



RESEARCH ARTICLE

Effect of attenuated parapoxvirus ovis on levels of some serum cytokines and antibody response in sheep

 
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Attenüe Parapoxvirus ovis'in koyunlarda bazı serum sitokin ve antikor seviyelerine etkisi

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Öz

Amaç: Parapoxvirus ovis (ORFV) enfeksiyonu küçük ruminantların önemli bulaşıcı viral hastalıklarından birisidir. Bu araştırma ile attenüe orf virus aşısı uygulanan koyunların kan serumlarında nötralizan antikor varlığı ile sitokin miktarlarının belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmada ORFV'ye karşı antikora sahip olmayan 6-8 aylık 10 adet Akkaraman erkek toklu kullanıldı. Tüm hayvanlara attenüe ORFV'nin 0,5 ml'si ile skarifikasyon işlemi yapıldı. Aşılama öncesi 0. saat (kontrol) ve sonraki 6., 12., 24., 48., 72., 96., 120. saat, 7., 14., 21., 30., 60., 90., 120., 150. ve 180. günlerde vena jugularis'ten serum örnekleri alındı. Elde edilen serumlarda nötralizan antikor varlığı araştırıldı. Buna ilave olarak toplanan serum örneklerinde proinflatuar özelliğe sahip (Th1) sitokinlerden IL-1 β , IL-12, IFN- γ , TNF- α , anti inflamatuar özelliğe sahip (Th2) sitokinlerden IL-4, ve IL-10 düzeyleri ELISA yöntemi kullanılarak belirlendi.

Bulgular: IL-1 β , IL-12, IFN γ , TNF- α ve IL-10 seviyelerinde kontrol serumlarına göre istatistiki açıdan önemli bir fark meydana gelmediği ($p>0,05$) görülmele beraber, IL-4 düzeyinde 30. günde istatistiki açıdan bir artış tespit edildi ($p<0,05$). Araştırma sonucunda attenüe canlı ORFV uygulamasının nötralizan antikor ve IL-4 hariç serum sitokin düzeylerinde bir değişime neden olmadığı belirlendi.

Öneri: İleride kullanılması düşünülen ORFV enfeksiyonuna karşı aşuların, bağışıklık sistemi üzerindeki etkileri net olarak gösterildikten sonra uygulanması gerektiği sonucuna varıldı.

Anahtar kelimeler: ORFV, attenüe suş, hücre kültürü, sitokin, koyun

Abstract

Aim: Parapoxvirus ovis (ORFV) infection is one of important contagious viral diseases of small ruminants. The aim of this study was to determine the concentration of cytokine and neutralizing antibody in blood serum of sheep that were carried out attenuated orf virus vaccine strain.

Materials and Methods: In this study, 10 male Akkaraman sheep which are 6-8 months of age and tested negative for antibodies against ORFV were used. Scarification was applied with 0.5 ml of attenuated ORFV to each animal. Serum samples were obtained from vena jugularis at 0. hour (control) before the inoculation and at the following 6., 12., 24., 48., 72., 96., 120. hours, 7., 14., 21., 30., 60., 90., 120., 150. and 180. days. Neutralizing antibody presence was investigated in obtained serums. In addition, levels of IL-1 β , IL-12, IFN- γ , TNF- α from proinflammatory cytokines (Th1) and IL-4 and IL-10 from anti-inflammatory cytokines (Th2) were determined by ELISA.

Results: ORFV caused fluctuations but no statistically significant change in levels of IL-1 β , IL-12, IFN γ , TNF- α and IL-10 were detected compared to control sera ($p>0,05$), and significant increase in IL-4 level was detected in the 30th day ($p<0,05$). As a result of the study, attenuated live ORFV administration and have no effect on levels of some serum cytokine except IL-4 and neutralizing antibodies.

Conclusion: It was concluded that the vaccines against ORFV infection considered to be used in the future should be applied after the effects on the immune system have been clearly demonstrated.

