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Research Article

Investigation of the immunomodulatory effect of inactive parapoxvirus (iPPVO) on infectious bovine rhinotracheitis (IBR) vaccine in cattle

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ABSTRACT

In the study, alterations in antibody titers, proinflammatory and antiinflammatory cytokine levels were determined in serum samples collected at various periods before and after administration of inactive IBR and iPPVO to cattle. It was aimed to investigate the immunomodulatory effects of inactivated parapoxvirus ovis (iPPVO) in cattle vaccinated with inactivated-IBR vaccine. In the study, 40 unvaccinated, clinically healthy cattle of different breeds aged older than 3 months were used. Three groups of cattle were formed as control group 1 (n=10), control group 2 (n=10) and experimental group (n=20). iPPVO was applied to the cattle in the control group 1 and experimental groups on the 0, 2nd, and 4th days. Blood samples were collected from all the animals after 6th hours of the injections applied on 0th and 4th days of the study. Commercially available ELISA kits were used to determine serum levels of of IL-2, IL-6, IL-12 and IFN-γ. Furthermore, virus neutralization test was also performed to detect virus neutralizing antibody titres. In the present study, serum levels of IL-2, IL-6 IL-12, and IFN-γ levels were found to be significantly higher in the experimental group compared to that of the control group 1 and control group 2 ($p<0,05$). The differences between control group 1 and control group 2 groups were not statistically significant.

In conclusion, iPPVO increased the levels of cytokines in IBR vaccinated cattle due to its immunomodulatory effects. In addition, virüs neutralizing antibody titers were found to be significantly higher in cattle that received vaccine and iPPVO.

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) is caused by Bovine herpes virus-1 (BoHV-1). Bovine herpes virus-1 (BoHV-1) causes fertility disorders, abortions and fatal systemic diseases in newborns by affecting the nervous and genital system, especially the respiratory system in cattle (Jones and Chowdhury, 2010; Raaperi et al., 2012). BoHV-1 infected cattle are latent throughout their lives. The virus may become reactivated due to stress or immunosuppressors (Kook et al., 2015; Winkler et al., 2000). In general, the elimination of stress factors and the use of immunomodulators in vaccination provide better immunization. Inactivated Parapoxvirus ovis D1701 strain (iPPVO) has been licensed as an immunostimulant drug in the veterinary field (Coskun, 2017). It has been declareted that iPPVO reduces susceptibility and has antiviral activity against herpes simplex and hepatitis B infection in mice by activating cytokine (TNF-α, IFN-γ, IL-12 and IL-18) induction against these viruses. In a study in mice, it was determined that iPPVO administration stimulated the synthesis of TNF-α (16th and 24th hours) and IL-6 (12th, 16th and 24th hours) and caused changes in IL-10 and IL-12 levels. It has been reported that the increase in cytokine levels can be attributed to the immunomodulatory activity of iPPVO (Dinarello, 2000). However, in the literature review, it was seen that there was no research on the use of iPPVO in vaccination with inactivated vaccines and its effects on the synthesis of cytokines, which is the first response mediator of the immune system after vaccination (Friebe et al., 2004; Weber et al., 2003).

In this study, it was aimed to investigate the immunomodulatory effect of iPPVO (Zylexis® flk) in cattle vaccinated with inactivated-IBR vaccine by determining the virus neutralizing antibody titers, proinflammatory and antiinflammatory cytokine levels.

MATERIALS and METHODS

Animals

In the study, 40 clinically healthy cattle, older than 3 months, various breeds and sexes were used. All the animals used in the study were not vaccinated with IBR vaccine before the study. Cattle were divided into 3 groups as follows; Control group-1 (n=10), Control group-2 (n=10) and Experimental group (n=20). This study was approved by the Local Ethics Committee for Animal Care of the Burdur Mehmet Akif Ersoy University (12/12/2018- decision number: 470). Consent forms were signed by the animal owners.

Administration of iPPVO (Zylexis® flk) and IBR vaccine (Rispoval®)

Contro group 1: A single dose of 2ml of iPPVO (Zylexis® flk) were administered intramuscularly on 0th, 2nd and 4th days of the study. Blood samples were taken at the 6th hour after the each injection.

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Control group 2: IBR vaccine (Rispoval®) was administered to the each animal subcutaneously at a dose of 2 ml on the 4th day of the study. Blood samples were taken at before and the 6th hour of the 4th days.

