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RESEARCH ARTICLE

The Effect of the Combination of Peste Des Petits Ruminants (PPR) Vaccine with Different Doses of Lentinan on Cytokines and Antibody Levels of Sheep

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A B S T R A C T

Peste des Petits Ruminants (PPR) is an acute contagious viral disease in small ruminants. New methods and adjuvants are needed to increase the immunological potential of the vaccines used and to prolong the duration of action. The aim of this research is to determine the effectiveness of the combination of different doses of lentinan and PPR vaccine on total antibody and some cytokines levels in sheep. For this purpose, 21 sheep were randomly divided into 3 equal groups and the groups were designed as PPR vaccine control, PPR vaccine+Low dose lentinan (5 mg dose, i.v.) and PPR vaccine+High dose lentinan (10 mg dose i.v.). The immunological and hematological parameters were analyzed in all groups before the first application (0. hour) and on the 7th, 14th, 21st, 30th, 45th, 60th, 75th and 90th days. Antibody levels against PPR appears to be positive after 7 days in all groups. However, the antibody level in the PPR+Lent5 group increased partially on the 30th, 60th and 90th days as compared to the PPR group. In addition, the antibody level in the PPR+Lent5 group tends to increase although the antibody level of PPR vaccine group tends to decrease on the 30th day. IL-6, IL-10, IL-4, IFN-γ levels were induced due to the stimulation of Th1 and Th2 cells by different doses of lentinan at different times. In conclusion, lentinan can be a beneficial adjuvant/supporting agent for vaccines. However, more detailed research is needed for different durations, and in different pharmaceutical forms and doses of lentinan.

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INTRODUCTION

Peste des Petits Ruminants (PPR) is a contagious viral disease with high morbidity and mortality in domestic and wild ruminants (especially sheep and goats). The disease is caused by PPRV from Paramyxoviridae family, Orthoparamyxovirinae subfamily, Morbillivirus genus (Zhao *et al.*, 2021). The causative agent of the disease is morbillivirus. Its shows symptoms similar to rinderpest and human measles (Njeumi *et al.*, 2020). Recently, it has been determined that PPR virus (PPRV) affects not only goats and sheep, but also some wild ungulate species such as camels, cattle, water buffalo, African buffalo and Nubian ibex (Zhao *et al.*, 2021).

PPRV has clinical manifestations such as fever, diarrhea, conjunctivitis, rhinotracheitis, ulcerative stomatitis, gastroenteritis, and pneumonia (Prajapati *et al.*, 2021). Morbidity rate can reach 100% and mortality rate can reach 90% (Pope *et al.*, 2013).

Vaccination against PPRV is of great importance due to the widespread impact and economic importance of sheep and goats (Baron *et al.*, 2017). Vaccination is necessary for the control and eradication of viral diseases. Many methods and drug trials are carried out to increase the immunological potential of the vaccine and to prolong the duration of immunity (Mohamed *et al.*, 2013). It was observed that the protective effects continued the 28th day after the challenge trial after PPRV vaccination in goats. In addition, it has been reported that antibody titer peaks 12 days after vaccination (Hosamani *et al.*, 2006). In another research, the goats that is vaccinated a single dose of PPR vaccine, was provided immunity after the 14th day and the protection continued throughout the year. However, the antibody titer has fluctuating and the level decreased after the 30th day and created new peaks on the 90th and 150th days (Singh *et al.*, 2004). In another research, it was determined that only the PPR vaccine stimulated the immune system for a short time and did not maintain the

antibody titer for a long time. However, it has been reported that Th 1 cells are stimulated earlier, and Th 2 cells are stimulated for a longer period and the release of interferon (IFN) and other cytokines has longer as a result of PPR vaccinated applied together with Corynebacterium cutis lysate (CCL). The CCL together with the PPR vaccine has provided immunity in a short time and generally increases the antibody level and duration (Dik *et al.*, 2016).