Keywords: ORFV, attenuated strain, cell culture, cytokine, sheep





Introduction

Orf virus (ORFV) is known as contagious pustular dermatitis, contagious pustular stomatitis, scabby mouth, contagious ecthyma or shortly ecthyma and it is classified within *Parapoxvirus* subfamily of *Poxviridae* family causing zoonotic infection in sheep, goats and humans (Çabalar et al 1996, Fleming et al 1997, Housawi and Abu Elzein 2000). ORFV has a size ranging from 140 to 300 nm and has ovoid shaped virion and linear dsDNA structure (Deane et al 2000). ORFV infection was first identified in sheep by Steeb in 1787 and specified in goats in 1879. Newson and Cross reported the first orf case for humans in 1934 (Saçar and Saçar 2012). While morbidity could reach 100% within the infectious herds, mortality is about 1%. However, the mortality of animals in suckling period might reach about 50% (Gökçe et al 2005).

Ecthyma is more commonly monitored in 3-6 month-old young animals whereas quite few mature ones could be affected as well. The risk of infection might highly increase especially in fattening lambs when the herd is expanded by participation of other animals. The incubation period of ORFV varies between 3-10 days. The disease clinically progresses in three forms; labial, podalic and genital (Fleming and Mercer 2007). The first study on the infection caused by ORFV in animals in Turkey was carried out in 1960 by Böğrün et al in the form of vaccine preparation with scabs taken from infected lambs (Çabalar et al 1996).

Poxviridae family encodes various virulence factors within their genomes in order to overcome infected host immune response. In some cases, it is believed that these virulence factors have homologous structure together with host immune proteins and during this period, poxviruses get hold of host genes. Poxvirus proteins which are similar to interleukin 1(IL-1), tumor necrosis factor (TNF), interferon gama (IFN- γ) and interferon alfa (IFN- α) and beta (IFN- β) receptor structure can be examples to these factors (Fleming et al 1997).

The interaction between immune system and ORFV has been a research subject for years (Weber et al 2003). Following ORFV infection, a natural immune response is firstly initiated by host immune system programmed to eliminate pathogen factors entering into organism (Fleming and Mercer 2007). This condition resulted of stimulation chemokine and cytokine release by various cells of immune system.

Viruses might affect immune system cells using various methods and cause the formation of cellular and humoral immune response against themselves. This research aims to reveal the correlation between the in vivo antibody kinetics formed by attenuated ORFV strain in sheep and cytokine profile and to determine its possible effects on immunity.

Material and Methods

Animals and samples

In this study, ten clinically healthy 6-8 month-old male yearling Akkaraman sheep had no bacterial or viral vaccines since its birth were used as research material. Before beginning of the study, blood serum of these selected animals was proved to carry no specific antibody against ORFV. Blood serum was timely obtained from the animals for six months and kept under necessary conditions.

Cell culture and virus

In the study, Madin Darby Bovine Kidney (MDBK) continuous cell cultures used in Selçuk University Faculty of Veterinary Science Virology Department were utilized. During the tests, *Parapoxvirus ovis* E(P)CK₂₂ attenuated vaccine strain was used in animal inoculations and serum neutralization tests.

Production of parapoxvirus ovis and detecting its titer

0,1 ml of commercially available attenuated Parapoxvirus ovis E(P)CK₂₂ vaccine strain was inoculated into 25 cm² flasks by using adsorption method and following the incubation for one hour at 37°C. Virus producing medium was added into cell producing flask at standard amount and left for incubation at 37°C. Viral cytopathic effect (CPE) was monitored by controls performed daily under a cell culture microscope (Olympus 1x71, Japan).

Titer of ORFV was calculated by using microtitration method (Frey and Liess 1971). For this purpose, ten folded dilutions of the virus as 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ and 10⁻¹⁰ were prepared with serum-free medium in 2 ml polystyren tubes. CPE formations depending on virus production were observed for five days. At the end of this period, the titer of the virus was calculated using (TCID₅₀) Kaerber method (1964).

Experimental design

0.5 ml scarification application was practised on hairless parts of inguinal areas of ten animals. Before the animals were given the vaccine strain, blood serum samples from all the animals were regarded as day 0 (control) samples in detecting cytokine levels and antibody titer profiles. Serum obtained from blood samples taken at 6th, 12th, 24th, 48th, 72nd, 96th, 120th hours and on days 7, 14, 21, 30, 60, 90, 120, 150 and 180 and brought to laboratory in cold chain was kept in -85°C deep freezer until testing in order to detect the effects of attenuated ORFV applications on cytokine and antibody profiles.

Serum neutralization test

After blood serum samples were taken periodically from the inoculated sheep (Double wells for a total of 170 serum samples from 10 animals), the method stated by Frey and Liess, (1971) was used to detect specific neutralising antibodies against ORFV.