Experimental group: 20 cattle in the experimental group were ricieved 2 ml iPPVO (Zylexis® flk) intramuscularly on the 0th and 2nd days, and then at the 4th days iPPVO and 2 ml of IBR vaccine (Rispoval®) were given simultaneously. Samples were taken at the 6th hour of the 0th and 4th days of the injections.

Collection of Blood Samples

Samples were collected from all the animals on 0th, 2nd, 4th days of the study into plane tubes. These samples were then used to measure cytokine levels (IL-2 IL-6, IL-12, IFN-γ). Serum samples were also collected on 0th, 7th, 21st days and used to determine virus neutralizing antibody titres. All the serum samples were kept at at -20°C until used. Samples were also collected on 0th and 4th days into tubes with EDTA and used to determine hematological parameters.

ELISA Tests

Commercial Bovine specific ELISA kits were used to determine (ELISA, YL biont, Shanghai, CHINA) IL-2, IL-6, IL-12 and IFN-γ concentrations in the collected serum samples. The ELISA was performed according to the manufacturer's instructions. The optical density (OD) of each well for parametres were defined with a micro-ELISA plate reader (MR-96A, Minray, China) at a test wave-length of 450 nm. IL-2, IL-6 and IL-12 and IFN-γ concentrations in the samples were calculated by obtaining the formula from each standart regression analysis.

Microneutralization Test (mNT)

Serum virüs-neutralization test was performed according to the methods of Frey and Liess (1971). Cytopathologic effect (CPE) formation was checked every day under tissue culture microscope, and the virus titer (tissue culture infective dose 50-DKID50) at the end of the 5th day was calculated according to the Kaeber (1964) method. The presence of neutralizing antibodies against BHV-1 in blood serum samples was determined according to the mNT method reported by Frey and Liess (1971).

Statistical Analysis

Differences between groups in measured-parameters were analyzed with independent t-test, and antibody levels were analyzed with ANOVA and post-hoc Duncan tests (SPSS 10.0 for Windows/SPSS® Inc., Chicago, IL, U.S.A.). In the evaluation, $p<0.05$ was acknowledged as the significance limit.

RESULTS

Hemogram and ELISA Results

IL-2, IL-6, IL-12 and IFN-γ values were found to be significantly higher in the experimental group compared to those of the control-1 and 2 groups ($p<0.05$).

Table 1. Hemogram and ELISA results of blood samples taken on the 0th day (Mean ± standard deviation).

0th DAY	CONTROL GROUP 1 (n=10)	CONTROL GROUP 2 (n=10)	EXPERIMENTAL GROUP (n=20)
IL2 (ng/l)	6,1±0,64^{b}	5,5±0,38^{b}	14,8±1,38^{a}
IL6 (ng/l)	45,3±1,82^{b}	50,0±3,35^{b}	95,9±9,33^{a}
IL12 (ng/l)	2,5±0,46^{b}	1,9±0,14^{b}	3,6±0,38^{a}
IFN-γ (pg/ml)	12,0±1,09^{b}	14,0±2,61^{b}	49,5±6,42^{a}
LYM (x10^{9}/L)	5,8±0,18^{b}	4,9±0,20^{ab}	5,5±0,25^{a}
WBC (x10^{9}/L)	8,7±0,35^{b}	6,8±0,31^{a}	9,2±0,39^{a}
GRAN (x10^{9}/L)	1,6±0,17^{b}	1,0±0,97^{a}	1,4±0,10^{a}
RBC (x10^{12}/L)	5,5±0,23^{b}	5,0±0,16^{b}	5,0±0,13b
HGB (g/dl)	10,9±0,41^{b}	10,5±0,40^{b}	10,9±0,27^{b}
HTC (%)	24,8±1,21^{b}	21,8±0,81^{b}	21,9±0,47^{a}
MCHC (g/dl)	44,3±1.39^{b}	48,6±1,92^{ab}	53,5±1,42^{a}
MCH (pg)	19,8±0,35^{b}	20,9±0,59^{ab}	22,0±0,51^{a}
MCV (fl)	44,9±0,59^{b}	43,3±0,49^{a}	41,9±0,31^{c}

IL2 (Interleukin 2), IL6 (Interleukin 6), IL12 (Interleukin 12), IFN-γ (Interferon Gamma), WBC (leukocyte), LYM (lymphocyte), GRAN (granulocyte), RBC (Red Blood Cells), HGB (hemoglobin), HTC (Hematocrit). MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), MCV (Mean Corpuscular Volume). The degree of statistical significance between the groups was indicated with letters, and the presence of different letters in the same line indicates the statistical significance ($p<0.05$) between the groups.

