporadically spread in China, and there are great differences in epidemic situations and vaccine immunization effects in different regions. The purpose of the study was to define the status of FMDV antibodies for serotypes O and A in Tongliao and provide a basis for the development of FMD prevention and control plans.

Material and methods

The study sites. The study was conducted in Tongliao city, Inner Mongolia Autonomous Region, China. Eight regions in Tongliao city were selected and marked as A, B, C, D, E, F, G and H (Fig. 2). The Inner Mongolia Autonomous Region is located in North China, bordering Mongolia and Russia. It is one of the major cattle-raising regions in China, and Tongliao city is one of the major representative cattle-raising regions in Inner Mongolia.

Samples. From January to December 2020, a total of 22902 cattle blood samples from farmers and farms were collected from 8 regions (marked with the letter A to H) in Tongliao city, Inner Mongolia Autonomous Region, China. Blood was collected by jugular venepuncture and allowed to stand at ambient temperature for 1 to 2 h. It was then transferred to an Esky container for several hours and centrifuged at 3000 rpm for 10 to 15 min to separate the serum. Sera were then transferred into two Eppendorf tubes for the subsequent ELISA tests.

Test procedure. The serum antibody levels of serotypes O and A FMDV were detected by liquid-phase blocking (LPB) ELISA. LPB ELISA is serotype-specific and is highly sensitive, and the virus or antigen used in the test was closely matched to the strain circulating in the field. The specific procedures were carried out according to the instructions of the improved liquid-phase blocking ELISA kits for detecting antibodies of serotypes O or A FMDV produced by Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

Interpretation. Four wells of virus antigen control were set in each 96-well ELISA plate, the highest and lowest D_{450nm} values were discarded, and the average D_{450nm} values of the remaining two wells were calculated and then divided by 2; i.e., the 50% control value, which was the critical value, indicated that the control D_{450nm} value blocked 50% of the reaction. If the D_{450nm} value of the tested serum was greater than the critical value, it was judged as negative, and if it was less than or equal to the critical value, it was judged as positive. If the critical value was the same as the D_{450nm} value of the positive well with the highest dilution ratio, the highest dilution ratio of the positive well was used as the antibody titer of the serum. If the critical value was between the D_{450nm} values of two dilution wells, the intermediate value of the antilog of dilution of multiple adjacent positive wells and negative wells was taken as the antibody titer. The specific calculation of the antibody titer is shown in Table 1.