Measuring cytokine levels

After the animals were provided with attenuated virus applications, blood serum samples taken periodically and kept at -85°C , cytokine levels were detected using commercial kits based on double antibody sandwich ELISA. From the obtained serum samples, proinflammatory (IL-1 β , IFN- γ , IL-12, TNF- α) [Sunred Biological Technology Co., Ltd.] Shanghai, China] and anti-inflammatory (IL-4, IL-10) cytokine levels were detected by the ELISA reader.

For this purpose, ELISA test was performed according to manufacturer's procedure. For the relation between the obtained OD values and their standard concentrations, standard curves of parameters were drawn separately with Microsoft Excel program by using linear regression equation. OD values seen in sections containing the samples were

transformed into cytokine concentration amounts obtained from standard curve graphics using regression equations.

Afterwards, profiles of IL-1 β , IFN- γ , IL-12, TNF- α , IL-4, and IL-10 levels were identified by days and intra-group statistical comparisons were stated.

Statistical analysis

Before performing significance tests, the obtained data were arranged by Shapiro Wilks in terms of normality from parametric test assumptions. Homogeneity of the variances were examined with the Levene's test. The statistical control of the difference among the variables was done with ANOVA. Tukey test was used for evaluation of differences between the groups. Descriptive statistics for each variable have been calculated and presented as "Mean \pm Standard Mean Deviation" (Mean \pm SD). All statistical analyzes were examined with a minimum error of 5%. Using the SPSS 22.0 package program, ($p < 0.05$) level was considered significant.

Results

During the titration process using microtitration method in MDBK continuous cell cultures of ORFV, virus titer was calculated as $\text{TCID}_{50} = 10^{-7,0}/0.1$ mL according to Kaerber (1964) method. The virus was diluted at the rate

Table 1. The effect of attenuated parapoxvirus ovis (0.5 ml, inguinal region, scarification) on the cytokine values of male Akkaraman sheep (mean \pm SE)