Table 2. Hemogram and ELISA results of blood samples taken on the 4th day (Mean ± Standard deviation).

4th DAY	CONTROL GROUP 1 (n=10)	CONTROL GROUP 2 (n=10)	EXPERIMENTAL GROUP (n=20)
IL2 (ng/l)	5,9±0,73^{b}	5,4±0,78^{b}	16,9±1,13^{a}
IL6 (ng/l)	49,6±3,15^{b}	45,2±2,43^{b}	89,7±8,62^{a}
IL12 (ng/l)	1,6±0,16^{b}	1,7±0,15^{b}	2,8±0,25^{a}
IFN (pg/ml)	17,7±3,88^{b}	9,9±1,92^{b}	62,6±5,45^{a}
LYM (x10^{9}/L)	5,7±0,25^{b}	5,2±0,15^{b}	7,6±0,42^{a}
WBC (x10^{9}/L)	9,5±0,37^{b}	7,9±0,44^{b}	12,6±0,57^{a}
GRAN (x10^{9}/L)	2,4±0,34^{b}	1,5±0,28^{b}	2,1±0,24^{b}
RBC (x10^{12}/L)	5,9±0,24^{b}	4,7±0,23^{a}	5,7±0,18^{a}
HGB (g/dl)	11,8±0,37^{b}	9,9±0,33^{a}	11,6±0,26^{a}
HTC (%)	28,8±2,62^{b}	20,9±1,36^{b}	23,6±0,70^{a}
MCHC (g/dl)	44,9±2,07^{b}	48,0±1,89^{ab}	52,5±1,31^{a}
MCH (pg)	20,0±0,59^{b}	20,9±0,52^{ab}	22,4±0,45^{a}
MCV (fl)	44,8±0,73^{b}	43,7±0,66^{b}	43,5±0,46^{b}

IL2 (Interleukin 2), IL6 (Interleukin 6), IL12 (Interleukin 12), IFN-γ (Interferon Gamma), WBC (leukocyte), LYM (lymphocyte), GRAN (granulocyte), RBC (Red Blood Cells), HGB (hemoglobin), HTC (Hematocrit), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), MCV (Mean Corpuscular Volume).
The degree of statistical significance between the groups was indicated with letters, and the presence of different letters in the same line indicates the statistical significance ($p<0.05$) between the groups.

Table 3. BHV-1 Antibody Titer Values of Cattle in the Control 2 group.

BHV-1 Antibody Titer	Number of Animals (n=10)	%	Day
1/2	1	10	7th day
1/4	-	-	7th day
1/8	8	80	7th day
1/16	1	10	7th day
1/128	-	-	7th day
1/2	1	10	14th day
1/4	9	90	14th day
1/8	-	-	14th day
1/16	-	-	14th day
1/32	-	-	14th day
1/64	-	-	14th day
1/128	-	-	14th day
1/2	-	-	21st day
1/4	1	10	21st day
1/8	1	10	21st day
1/16	8	80	21st day
1/32	-	-	21st day
1/64	-	-	21st day
1/128	-	-	21st day

WBC values determined on day 0 were found to be high in experimental group, whereas low in control groups-1 and 2. Lymphocyte (LYM) values were detected to increased in all the animals after vaccination and iPPVO applications. IL-2, IL-6, IL-12, IFN-γ and lymphocyte values were statistically similar in the experimental and control groups-2, while the control-1 group was different ($p<0.05$, Table 1).

When the samples taken on the 4th day in the control 1, control 2 and experimental groups were examined, it was determined that IFN-γ, IL-2, IL-6, IL-12 increased 6th hours after the 4th day of the vaccination in experimental group ($p<0.05$). WBC and lymphocyte values were similar in control 1 and 2 groups, but statistically different in the experimental group ($p<0.05$).

sera of animals in the control 2 group; It was defined as 1 (10%) animal at 1/2 (dilutions based on log2) titer, 1 (10%) animal at 1/4 titer, and 8 (80%) animals at 1/16 titer (Table 3).