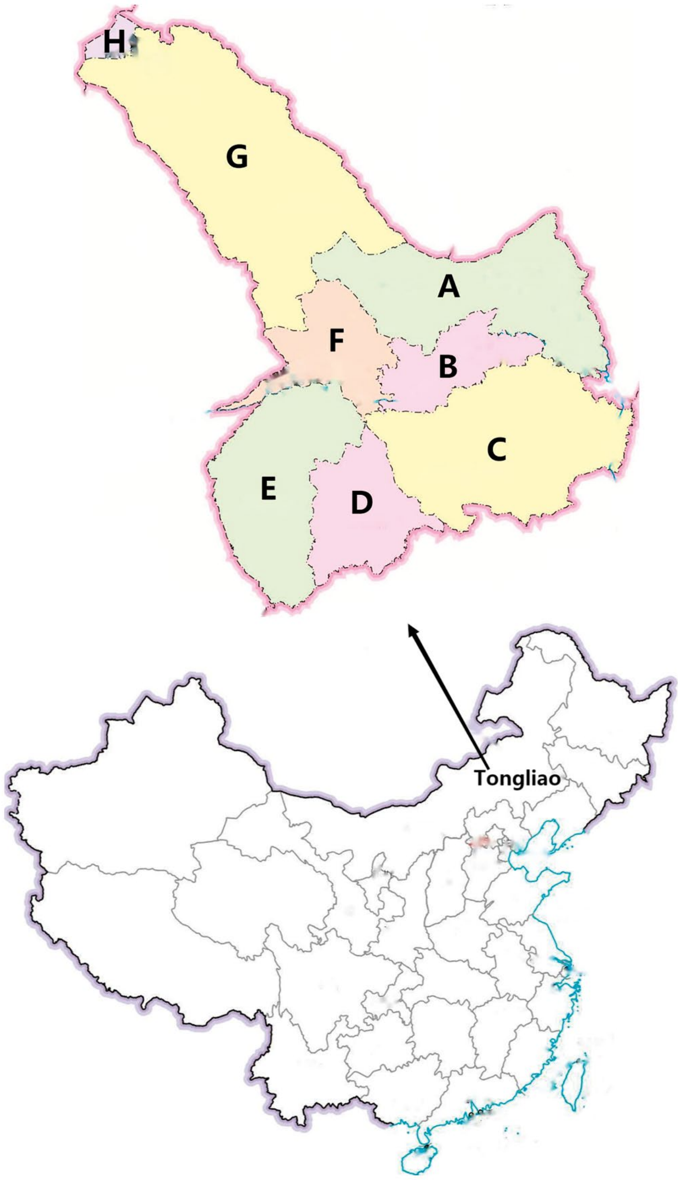

Fig. 2. Diagrammatic sketch of the geographic location of collection sites

If the antibody titer was greater than or equal to 1 : 128, the sample was judged to be positive for serotype O or A FMDV antibody.

If the antibody titer was less than 1 : 64, the sample was judged to be negative for serotype O or A FMDV antibody.

Tab. 1. Comparison table of antibody titer calculation

Antibody titer	Log_{10} (titer)	Antibody titer	Log_{10} (titer)	Antibody titer	Log_{10} (titer)
1 : 4	0.6	1 : 32	1.5	1 : 256	2.4
1 : 6	0.75	1 : 45	1.65	1 : 360	2.55
1 : 8	0.9	1 : 64	1.8	1 : 512	2.7
1 : 11	1.05	1 : 90	1.95	1 : 720	2.85
1 : 16	1.2	1 : 128	2.1	1 : 1024	3
1 : 22	1.35	1 : 180	2.25	1 : 1440	3.15

If the antibody titer was between 1 : 64 and 1 : 128, the sample was considered suspicious for serotype O or A FMDV antibody. Suspicious samples were retested. If the antibody titer was greater than or equal to 1 : 128 after retesting, the sample was judged to be positive for serotype O or A FMDV antibody. When the antibody titer was less than 1 : 128, the sample was judged to be negative for serotype O or A FMDV antibody.

Data analysis. Excel 2016 and GraphPad Prism 8.0.1 were used for the data summary and descriptive analysis of the detected results.

Results and discussion

Overview. As detailed in Table 2, from January to December 2020, a total of 22,902 blood samples of cattle were collected from 8 regions (marked with the letter A to H) in Tongliao city. The data are classified by season and breeding scale. The share of household samples is 56.22%, and the share of farm samples is 43.78%, as shown in Figure 3. The serotypes O and A FMDV antibody-positive rates were 91.21% and 90.18% of the households and farms, respectively.

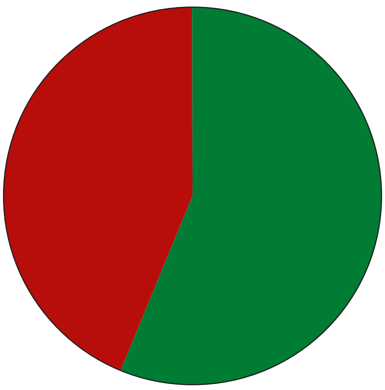

Fig. 3. Sample composition by source
Explanations: A total of 22,902 serum samples were detected in 8 different regions of Tongliao, Inner Mongolia, China. The sample quantity and percentage from different sources are shown. The red colour in the figure represents the samples from households, and the blue colour represents the samples from farms. The number 1 represents that the cattle are from households, and 2 represents that the cattle are from farms

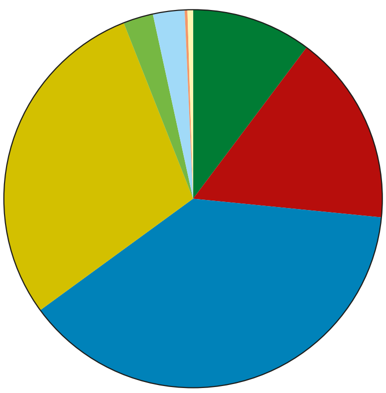

Fig. 4. Sample composition by region
Explanations: A total of 22,902 serum samples were collected from 8 different regions of Tongliao, Inner Mongolia, China. The sample quantity and percentage in each region are shown. The letters A to H represent 8 regions, marked with different colours