Parameters	IL 1- β (pg/mL)	IL-12 (pg/mL)	IFN- γ (pg/mL)	TNF- α (pg/mL)	IL-4 (pg/mL)	IL-10 (pg/mL)
0 hour/ control	22,4 \pm 1,20 ^{ab}	31,5 \pm 4,44 ^{ab}	69,8 \pm 9,31 ^{ab}	46,6 \pm 3,40 ^{ab}	33,3 \pm 3,00 ^{ab}	92,5 \pm 4,57 ^{ab}
6 hours	21,0 \pm 2,53 ^{ab}	28,5 \pm 5,58 ^{ab}	79,2 \pm 11,0 ^{ab}	51,7 \pm 2,75 ^{ab}	30,1 \pm 2,16 ^a	78,7 \pm 4,12 ^{ab}
12 hours	22,1 \pm 0,73 ^{ab}	25,6 \pm 3,68 ^{ab}	54,3 \pm 11,2 ^{ab}	49,3 \pm 2,00 ^{ab}	39,8 \pm 2,34 ^{abc}	88,4 \pm 6,73 ^{ab}
24 hours	22,5 \pm 0,99 ^{ab}	27,5 \pm 2,62 ^{ab}	31,2 \pm 5,90 ^b	32,6 \pm 3,99 ^b	42,2 \pm 2,03 ^{abc}	74,1 \pm 7,39 ^{ab}
48 hours	21,5 \pm 1,15 ^{ab}	28,9 \pm 3,50 ^{ab}	48,2 \pm 8,25 ^{ab}	34,9 \pm 5,26 ^{ab}	36,3 \pm 5,41 ^{ab}	81,2 \pm 7,75 ^{ab}
72 hours	20,5 \pm 1,28 ^{ab}	31,1 \pm 4,69 ^{ab}	32,6 \pm 8,64 ^b	48,5 \pm 2,16 ^{ab}	41,2 \pm 2,74 ^{abc}	72,6 \pm 7,80 ^{ab}
96 hours	14,6 \pm 2,08 ^b	29,1 \pm 3,53 ^{ab}	44,1 \pm 11,7 ^{ab}	44,1 \pm 3,89 ^{ab}	40,6 \pm 4,48 ^{abc}	79,1 \pm 5,50 ^{ab}
120 hours	17,7 \pm 1,43 ^{ab}	25,9 \pm 3,18 ^{ab}	47,1 \pm 8,98 ^{ab}	52,4 \pm 3,64 ^{ab}	37,8 \pm 3,42 ^{abc}	62,8 \pm 8,66 ^b
7 days	18,4 \pm 1,93 ^{ab}	28,2 \pm 2,37 ^{ab}	62,0 \pm 12,4 ^{ab}	51,3 \pm 6,08 ^{ab}	38,2 \pm 2,57 ^{ab}	95,6 \pm 12,8 ^{ab}
14 days	23,3 \pm 0,94 ^{ab}	15,5 \pm 3,21 ^b	66,8 \pm 12,9 ^{ab}	44,3 \pm 4,81 ^{ab}	48,4 \pm 5,28 ^{abc}	110 \pm 12,8 ^{ab}
21 days	23,7 \pm 1,85 ^{ab}	32,3 \pm 4,83 ^{ab}	99,1 \pm 4,55 ^a	40,3 \pm 8,84 ^{ab}	53,2 \pm 8,23 ^{abc}	122 \pm 15,5 ^{ab}
30 days	27,2 \pm 4,25 ^{ab}	50,7 \pm 15,0 ^{ab}	81,6 \pm 7,08 ^{ab}	52,6 \pm 12,1 ^{ab}	75,2 \pm 16,5 ^c	148 \pm 43,7 ^{ab}
60 days	25,4 \pm 1,72 ^{ab}	25,5 \pm 5,48 ^{ab}	88,7 \pm 7,08 ^a	54,0 \pm 5,55 ^{ab}	65,5 \pm 2,54 ^{bc}	131 \pm 9,77 ^{ab}
90 days	27,5 \pm 5,53 ^{ab}	60,8 \pm 17,9 ^a	67,9 \pm 16,4 ^{ab}	58,3 \pm 15,2 ^{ab}	67,7 \pm 13,4 ^{bc}	139 \pm 46,4 ^{ab}
120 days	26,1 \pm 1,78 ^{ab}	40,9 \pm 3,54 ^{ab}	46,5 \pm 10,7 ^{ab}	62,0 \pm 5,61 ^{ab}	68,7 \pm 5,60 ^{bc}	151 \pm 11,4 ^{ab}
150 days	28,6 \pm 4,85 ^a	51,6 \pm 10,4 ^{ab}	53,8 \pm 12,3 ^{ab}	68,6 \pm 10,1 ^{ab}	68,5 \pm 12,6 ^{bc}	153 \pm 34,0 ^{ab}
180 days	22,8 \pm 3,86 ^{ab}	59,7 \pm 10,8 ^a	80,4 \pm 19,6 ^{ab}	70,4 \pm 13,1 ^a	68,2 \pm 9,32 ^{bc}	168 \pm 34,8 ^a

Values are given in mean \pm SEM

Different letters in the same line (^{abc}) denote statistical significance ($p < 0.05$)





of 100 TCID₅₀ and used in serum neutralization test. With microneutralization test, no neutralized antibody presence against ORFV could be detected in 170 serum samples from ten animals. In blood serum samples used in the study, cytokine levels (IL-1β, IFN-γ, TNF-α, IL-4, IL-10, IL-12) were detected using commercial ELISA kits.

Samples taken from animals before attenuated ORFV vaccine application (control) and serum IL-1β levels found in samples taken periodically (6th, 12th, 24th, 48th, 72nd, 96th, 120th, hours and days 7, 14, 21, 30, 60, 90, 120, 150 and 180) 180 days after the application were shown in (figure

1), IL-12 levels in (figure 2), IFN-γ levels in (figure 3), TNF-α levels in (figure 4), IL-4 levels in (figure 5) and IL-10 levels in (figure 6) and results were presented in (Table 1). IL-1β, IL-12, IFN-γ and TNF-α levels of cytokines with proinflammatory characteristics (Th1) had a fluctuant progress, reached peak concentration on days 150, 90, 21 and 180 respectively, but changes in cytokine levels did not display statistical differences (p>0,05). IL-4 level of cytokines with antiinflammatory characteristics (Th2) had a statistical peak on day 30 (p<0,05) and IL-10 level tended to increase from day 7, had a peak on day 180, however these changes did not display statistical differences. (p>0.05).

