Evaluation of the Immunization Effect of a Peste des Petits Ruminants-Goatpox Combined Live Vaccine in Large-Scale Sheep Farms in Northern Shandong

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Abstract [**Objectives**] This study was conducted to enhance the prevention and control of peste des petits ruminants (PPR) and goatpox (GTP). [**Methods**] Two experimental sheep farms in Northern Shandong were selected to conduct a comparative experiment between separate vaccinations for PPR and GTP and immunization with a combined live vaccine for both diseases. Antibody levels were measured to assess immunization effect on days 7, 14, 21 and 28 after vaccination. [**Results**] The qualified rates of group immune antibodies in both the experimental and control groups exceeded 75%, achieving the goal of preventing both PPR and GTP through a single immunization. [**Conclusions**] This study provides clinical application references for the prevention of PPR and GTP in the local area.

Key words Peste des petits ruminants; Goatpox; Combined live vaccine

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Peste des petits ruminants (PPR) is an acute contact infectious disease caused by the PPR virus (PPRV) infecting small ruminants such as goats and sheep[1-2]. After infection, it primarily presents with stomatitis, fever, and diarrhea, characterized by high morbidity and mortality rates. PPR was first identified in Côte d'Ivoire, Africa, in 1942 and initially entered China in 2007 via the Ali region of Tibet, where it was promptly controlled and eradicated[3-4]. At the end of 2013, PPR reappeared in China and outbreaks occurred in multiple provinces including Hunan, Shandong, Liaoning, and Inner Mongolia. These outbreaks inflicted significant economic losses on the sheep farming industry[5]. Consequently, PPR has been classified as a Category I animal disease in China and is listed by the World Organisation for Animal Health (WOAH) as a notifiable major animal infectious disease.

Goatpox (GTP) is an acute, febrile, and highly contagious infectious disease caused by infection with the goatpox virus (GTPV). It leads to symptoms such as pox lesions on the skin, digestive tract mucosa, and respiratory tract of infected goats, making it a seriously threatening animal pox disease[6]. Infected animals exhibit fever, widespread papules or nodules on the skin, mucosa and organ surfaces, skin edema, lymph node enlargement, emaciation, a significant decrease in milk production, and in severe cases, death. GTP was first identified in Europe around 200 BC, while sheeppox was initially reported in the United Kingdom during the 13th century. The morbidity rate ranges from 50% to 80%, with a case fatality rate of 20% to 80%. Notably, the mortality rate in lambs can reach 100%[7], posing a severe threat to the sheep farming industry. In China, it is classified as a Category II animal disease and is also listed as a notifiable disease by the World Organisation for Animal Health (WOAH).

Currently, the most effective method for preventing and controlling PPR and GTP is vaccination, the efficacy of which directly impacts both the economic returns of the sheep industry and biosecurity. Existing vaccination protocols require separate immunizations administered at different times. Multiple rounds of immunization injections not only impose significant physiological stress on animals, but also increase the workload for farmers and disease control personnel. Therefore, under the premise of maintaining the same types of vaccines, optimizing the immunization protocol to reduce stress reactions in sheep and minimize labor intensity while still achieving the nationally required immunization effects has become an issue that requires further research. In this study, two large-scale sheep farms were selected in Binzhou and Dongying for immunization trials, and through post-immunization clinical observation and detection of antibody levels, the effect of the combined immunization was scientifically evaluated and compared with that of conventional immunization. This study provides practical clinical references for farmers in developing effective sheep immunization protocols.

Materials and Methods

Experimental materials

Experimental animals Two large-scale sheep farms were selected

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for the experiment: experimental flock 1, located in the Development Zone of Binzhou City, with a herd size of 1 000 sheep, and experimental flock 2, situated in Lijin County, Dongying City, with a herd size of 2 000 sheep. Both flocks underwent clinical health examinations prior to vaccination. From each flock, 90 immunologically-naive sheep aged over two months and in good physical condition were selected for the experiment. Each flock was randomly divided into 3 groups, with 30 sheep per group, resulting in a total of 6 experimental groups.

Main reagents Goatpox live vaccine (AV41 strain), purchased from Shandong Lvdu Bio-Sciences & Technology Co., Ltd., batch number: 202405003; peste des petits ruminants live vaccine (Clone 9 strain), purchased from Tecon Biology Co. Ltd., batch number: 2024007-2; peste des petits ruminants-goatpox combined live vaccine (Clone 9 strain + AV41 strain), purchased from Huapai Biological Group Co., Ltd., batch number: 2022010-1; PPR competitive ELISA antibody detection kit, purchased from Qingdao Lijian Biological Co., Ltd., batch number: 20172115; goatpox indirect ELISA antibody detection kit, purchased from Lanzhou Veterinary Research Biological Technology Co., Ltd., batch number: 20240110119.

Main instruments Benchtop centrifuge (FC5513), purchased from OHAUS Instruments (Shanghai) Co., Ltd.; constant temperature incubator (DHP-9162B), purchased from Shanghai Yiheng Technology Co., Ltd.; automatic microplate washer (SAF-505H), purchased from Shanghai Bajiu Industrial Co., Ltd.; microplate reader (Cmax Plus), purchased from Molecular Devices (Shanghai) Co., Ltd.

Experimental methods

Immunization The experiment at each sheep farm was conducted with three groups. Except for the different vaccines administered, all other rearing conditions were identical for various groups. Experimental sheep in various groups were marked with different colored back tags. Specifically, the experimental group was marked with red back tags and received the PPR-GTP combined live vaccine. The control group 1 was marked with yellow back tags and received the PPR live vaccine. The control group 2 was marked with blue back tags and received the GTP live vaccine. The vaccine injection methods and dosages strictly followed the manufacturers' instructions.