Tab. 2. Number of serum samples from regions of Tongliao, Inner Mongolia, China in 2020

Region	Source	Number of serum samples				Total
		Spring	Summer	Autumn	Winter	
A	A1	7	580	508	457	1552
	A2	5	543	93	139	780
B	B1	451	1049	1349	484	3333
	B2	20	281	122	0	423
C	C1	894	2930	2089	812	6725
	C2	60	1776	220	6	2062
D	D1	0	51	311	8	370
	D2	0	3193	2707	380	6280
E	E1	81	77	0	74	232
	E2	0	0	333	0	333
F	F1	67	0	432	50	549
	F2	138	0	0	10	148
G	G1	0	0	0	22	22
	G2	0	0	0	0	0
H	H1	93	0	0	0	93
	H2	0	0	0	0	0
Total		1816	10480	8164	2442	22902

Explanations: the different numbers after the capital English letters represent the different sources of cattle. The letters A to H represent 8 regions in Tongliao city. The number following the letter represents the breeding scale, where 1 represents the cattle from households and 2 represents the cattle from farms

The positive rates fulfilled the requirement of ≥ 70% set by the National Compulsory Immunization Plan of Animal Epidemic Diseases in 2020.

The FMDV antibody-positive rates analysed by source and region. The sample composition by source and region are shown in Figure 3 and Figure 4. The serotype O and A FMDV antibody test results by source and region are shown in Figure 5 and 6. The positive rate of serotype O FMDV antibody in cattle from 8 regions of Tongliao city ranged from 85.80% to 98.41%. The lowest positive rate was 85.80% in region F, and the highest was 98.41% in region E. The positive rates of serotype O FMDV antibody in cattle from households and farms were 88.94% and 94.12%, respectively. The positive rate of serotype O FMDV antibody in cattle from households was lower than that from farms. The positive rate of serotype O FMDV antibody in region F was the highest among cattle from farms in the 6 sampling regions, while the positive rate of serotype O FMDV antibody in region F was the lowest in cattle from households among the 8 sampling regions. The positive rate of serotype A FMDV antibody in 8 regions of Tongliao city ranged from 84.48% to 96.77%, also exceeding the national requirements of more than 70%. The lowest positive rate was 84.48% in region B, and the highest was 96.77% in region H. The positive rates of serotype A FMDV antibody in cattle from households and farms were 88.47% and 92.38%,

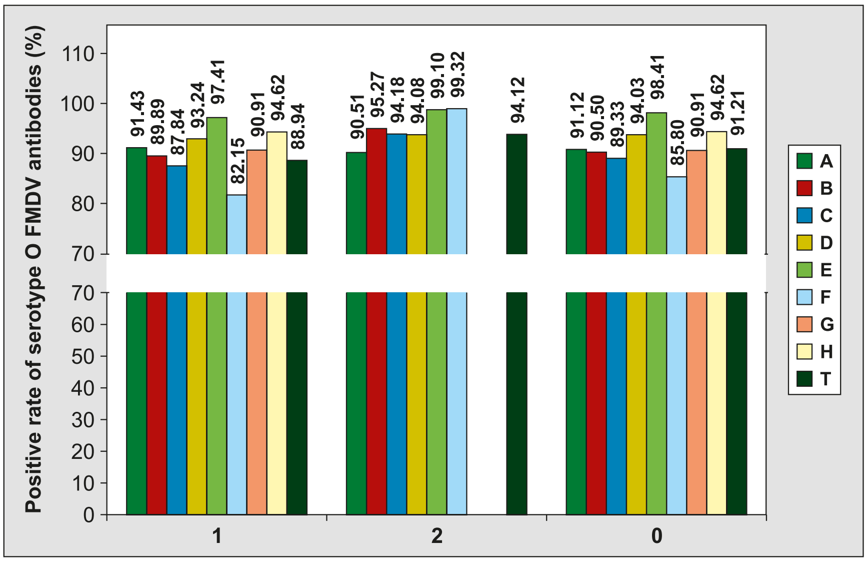

Fig. 5. Positive rate of serotype O FMDV antibody in samples from different regions and sources

Explanations: In the legend, the letters A to H represent 8 different regions in Tongliao city, and the letter T represents all the dates of the 8 regions. On the horizontal axis, 1 represents cattle from households, 2 represents cattle from farms, and 0 represents the sum of cattle from households and farms