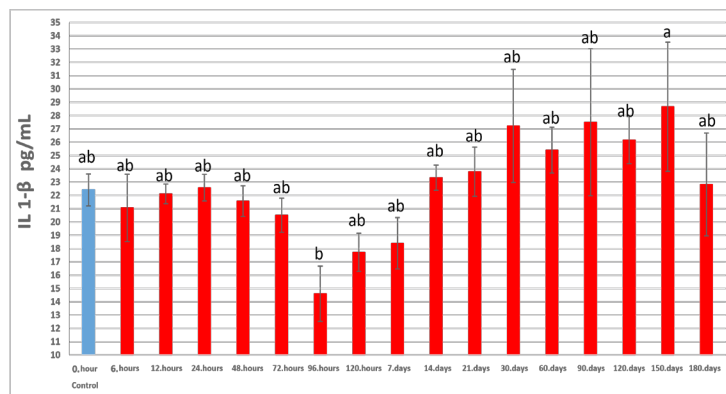


Figure 1. Akkaraman male sheep attenuated after parapoxvirus ovis administration (0.5 ml, inguinal region, effect of scarification IL-1β (pg / mL) values (mean ± SE)

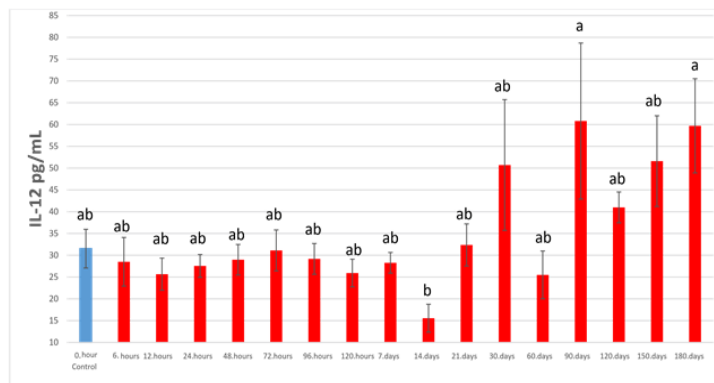


Figure 2. Akkaraman male sheep attenuated after parapoxvirus ovis administration (0.5 ml, inguinal region, effect of scarification IL-12 (pg / mL) values (mean ± SE)

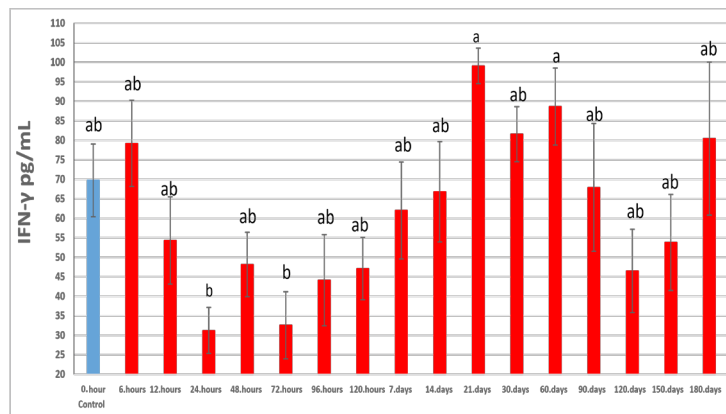


Figure 3. Akkaraman male sheep attenuated after parapoxvirus ovis administration (0.5 ml, inguinal region, effect of scarification IFN-γ (pg / mL) values (mean ± SE)





information on whether the animals with high seropositivity rates slaughtered in slaughterhouses had an infection story in their past.

Though it differs from country to country, attenuated tissue culture vaccines, non-attenuated live vaccines or live vaccines produced in sheep have been used to prevent the disease. It varies according to the vaccine used. Although it is thought that immunity is usually formed three weeks after vaccination, there are doubts whether they provide a full protection. Vaccine applications are generally performed subcutaneously or cutaneously by scarification method. In order to get sufficient immune response in controlling the infection, preferring attenuated and live vaccines might cause the virus spread around. (Haig and McInnes 2002) Therefore, attention must be paid since using the vaccines in herds and areas where the disease is not seen might be a risk factor.

In a study performed by Çabalar et al (1996) in our country, a double blood serum sampling was carried out every four weeks in 8-10 month-old lambs found in a herd with ORFV infection. Specific antibody detection was performed by serum neutralization test and neutralized antibody titers were monitored; according to the results, 1/151,72 titer was averagely found in the first samples taken after 8-10 days following the onset of the illness in the herd while 1/37,30 neutralized antibody titer was observed in the second samples taken four weeks after the first sampling (about 40 days following the onset of the infection). This case revealed that there was a crucial decrease in antibody titers statistically in only four weeks even in cases of natural infection.