Lentinan (LNT) is a β-(1,3)-D-glucan isolated from Lentinus edodes, a mushroom species that is widely grown and consumed in Asian countries. It has biologically active macromolecule that activates the immune system, and has anticoagulant, anti-viral, anti-tumor, anti-cancer and immunomodulatory properties (Zhang *et al.*, 2011; Zi *et al.*, 2020). Lentinan was approved in Japan in 1985 and in China in 1995 as an adjuvant in cancer chemotherapy (Barton *et al.*, 2016). Intranasal administration of lentinan in combination with a vaccine against Bacillus Calmette-Guerin (BCG) induced high-level activation of immune cells in lung tissue. The combination of BCG with lentinan has increased the local immune response to BCG in the lung, while reducing the vaccine-related side effects (Drandarska *et al.*, 2005). It was emphasized that co-administration of lentinan with Newcastle disease virus vaccine increased the antibody titer of the vaccine and lentinan can be a new T-cell-directed adjuvant candidate (Chihara *et al.*, 1987; Guo *et al.*, 2009).

Recombinant vaccines are being developed for the eradication of the disease and it needs more effective vaccines (Parida *et al.*, 2015). Therefore, aluminum adjuvants, which can produce long-lasting and effective antibody reactions, have been widely used for many years. The aluminum adjuvants could not sufficiently generate Th1-type immunity, which is important in intracellular infections. However, lentinan could increase Th1-type and Th2-type immune responses in many studies. In addition, this immune modulator effect has long-lasting and strong. It can be a safe and appropriate adjuvant against intracellular and extracellular infections (Jiao *et al.*, 2022).

For many years, many studies have been carried out to increase the effectiveness of vaccines and to discover new adjuvant substances. Today, it has also been seen in the Corona virus (COVID-19) pandemic that continuous booster applications may be required for the vaccines to provide sufficient effectiveness for a long time. In addition, the effectiveness and duration of vaccines against mutations in viral diseases can be shortened. The effective and continuous vaccination is required for the eradication of PPRV infection and the prevention of economic losses. Vaccination studies have been carried out on this disease, but they have still not sufficient.

Lentinan has not clearly known to affect the vaccine efficacy and dosage regimen in mammals. For these reasons, lentinan was used as an adjuvant in the present study. In this study, the effects and effective duration of lentinan administered together with the PPR vaccine on total antibodies and cytokines (IFN-γ, IL-6, IL-4, IL-10) were determined.

MATERIALS AND METHODS

Animals and experimental design: The sheep was held and the experiments were carried out at Selcuk University Prof. Dr. Hümeyra ÖZGEN Research and Application Farm. In the study, 21 Central Anatolian Merino sheep (6-9 months old) were used. The sheep were determined to be negative for PPRV antibodies with a commercial Enzyme Immunosorbent Assay (ID Screen® PPR Competition ELISA kit, Catalogue no: PPRC ver 0821 DE, ID-Vet, France) kit. The animals were fed and water as adlibitum during the experimental period. The sheeps were randomly divided into 3 equal groups after general health checks were performed.

Group one (PPR Vaccine Control, n=7): PPR vaccine (Pestdoll-S® Lyophilized PPR vaccine, Dollvet, Turkey) containing 10^3 concentrations of virus was applied as 1 mL to the hairless area under the skin.

Group two (PPR Vaccine+Low dose lentinan, n=7): PPR vaccine (Pestdoll-S® Lyophilized PPR vaccine, Dollvet, Turkey) containing 10^3 concentrations of virus was applied as 1 mL to the hairless area under the skin and simultaneously applied lentinan (Catalog no: CS-AD-00417, Clearsynth, India) at a dose of 5 mg intravenously (Gordon *et al.*, 1998).

Group third (PPR Vaccine+High dose lentinan, n=7): PPR vaccine (Pestdoll-S® Lyophilized PPR vaccine, Dollvet, Turkey) containing 10^3 concentrations of virus was applied as 1 mL to the hairless area under the skin and simultaneously applied lentinan (Catalog no: CS-AD-00417, Clearsynth, India) at a dose of 10 mg intravenously (Gordon *et al.*, 1998).

In all groups, blood samples were taken from the vena jugularis just before the first application (0 hour) and on the 7^{th}, 14^{th}, 21^{st}, 30^{th}, 45^{th}, 60^{th}, 75^{th}, and 90^{th} days after the first application and portioned into EDTA and gel tubes. Blood samples into gel tubes were centrifuged at 4000 rpm. The serum obtained from blood samples were portioned into eppendorf tubes and stored at -80°C until biochemical and protein analyzes were performed.