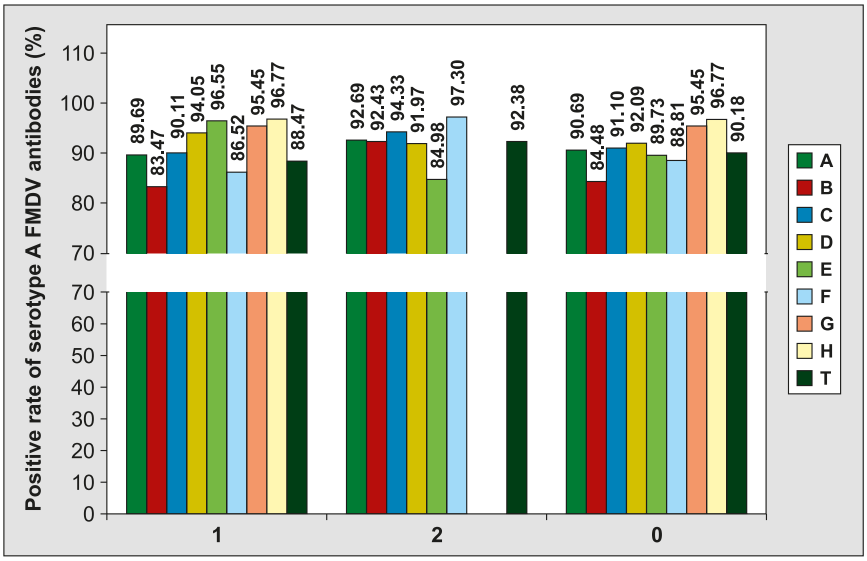

Fig. 6. Positive rate of serotype A FMDV antibody in samples from different regions and sources

Explanations: as in Fig. 5.

respectively. The positive rate of serotype A FMDV antibody in cattle from households was lower than that in cattle from farms. The positive rates of serotypes O and A FMDV antibody in cattle from households in different regions were almost consistent with the trend of that in different regions throughout the year, indicating that immunization and antibody testing of cattle from farmers need to be further strengthened. The positive rate of serotype A FMDV antibody in cattle from farms in region F was the highest among the 6 sampling regions, but the positive rate of serotype A FMDV antibody in cattle from farmers in region F was 86.52%, which was only higher than 83.47% in region B among the 8 sampling regions. The results indicated that immunization against serotype A FMDV should be strengthened in both regions B and F.

The FMDV antibody-positive rates analysed by region and season. The sample compositions by season are shown in Figure 7. The serotype O and A FMDV antibody test results by region and season are shown in Figure 8 and 9. The positive rates of serotype O FMDV antibody in Tongliao city in spring, summer, autumn and winter were 92.68%, 94.40%, 88.84% and 84.32%, respectively.

Comprehensive analysis shows that the immunization against serotype O FMDV in autumn and winter should be strengthened in Tongliao city, especially the immunization, antibody detection and revaccination; these should be performed extensively in region C to further improve the positive rate of serotype O FMDV antibody. The positive rates of serotype A FMDV antibody in Tongliao city in spring, summer, autumn and winter were 93.50%, 92.19%, 87.26% and 88.86%, respectively. The positive rate of serotype A FMDV antibody in region B in autumn was very low, almost approaching the minimum requirement of 70%. Comprehensive analysis shows that Tongliao city needs to further strengthen the immunization of serotype A FMDV in autumn and winter, especially in region B, which needs to strengthen immunization, antibody detection and revaccination to further improve the positive rate of serotype A FMDV antibody.

Cattle raising is the characteristic leading industry of Tongliao city, which plays an important role in Tongliao's

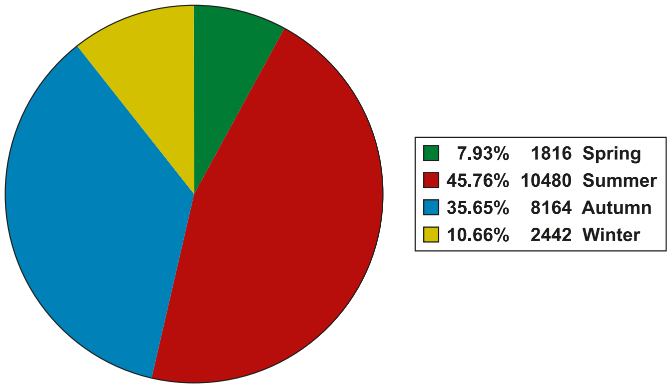

Fig. 7. Sample composition by season

Explanations: A total of 22902 serum samples in spring, summer, autumn and winter were collected. The sample quantity and percentage in each season are shown