Çabalar et al (1996) stated the neutralized antibody presence until day 40 when they ended the research for naturally infected lambs and Hausawi et al (2008) until day 28 in their experimental study, however unlike these researchers, Pye (1990) couldn't detect any antibody response in lambs vaccinated by ORFV strain with TCID₅₀ 10⁻⁵/ml titer adapted to cell culture in his immunity studies in lambs and Ergin and Köklü (1975) stated that ORFV was produced in sheep thyroid cell culture at TCID₅₀ 10^{-5.4} and 10^{-6.2}/ml titers and neutralization did not actualize in neutralization tests carried out with the producing virus.

Gülyaz et al (2012) also determined that they could not observe any neutralized antibody activity in lambs experimentally infected by attenuated vaccine strain during their study and they attributed this case to the fact that the virus stimulated cellular immune response rather than humoral immunity. In this study where the attenuated strain was used at TCID₅₀ 10⁻⁷/0,1ml titer by Gülyaz et al (2012), neutralized antibody detection could not be performed in all serum samples from lambs sampled until day 180. In

accordance with this finding, Haig and Mercer (1998) stated that serum neutralization test is not a reliable method in especially primary diagnosis, ORFV basically stimulates cellular immunity and might cause neutralized antibody stimulating only in low concentrations. Likewise, in antibody detection, there are numerous researches emphasizing that ELISA test facilitating for the detection of antibodies with no neutralized features in serum samples is more sensitive and is used successfully (Mckeever et al 1987, Yirrell et al 1991, 1994). Since the ELISA test was not used in this study, it could not be determined whether there was any antibody activation other than neutralized antibodies at the end of the performance.

Anziliero et al (2014) investigated the effect of IL-1 β by PCR on cytokine expression as a result of intraperitoneal inoculation of iORFV into mice at 6-8 weeks of age in a study in which they investigated the effect of IL-1 β on 24 hours reached a peak level of TNF- α and IL-8 mRNA at 48 hours. They found that there was a 15-fold increase in IFN- γ at 48 hours, a 6-fold increase in IL-12 at 24 hours, IL-10 at 72 hours, and IL-4 expression at 96 hours. The increase detected by ELISA in serum in IL-1, IFN- γ , TNF- α , IL-10 and IL-12 levels showed a kinetic resemblance to the amounts detected by PCR and the increase especially in IL-12 and IFN- γ amounts was remarkable. At the end of the study, it was revealed that ORFV inoculation caused a temporary and complex cytokine response and Th1 cytokines were observed at the beginning and then Th2 cytokines followed.

In a study conducted by Anderson et al (2001) with 4-6 month-old Suffolk crossbred sheep determined to be negative in terms of specific antibodies. Skin biopsy materials taken from animals with primary infection using the scarification method with the Moredun reference strain of ORFV were examined by in situ hybridization method. Although cells expressing IFN- γ and IL-4 mRNA were not found, it was reported that cells expressing TNF- α mRNA were found on the 5th, 8th and 20th days after infection. In the same study, 8 weeks after primary virus inoculation was performed, cells expressing TNF- α mRNA and IFN- γ were seen in the second group animals reinfected by the same virus. However, cells expressing mRNA belonging to IL-4 could not be detected. No cells expressing IFN- γ mRNA were detected in samples taken from animals just before the infection while they appeared on day 2 after reinfection.

Human (Friebe et al 2004), mouse (Avci et al 2016), guinea pig (Weber et al 2003), such as sheep, except for species with usually inactive in many research studies as a stimulant of the virus paraimmunity ORFV IL-12, IL-18, IFN- γ and other Th1 cytokines showed it is suggested to increase regulation of a character. However, in our study, there was a fluctuation in the amounts of IL-1 β , IL-12, IFN- γ and TNF- α selected as Th1 cytokines intended to be measured as a result of vaccine