Hematology and biochemical parameters analysis: Hematology parameters (RBC, hematocrit, thrombocyte) were analyzed on the hematology analyzer device (Mindray Bio-Medical Electronics, Shenzhen, China) from the blood samples collected in the EDTA tube.

The serum samples were analyzed in terms of biochemical parameters by an autoanalyzer (BT-3000 plus, Biotecnica Instruments Roma, Italy). Immunoglobulin G (Sheep immunoglobulin G ELISA kit, Catalogue no: E0019Sh, BT LAB, China), IFN-γ (Catalogue no: E0049Sh, BT LAB, China), IL-6 (Catalogue no: E0072Sh, BT LAB, China), IL-4 (Catalogue no: E0079Sh, BT LAB, China), IL-10 (Catalogue no: E0053Sh, BT LAB, China) levels were determined through commercial ELISA kits by ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200).

Statistical analysis: The data were analyzed on SPSS 25.0 (SPSS, Inc., Chicago, IL, USA) software. The biochemical data were evaluated as mean ± standard deviation. The statistical significance between groups was tested using one-way ANOVA followed by a posthoc Tukey test. The $P<0.05$ was statistically significant.

Ethical approval: All procedures were approved by Selcuk University Veterinary Faculty Ethics Committee (Ethical approval number 2021/96 on 05/08/2021).

RESULTS

Antibody levels of PPR and different doses of lentinan groups are shown in Fig. 1. Antibody level against PPR appears to be positive after 7 days in all groups. However, the antibody level increased partially in the PPR+Lent5 group on the 30, 60 and 90 days compared to the PPR group. In addition, the antibody level remains stable in the PPR+Lent5 group, although the level in PPR group tends to decrease on 30 days.

Serum levels of IL-6, IL-10, IL-4, IFN-γ parameters are presented in Table 1, 2, 3, and 4, respectively. IL-6 level was statistically higher in the PPR+Lent5 group on 14 days, and in the PPR+Lent5 and PPR+Lent10 groups on 30 days compared to the PPR group. The IL-10 level was statistically lower in the PPR+Lent10 group on 14 and 30 days compared to the PPR group. This decrease did not occur in the PPR+Lent5 group and it was statistically higher on 75 days in PPR+Lent5 group compared to the PPR group. IL-4 level was statistically higher in the PPR+Lent5 and PPR+Lent10 groups compared to the PPR group on 30 days. The IFN level was statistically higher in the PPR+Lent5 group than in the PPR group on 7 days, it was statistically lower in the PPR+Lent5 and PPR+Lent10 groups compared to the PPR group on 14 days. It was statistically lower in the PPR+Lent10 group than in the PPR group on 30 days.

The hematological (WBC, RBC, PLT) results are presented in Table 5, 6 and 7. The RBC level was statistically higher in the PPR+Lent5 group compared to the PPR group on 7 days.

DISCUSSION

In a study, goats vaccinated with PPR vaccine have reached seropositive antibody level after the 14 days and this level has maintained throughout the year (Balamurugan *et al.*, 2012). Although similar results have been obtained in another study, the antibody titer fluctuated and the level decreased after 30 days and occurred new peaks on 90 and 150 days (Singh *et al.*, 2004). The antibody titer has more induced in animals treated with PPR+ Corynebacterium cutis lysate (CCL) compared to administered PPR vaccine in sheep 14 days after the vaccination (Dik *et al.*, 2016). It has been emphasized that the application of lentinan at different doses together with the Newcastle virus vaccine increases the antibody titer of the vaccine and ensures lymphocyte proliferation (Guo *et al.*, 2009). In the present study, seropositive antibody levels were reached in all groups after the 7th day, however, the antibody level increased statistically in the PPR+Lent5 group on the 30, 60, and 90 days compared to the PPR group. The low-dose lentinan administration may have induced the antibody of the PPR vaccine by providing lymphocyte proliferation.