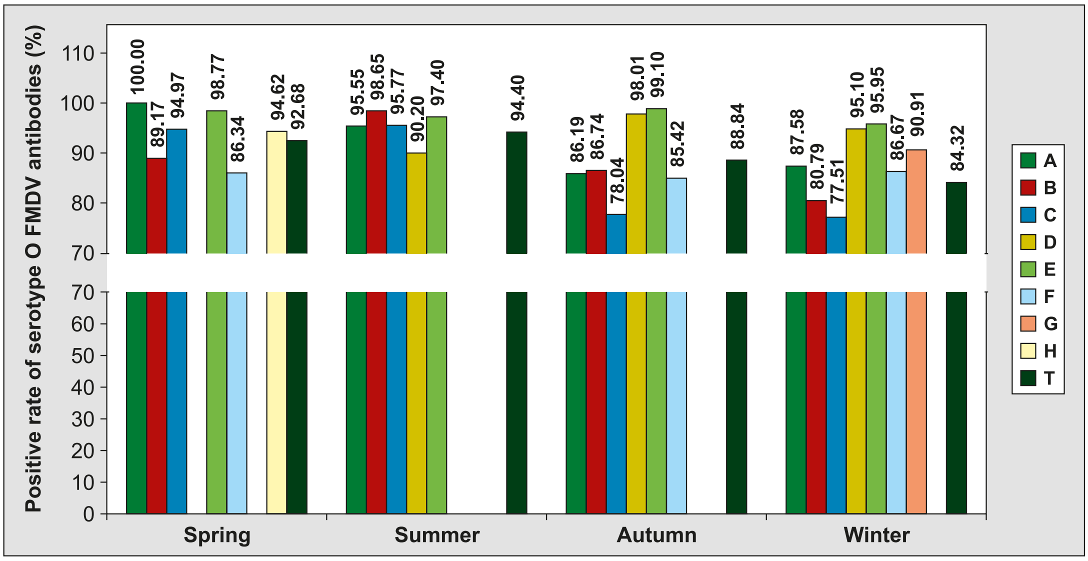

Fig. 8. Positive rate of serotype O FMDV antibody in samples from different seasons and regions
Explanations: In the legend, the letters A to H represent 8 different regions in Tongliao city, and T represents entire regions in each season

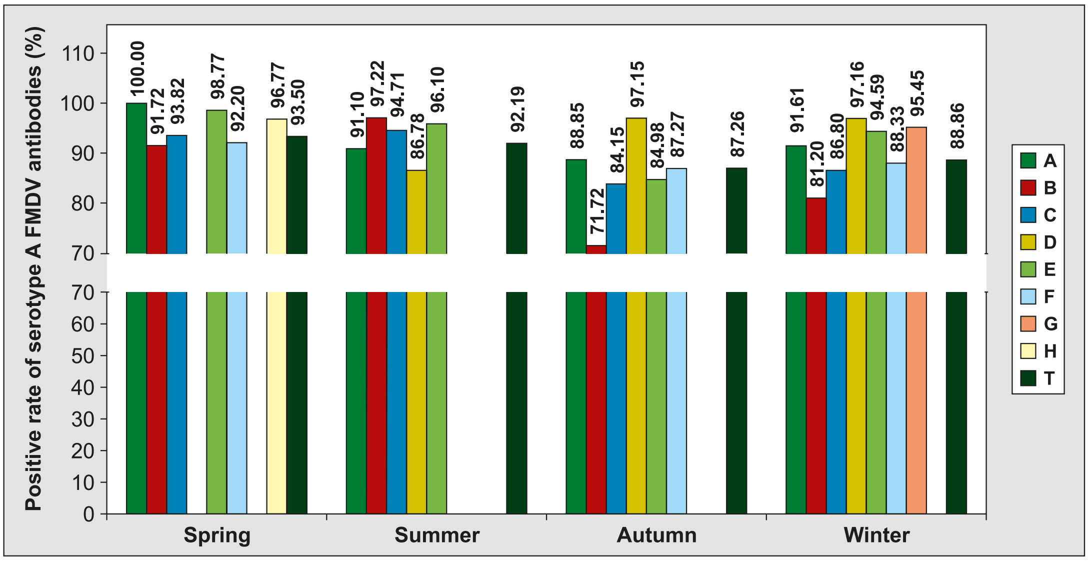

Fig. 9. Positive rate of serotype A FMDV antibody in samples from different seasons and regions
Explanations: as in Fig. 8.