Clinical symptom observation Within 48 h after vaccination, the health status of the sheep was closely monitored, and stress responses such as mental state, appetite, feces, and body temperature in each experimental group were recorded.

Antibody detection Blood samples were collected on days 7, 14, 21 and 28 after immunization. Serum was separated, and each sample was labeled with a corresponding identification number. Antibody levels in the serum samples from various experimental groups were measured using commercial ELISA kits, strictly following the kit's operation instructions. Results were interpreted according to the instructions.

Results and Analysis

Clinical symptom observation

Observations of post-vaccination reactions in each experimental group showed that within 24 h of immunization, individual sheep in some groups exhibited adverse reactions such as depression and loss of appetite. No human intervention was applied. By 48 h after immunization, all adverse reactions in the experimental groups had resolved.

PPR antibody detection results

The PPR immunization antibody results from the two sheep farms are shown in Table 1. Except for the blank control group 2, all groups developed PPR antibodies 7 d after immunization. Subsequently, both antibody level and positive rate continued to rise. After 21 d, the positive rates for immunization antibodies in the experimental group and control group 1 exceeded 80%, meeting the national standards for qualified immunization antibody rates. After 28 d, the antibody positive rates in both flocks exceeded 90%, with no significant difference between flock 1 and flock 2 ($P>0.05$). Within the same flock, the PPR antibody positive rate in control group 1 was slightly higher, but showed no significant difference compared with the experimental group ($P>0.05$).

Table 1 Statistical results of PPR antibody detection

Experimental flock	Group	7 d			14 d			21 d			28 d		
		Number of samples	Number of positive samples	Positive rate//%	Number of samples	Number of positive samples	Positive rate//%	Number of samples	Number of positive samples	Positive rate//%	Number of samples	Number of positive samples	Positive rate//%
1	Experimental group	30	16	53	30	20	67	30	25	83	30	28	93
	Control group 1	30	18	60	30	23	76	30	27	90	30	30	100
	Control group 2	30	0	0	30	0	0	30	0	0	30	0	0
2	Experimental group	30	14	47	30	19	63	30	24	80	30	27	90
	Control group 1	30	16	53	30	21	70	30	25	83	30	30	100
	Control group 2	30	0	0	30	0	0	30	0	0	30	0	0

GTP antibody detection results

The GTP immunization antibody results from the two sheep farms are shown in Table 2. In both flocks, except for the blank control group 1, the antibody level and positive rate increased

rapidly from 7 to 14 d after immunization, and from 21 to 28 d, the antibody levels in various experimental groups increased at a slower rate, with the positive rate ranging from a minimum of 70% to a maximum of 87%. After 28 d, there was no significant difference between the two flocks ($P>0.05$). Within the same flock, no significant difference in GTP antibody positive rate was observed between the experimental group and control group 2 ($P>0.05$).

Table 2 Statistical results of GTP antibody detection

Experimental flock	Group	7 d			14 d			21 d			28 d		
		Number of samples	Number of positive samples	Positive rate//%	Number of samples	Number of positive samples	Positive rate//%	Number of samples	Number of positive samples	Positive rate//%	Number of samples	Number of positive samples	Positive rate//%
1	Experimental group	30	12	40	30	16	53	30	21	70	30	23	77
	Control group 1	30	0	0	30	0	0	30	0	0	30	0	0
	Control group 2	30	16	53	30	20	67	30	24	80	30	26	87
2	Experimental group	30	13	43	30	17	57	30	22	73	30	224	80
	Control group 1	30	0	0	30	0	0	30	0	0	30	0	0
	Control group 2	30	15	50	30	19	63	30	23	76	30	25	83

Conclusions and Discussion

The Lubei region is home to the Yellow River Estuary Tan Sheep Industrial Park, which has the largest standardized meat sheep farming base in China. The primary breeds include Lubei White Goats, Boer Goats, and Wadi Sheep, with an annual slaughter volume exceeding 3 million head. Peste des petits ruminants (PPR) and goatpox (GTP) are currently major infectious diseases jeopardizing the development of the Yellow River Estuary Tan Sheep farming industry. Effectively implementing prevention and control measures against PPR and GTP is of great importance for the sustainable and healthy development of the sheep industry. Currently, vaccination remains the most effective control strategy. Based on the prevalence of regional infectious diseases, compulsory immunization against both diseases has been implemented in this area. However, using separate live vaccines for PPR and GTP undoubtedly increases production costs, such as adding to the workload of frontline animal health personnel and consuming more human and material resources. Additionally, frequent vaccination can also heighten physiological stress responses in animals. To further enhance the efficiency of animal disease prevention and control, and drawing on successful experiences of combined vaccination strategies for other diseases, in this study, we conducted a comparative experiment between separate vaccinations for PPR and GTP and immunization with a combined live vaccine for both, as well as an evaluation of the immunization effect.

Observation during the experiment showed that no adverse vaccine reactions occurred in any of the experimental groups in the two test flocks, indicating that both vaccines are safe and effective. The results of the immunization antibody tests demonstrated that under all three immunization protocols, the positive rates for group immune antibodies against both diseases exceeded 75%, meeting national standards, with no evidence of immune interference. Specifically, no significant differences were observed between flock 1 and flock 2 ($P>0.05$). Within the same flock, there were also no notable differences between the experimental group and the control group ($P>0.05$). Overall, the qualified antibody rate for PPR was significantly higher than that for GTP ($P<0.05$). Furthermore, the combined immunization with the two vaccines can reduce physiological stress in animals, significantly lessen the workload for disease prevention personnel and farmers, and markedly improve work efficiency. Therefore, adopting a combined immunization strategy is both effective and feasible.

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