The expression of IFN-β, IFN-γ, IL-4, IL-1β, IL-8, IL-10, IL-6 and IL-12 increased due to PPRV infection (Baron *et al.*, 2014). However, IFN-γ and IL-4 synthesis are stimulated in the early period as an immune system response of viral diseases such as PPR and measles. In the later stages, Th2 response develops (Howe *et al.*, 2005; Patel *et al.*, 2012). Interferons play an important role in the primary immune response (Dittmer *et al.*, 2001; O'Garra, 1998). Th1 cells cause the destruction of the infectious pathogen. The level of cytokines after vaccination varies with induced cell type and immunity (Patel *et al.*, 2012).

Th1 cells secrete IFN-γ and IL-2, while Th2 cells secrete cytokines IL-4, IL-5 and IL-6. In addition, IL-12 is secreted by macrophages and dendritic cells (Choi and Reiser, 1998). IFNs released by T and natural killer (NK) cells are involved in the anti-viral immune response via the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway (Kumar *et al.*, 2014).

TNF-α, IL-1β, IL-6 and IFN-α, which are proinflammatory cytokines, are closely associated with inflammation (Atmaca and Kul, 2012; Dik *et al.*, 2018). They also have a key role in coordinating and activating the adaptive immune response against diseases (Atmaca and Kul, 2012). Although IL-10 is a versatile cytokine, it suppresses increased inflammation. IL-6 and IL-10 have an

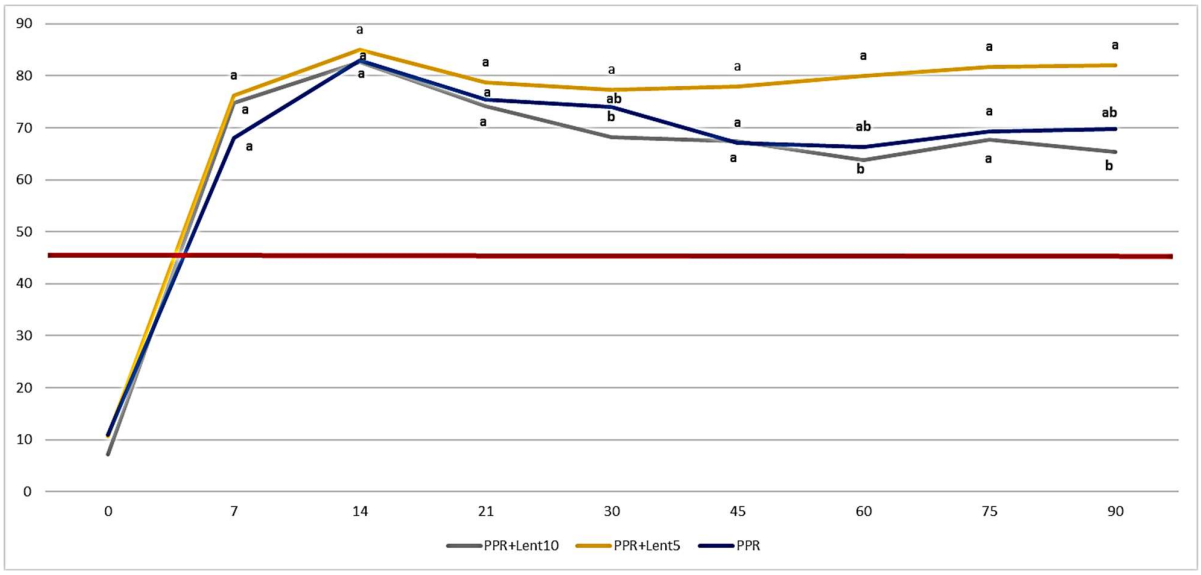

Fig. 1: Seromonitoring of sheep vaccinated with PPR (10^3TCID$_{50}$/animal) and lentinan adjuvants (5 and 10 mg): Red Line: If the percentage of inhibition was ≤45, the group have specific antibody against PPRV.

Table 1: The IL-6 cytokine levels in post-vaccination with PPR vaccine (10^3TCID_{50}/animal) and lentinan adjuvants (5 and 10 mg) (mean ± SEM).