strain administration, but there was no statistically significant change in the direction of increase compared to control sera ($p > 0.05$), the IL-1 β level was on day 150 (28.66 ± 4.85 pg/ml), the level of IL-12 was on day 90 (60.8 ± 17.9 pg/ml), the IFN γ level was on day 21 (99.1 ± 4.55 pg/ml), and the level of TNF- α was again on day 180, it was determined that it reached the highest levels (70.4 ± 13.1 pg/ml) (Table 1). It has been observed that the attenuated ORFV strain does not have any effect on Th1 cytokines that are expected to be labeled early in the immune response to viral antigens and peak at a high level in a short time. In particular, it seems to be the opposite situation that attenuated live vaccine administration, which is considered to increase the level of Th1 cytokines responsible for the cellular immune response and, accordingly, to create an adequate immune response, does not lead to a statistically significant increase and causes an increase in Th1 cytokines in studies using inactivated ORFV. The most likely reason for this, some live cells escape from the immune system as a method of replication of the virus in the IL-10 homologues proteins (viral IL-10, vIL-10) encoding (Deane et al 2000, Weber et al 2013) in their analysis of cytokines and Th1 cellular immunity responsible for this protein, inactivated virus may be the result of the lack of this mechanism. In fact, vIL-10, sheep IL-10 cytokines from macrophages such as TNF- α production, peripheral blood cells from IFN- γ production inhibits co-stimulator feature and IL-3 and IL-4 secretion has been reported to stimulate the proliferation of mast cells that play a role in (Haig and Mercer 2010). The vIL-10 detected in the NZ2 strain of ORFV showed 80% similarity with sheep-derived IL-10, and 75%, 67% and 64% similarity with bovine, human and mouse-derived IL-10, respectively (Fleming et al 1997), mouse modeling (Imlach et al 2002) demonstrated that it plays an important role immunosuppression by inhibiting cytokine synthesis from macrophages. It has also been determined that it inhibits the maturation and functions of human and mouse dendritic cells (Lateef et al 2003). However, since the ELISA kits used to measure IL-10 cytokine levels in this study were sheep specific, although an increase was observed from the 7th day onwards, it was determined that it was not statistically significant; It was concluded that a detailed study should be done on the level of presence. In addition to all these, it should be kept in mind that another reason for the increase in Th1 cytokine levels in studies using iORFV may be the fact that the iORF preparations offered for sale in the market contain carrier substances such as polygeline, which may create a different stimulation in terms of immune response than the attenuated vaccine strain.

In this study, it was revealed that IL-4 level was at its peak (75.2 ± 16.5 pg/ml) to form a statistical difference on day 30 compared to control serum (33.3 ± 3.00 pg/ml) and later on showed a fluctuant progress with no statistical difference (Chart 1, Figure 5). IL-4, triggered in later periods rather than early synthesizing periods by Th1 subgroup cells in viral

infections, is a cytokine causing Th2 cells of non-stimulate CD4+ precursors to differentiate, the transformation of B cells into plasma cells and the suppression of macrophage functions dependent on IFN- γ . (Abbas and Lichtman 2007) Even though a peak was observed on day 30 in IL-4 cytokine level, antibody detection could not be performed in the study that might result from the fact that this increase in the cytokine did not reach threshold level to enable B cells to transform into plasma cells. Another reason of this could be that serum neutralization test used to reveal antibody detection in the study was insufficient in detecting antibodies with no neutralized features and appearing in low levels as a result of virus stimulating.

Conclusion

In this study, the effects of attenuated E(P)CK₂₂ strain used in preventing ORFV infection was investigated in terms of neutralized antibodies and primary cytokine activity because it has endemic characteristics for sheep and goat industry both in our country and the world. With the data obtained in the study, it is also aimed to gain an idea about how the cytokines, which play an active role in the humoral and cellular immune response, affect the effectiveness of the vaccine in the field.

However, in this study, no neutralized antibody could be detected in any of the serum samples obtained by the application on lambs, no crucial statistical change was seen in IL-1 β , IFN- γ , IL-12, TNF- α and IL-10 levels by ELISA compared to control serum but only an increase in IL-4 level was found on day 30 to form a statistical difference. In the light of these findings, using a single dose attenuated vaccine strain in the study might not be sufficient in immune response as well as the ability to develop a cellular immune response. Correspondingly, it is unignorable that herd problems depending on the infection might be observed although vaccines are applied in field.

Consequently, in detecting the effects of ORFV on IL-1 β , IFN- γ , IL-12, TNF- α , IL-4 and IL-10 levels, assuming that more different data could be obtained, more sensitive methods may be used such as molecular methods based on the detection of cells expressing mRNA pertaining to cytokines.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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