economy. In 2020, the stock of cattle in Tongliao was approximately 3 million, and more than 1.5 million live cattle were traded in the city's 7 large cattle trading markets, with a turnover of more than 20 billion yuan. Tongliao city attaches great importance to the prevention and control of FMD. To ensure that the immunization density of cattle reached 100%, centralized immunization was arranged for cattle raised by households from March to May and from September to October, and supplementary immunization was carried out in a timely manner. Programmed immunization was carried out for cattle on farms. In recent years, the pass rate of immune antibodies against FMDV in China has generally been over 85%. For example, in 2018 and 2020, the pass rates of immune antibodies against serotype O FMDV in cattle and sheep in Changsha city in Hunan Province were 86.05% and 90.05%, respectively (23); in 2020, the pass rates of immune antibodies against serotype O and type A FMDV in cattle in Fuxin city in Liaoning Province were 89.43% and 90.97%, respectively (6); and in 2020, the pass rates of immune antibodies against serotype O and type A FMDV in cattle and sheep in Sichuan Province were 89.87% and 88.42%, respectively (19). In 2016, the pass rate of immune antibody against serotype O FMDV in cattle in a district of Beijing was 90.1% (17). In 2020, the pass rates of immune antibody against serotype O and type A FMDV in cattle in Tongliao city were 91.21% and 90.18%, respectively: both higher than the requirements of the National Compulsory Immunization Plan of Animal Epidemic Diseases in 2020; that is, the pass rate of immune antibody remained above 70% all year round. This indicates that the immunization level of FMD in Tongliao city is relatively high, and the immunization effect of FMD is better in the whole country.

In general, the positive rates of serotypes O and A FMDV antibodies in cattle from farms in Tongliao were higher than those of households, indicating that the FMD immune effect of farms was better than that of households. Similar situations were also found in other serological surveillance. In 2018, Sichuan Province conducted serotype O FMDV antibody detection in cattle and sheep, and it was found that the pass rate of farms was higher than that of households, 85.76% and 84.12%, respectively (22). In 2019, the pass rates of serotype O FMDV antibody in pigs of farms and households in Mengyin County, Shandong Province, were 96.55% and 90.45%, respectively (5). The farms generally have a strong awareness and ability of disease prevention and control and attach importance to the establishment of relevant systems of disease prevention and control, such as immunization and antibody detection. They can conscientiously implement immunization and antibody detection and carry out revaccination in a timely manner. However, some households have a fluke mentality, are unwilling to vaccinate or do not vaccinate according to the requirements of the regulations. In addition, although some households attach great importance to the prevention and control of FMD in the raising process and vaccinate cattle against FMD, they do not carry out immune antibody detection. As a result, cattle cannot be revaccinated in a timely manner when their immune antibody levels are low. The compulsory immunization of cattle raised by households is generally carried out by part-time epidemic prevention personnel. Due to the differences in culture, age and other aspects, there is a certain gap in the understanding and application of immunization knowledge, and there are incorrect operations of vaccine preservation and injection, leading to poor immunization effects. Moreover, in the FMD endemic and immunized areas or countries, it is necessary to consider distinguishing infected from vaccine animals (DIVA). The differentiation of antibodies induced by vaccination or infection mainly depended on the detection of antibodies of nonstructural protein (NSP). In general, NSP antibodies could only detected from the infected animals. For further study, the detection of FMDV NSP antibodies should be considered.

The results showed that the immunization level of FMD in Tongliao city was relatively high, and the positive rates of serotypes O and A FMDV antibodies met the requirements of the National Compulsory Immunization Plan of Animal Epidemic Diseases in 2020; that is, the pass rate of immune antibody remained above 70% year-round. However, to improve the overall immunization effect of FMD in Tongliao city, it is especially necessary to strengthen the vaccination, antibody detection and revaccination of cattle raised by households in autumn and winter.

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