GroupGroup/ TimeTime	0 Day (CControl)	7. Day	14. Day	21. Day	30. Day	45. Day	60. Day	75. Day	90. Day
PPR	86,18±9,23[a]	83,21±11,08[a]	100,84±11,02[b]	114,14±14,36[a]	64,90±6,60[c]	86,54±13,89[a]	60,41±5,25[a]	80,03±6,04[a]	115,92±7,93[a]
PPR+Lent5	106,90±11,56[a]	127,60±17,99[a]	146,65±6,97[a]	114,14±12,79[a]	93,62±4,26[b]	91,56±7,50[a]	88,01±14,55[a]	94,64±9,22[a]	116,51±11,68[a]
PPR+Lent10	118,62±20,94[a]	92,83±5,94[a]	96,68±7,82[b]	85,63±11,14[a]	145,51±10,69[a]	85,86±6,65[a]	78,45±4,82[a]	73,37±10,74[a]	91,11±16,99[a]

Different letters in the same column ([a,b,c]) denote statistical significance ($p < 0.05$).

Table 2: The IL-10 cytokine levels in post-vaccination with PPR vaccine (10^3TCID_{50}/animal) and lentinan adjuvants (5 and 10 mg) (mean ± SEM).

Group/Time	0 Day (Control)	7. Day	14. Day	21. Day	30. Day	45. Day	60. Day	75. Day	90. Day
PPR	3,31±2,11[a]	12,14±5,35[a]	11,15±4,42[ab]	6,13±2,79[a]	116,34±9,33[a]	208,01±31,49[a]	219,46±11,39[ab]	184,27±18,33[b]	176,96±7,55[a]
PPR+Lent5	10,81±4,01[a]	8,59±7,07[a]	18,03±5,39[a]	26,83±15,34[a]	129,78±8,50[a]	191,34±8,17[a]	246,45±4,67[a]	264,13±7,05[a]	180,68±10,39[a]
PPR+Lent10	25,51±10,66[a]	5,48±1,69[a]	1,07±1,07[b]	10,90±5,22[a]	23,33±17,11[b]	151,74±14,42[a]	193,86±7,78[b]	201,37±20,62[b]	190,13±20,45[a]

Different letters in the same column ([a,b]) denote statistical significance ($p < 0.05$).

Table 3: The IL-4 cytokine levels in post-vaccination with PPR vaccine (10^3TCID_{50}/animal) and lentinan adjuvants (5 and 10 mg) (mean ± SEM).

Group/Time	0 Day (Control)	7. Day	14. Day	21. Day	30. Day	45. Day	60. Day	75. Day	90. Day
PPR	24,57±4,21[a]	26,43±5,19[a]	33,83±4,48[a]	24,52±6,76[a]	24,92±8,48[b]	36,31±6,25[a]	28,65±3,05[a]	30,15±6,65[a]	35,26±3,21[a]
PPR+Lent5	33,22±6,86[a]	34,40±5,61[a]	31,29±4,91[a]	20,74±3,05[a]	78,43±10,40[a]	29,34±3,44[a]	32,37±4,81[a]	34,75±2,38[a]	39,52±2,21[a]
PPR+Lent10	26,97±7,65[a]	32,23±3,53[a]	34,65±3,94[a]	28,67±3,69[a]	72,65±7,64[a]	40,15±12,79[a]	27,08±2,34[a]	27,96±2,65[a]	41,33±4,22[a]

Different letters in the same column ([a,b]) denote statistical significance ($p < 0.05$).

Table 4: The IFN-γ cytokine levels in post-vaccination with PPR vaccine (10^3TCID_{50}/animal) and lentinan adjuvants (5 and 10 mg) (mean ± SEM).

Group/Time	0 Day (Control)	7. Day	14. Day	21. Day	30. Day	45. Day	60. Day	75. Day	90. Day
PPR	12,77±1,79[a]	9,42±1,80[b]	14,17±1,44[a]	14,12±2,91[a]	20,62±1,21[a]	22,80±6,43[a]	25,90±1,69[a]	31,67±1,36[b]	32,77±2,44[a]
PPR+Lent5	17,97±1,62[a]	21,89±2,61[a]	7,64±0,85[b]	22,58±2,11[a]	19,75±0,80[a]	31,48±1,84[a]	29,16±1,69[a]	34,95±1,56[a]	35,60±2,24[a]
PPR+Lent10	25,05±5,11[a]	10,57±3,58[b]	8,88±1,80[b]	16,86±3,27[a]	8,55±2,56[b]	27,81±1,73[a]	28,21±1,77[a]	28,95±0,99[b]	33,41±1,91[a]

Different letters in the same column ([a,b]) denote statistical significance ($p < 0.05$).

Table 5: The effects of PPR vaccine (10^3TCID_{50}/animal of each. SC) with lentinan adjuvants (5 and 10 mg) on WBC parameters of ewes (mean±SEM).

Group/Time	0 Day (Control)	7. Day	14. Day	21. Day	30. Day	45. Day	60. Day	75. Day	90. Day
PPR	6,27±1,70[a]	5,40±1,29[a]	5,17±2,49[a]	0,89±0,89[a]	6,93±1,95[a]	6,54±1,19[a]	8,67±0,77[a]	10,31±0,81[a]	7,11±1,83[a]
PPR+Lent5	6,34±2,32[a]	3,38±2,81[a]	6,00±3,19[a]	3,90±1,44[a]	4,92±1,62[a]	7,89±1,33[a]	8,33±1,16[a]	7,94±2,20[a]	8,01±1,47[a]
PPR+Lent10	4,96±2,28[a]	3,62±1,45[a]	5,30±1,57[a]	3,06±1,56[a]	8,56±0,71[a]	5,13±1,28[a]	6,14±1,02[a]	6,14±1,51[a]	7,03±1,18[a]

Different letters in the same column ([a,b]) denote statistical significance ($p < 0.05$).

Table 6: The effects of PPR vaccine (10^3TCID_{50}/animal of each. SC) with lentinan adjuvants (5 and 10 mg) on RBC parameters of ewes (mean±SEM).

Group/Time	0 Day (Control)	7. Day	14. Day	21. Day	30. Day	45. Day	60. Day	75. Day	90. Day
PPR	12,94±3,01[a]	7,73±1,77[b]	12,95±0,92[ab]	10,07±1,72[a]	10,77±1,08[a]	9,16±0,83[a]	10,82±0,44[a]	11,85±0,41[a]	11,61±0,49[a]
PPR+Lent5	17,25±1,03[a]	17,71±2,54[a]	13,85±0,77[a]	11,91±1,04[a]	11,33±1,29[a]	10,70±1,57[a]	10,10±1,19[a]	9,99±1,54[a]	10,56±1,23[a]
PPR+Lent10	10,99±3,04[a]	15,17±2,49[ab]	9,96±1,33[b]	11,82±0,99[a]	11,93±0,25[a]	12,49±1,51[a]	9,27±1,51[a]	11,76±0,35[a]	11,22±0,39[a]

Different letters in the same column ([a,b]) denote statistical significance ($p < 0.05$).

Table 7: The effects of PPR vaccine (10^3TCID_{50}/animal of each. SC) with lentinan adjuvants (5 and 10 mg) on PLT parameters of ewes (mean±SEM).

Group/Time	0 Day (Control)	7. Day	14. Day	21. Day	30. Day	45. Day	60. Day	75. Day	90. Day
PPR	228,00±47,11[a]	219,57±53,46[a]	311,00±47,02[a]	296,57±55,31[a]	342,86±38,50[a]	459,8±158,7[a]	321,71±18,51[a]	294,64±50,76[a]	271,43±30,62[a]
PPR+Lent5	282,29±36,80[a]	321,29±26,52[a]	272,19±65,66[a]	284,57±41,77[a]	306,00±40,44[a]	247,57±33,76[a]	248,33±21,02[a]	276,71±39,37[a]	196,00±15,73[a]
PPR+Lent10	227,43±63,84[a]	279,86±19,41[a]	324,43±51,10[a]	299,00±26,56[a]	280,57±39,62[a]	272,29±21,18[a]	239,43±46,12[a]	306,86±28,01[a]	291,43±43,14[a]

Different letters in the same column ([a,b]) denote statistical significance ($P<0.05$).

important role in protection against the disease after viral vaccination (Strestik *et al.*, 2001). It has been reported that lentinan (orally at a dose of 10 mg/kg) decreased significantly IL-10 level compared to the control group in rabbits (Alkenany and Khalil, 2022). While lentinan caused an increase in white blood cells, monocytes and circulating cytotoxic T-cells in the acute myeloid leukemia model, it caused a decrease in the levels of anti-inflammatory cytokines IL-4, IL-10 and IL-6 (McCormack *et al.*, 2010). However, IL-6 level was statistically higher in the PPR+Lent5 group on 14 and 30 days, and in the PPR+Lent10 group on 30 day compared to the PPR group in the current study. It is thought that this increase contributes to the protection and anti-inflammatory effects of the PPR vaccine. On the other hand, IL-10 level was found to be statistically higher only in the PPR+Lent5 group on 75 days compared to the PPR group. The increased IL-10 level may have suppressed proinflammatory cytokines due to the chronic stimulation of the immune system by the PPR vaccine.

IFN-γ expression has decreased and IL-4 expression has induced a biphasic response after 1 day in animals vaccinated with PPRV. However, the expression of IFN-γ and IL-4 reached the maximum level in the acute period of the disease in sick animals, and their levels decreased rapidly before death in animals dying of the disease. PPRV in the live vaccine replicates in the host and chronically mimics the presence of the disease. Therefore, IFN-γ expression increases and IL-4 is expressed biphasically after vaccination. Moreover, the expression of IL-4 through Th2 increase to neutralize the viral antigen (Patel *et al.*, 2012). However, increased IFN secretion inhibits Th2 cell proliferation (Oriss *et al.*, 1997). IL-4 is a cytokine that inhibits activation of monocyte by IFN-γ. IL-4 level is similar compared to healthy animals, although IFN-γ level has statistically increased in the lungs, buccal mucosa and tongue tissue in PPRV-infected sheep. IFN-γ released through Th1 has been reported to have a significant effect on the response to PPRV (Atmaca and Kul, 2012). In the in vitro study, lentinan treatment more induced Th1 cells, and

so it has increased IFN-γ response, while decreased in the IL-4 level (Murata *et al.*, 2002). It is reported that lentinan as an adjuvant in an anthelmintic vaccine, induced IFN and IL-4 levels, and this is important for protection (Jin *et al.*, 2020). Co-administration of lentinan with ovalbumin has stimulated T cells and increased secretion of TNF-α, IFN-γ, IL-4, and IL-6 on the 35th day. However, the administration lentinan with poly lactic-co-glycolic acid (PLGA) polymer, which is a carrier, has shown a more pronounced effect on these cytokines. Moreover, it has created a strong immune response by release over time (Jiao *et al.*, 2022). In the current study, IL-4 showed biphasic release and IL-4 level decreased on the 30th day by PPRV vaccine. Lentinan treatment can have stimulated Th1 and Th2 cells at varying levels depending on the dose and duration of action. Contrary to results of the Murata *et al.* (2002), it can be speculated in the present study that lentinan may have induced more Th2 proliferation, especially at day 30, and prevented the decreased IL-4 level. In addition, high dose of lentinan may have induced Th2 proliferation more than Th1. Because the IFN level on the 30^{th} and 75^{th} days was statistically lower in the PPR+Lent10 group than in the PPR+Lent5 group.

Hematology values were fluctuating in the current study. However, it was determined that the levels of hematological parameters fluctuated within the reference range for sheep (Frye *et al.*, 2022). Lentinan treatment at a dose of 0.03 mg/kg/day for 5 weeks has decreased the RBC level in subacute toxicity research in rats (Ishıı *et al.*, 1980). Although the hematological values in the current study were within the hematological reference range, RBC level may have decreased on day 14 as side effect of lentinan.

In conclusion, lentinan may be a beneficial adjuvant for vaccines. The results were more stable in the lentinan at a dose of 5 mg/sheep, and the antibody level against PPR vaccine was high throughout the study at this dose. The effects of lentinan on Th1 and Th2 cells vary according to the dose of lentinan and duration. The lower than expected induction of PPR induction of lentinan may be related to the pharmaceutical form and dosage regimen. In future studies, lentinan should be investigated in different and repeated doses or with a controlled-release carrier.

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