BHV-1 antibody titers in the blood serum of Zylexis+vaccinated animals in the experimental group; At day 7, it was defined as 2 (10%) animals at 1/2 (dilutions based on log2) and 18 (80%) animals at 1/8 titer. BHV-1 antibody titers in the blood sera of the animals in the experimental group were determined as 20 (100%) animals at 1/32 titer (log2-based dilutions) on the 14th day. BHV-1 antibody titers in the blood serum of the animals in the experimental group; On day 21, it was determined as 20 (100%) animals at a titer of 1/64 (dilutions based on log2) (Table 4).

Table 4. BHV-1 Antibody Titer Values of Cattle in the Experimental Group.

BHV-1 Antibody Titer	Number of Animals (n=20)	%	Day
1/2	2	10	7th day
1/4	-	-	7th day
1/8	18	90	7th day
1/16	-	-	7th day
1/128	-	-	7th day
1/2	-	-	14th day
1/4	-	-	14th day
1/8	-	-	14th day
1/16	-	-	14th day
1/32	20	100	14th day
1/64	-	-	14th day
1/128	-	-	14th day
1/2	-	-	14th day
1/4	-	-	21st day
1/8	-	-	21st day
1/16	-	-	21st day
1/32	-	-	21st day
1/64	20	100	21st day
1/128	-	-	21st day

BHV-1 Antibody Titer Results

The presence of BHV-1 antibodies was not detected in the blood sera of the animals in the control-1 group, which were administered only Zylexis, on the 7th, 14th and 21st days.

BHV-1 antibody titers in the blood serum of animals in control-2 group on day 7th, 1 animal (10%) at 1/2 (dilutions based on log2), 8 (80%) animals at 1/8 titer, 1/16 titer 1 (10%) animal was detected. BHV-1 antibody titers in blood serum of control group-2 animals were identified on day 14th, 1 animal (10%) at 1/2 titer (log2-based dilutions) and 9 (90%) animals at 1/8 titer. On day 21th, BHV-1 antibody titers in the blood

DISCUSSION

Infectious Bovine Rhinotracheitis (IBR) causes great economic losses. It effects nervous, respiratory and genital system in cattle, causing fertility disorders, abortions and fatal systemic diseases in newborns (Jones and Chowdhury, 2010; Raaperi et al., 2012). It has been reported that eradication and vaccination program should be initiated due to the prevalence of IBR infection in Turkiye (Alkan et al., 2018). It is known that the best way to prevent the disease is vaccination. Establishing a high antibody titer in vaccination provides long-term and safe protection. Therefore, to increase antibody titres and protectivity

of vaccine, using immunomodulatory agents such as iPPVO in combination with vaccine is important. Inactive parapoxvirus ovis (iPPVO) (Zylexis®) is a nonspecific immunomodulator used to stimulate innate immunity (non-specific, innate immunity) against infectious viral diseases (Schütze et al., 2009).

A study observed that in vitro phagocytic activities of peritoneal macrophages collected from mice vaccinated with iPPVO significantly increased at different time periods after vaccination. Furthermore, increased expression of IL-12 and IFN-γ, mRNA has been reported to be related to advanced in vitro and in vivo phagocytic activity of macrophages (Ons, 2014). It has been reported that intraperitoneal administration of iPPVO causes stimulation of IL 6 synthesis and fluctuations in IL-10 and IL-12 concentrations in rats (Avcı et al., 2016). In present study, increases in IFN-γ and interleukin activities were also detected.

Kyriakis et al. (1998) found that iPPVO was effective to prevent IBR infection in healthy calves kept with infected calves. Kyriakis et al. (1998) found a three-fold reduction in diarrhea incidence and a six-fold reduction in mortality between iPPVO-treated and non-iPPVO-treated piglets. The study reveals the protective feature of iPPVO against diseases. In this study, the increase in IFN-γ and interleukin activity shows that iPPVO increases protection against IBR and supports both studies.

A small pilot study in bovine herpes virus-1-infected calves showed that the use of iPPVO as an immunomodulator after the onset of an outbreak of respiratory disease can significantly help to reduce clinical manifestations in animals that may subsequently become infected (Weber, 2013). In this study, it was revealed that the use of iPPVO together with the marker IBR vaccine is beneficial for the protection of herd health.

iPPVO is also reported to induce a predominant Type 1 T helper (Th1) immune response in several species. This hypothesis is explained by the ability of iPPVO to induce immunomodulatory activity in vitro as well as in vivo (Weber et al., 2013). In this study, the increase in lymphocytes in the IBR vaccine and iPPVO groups confirms this hypothesis.

Kyriakis et al. (1998) administered iPPVO for prophylactic or metaphylactic purposes in cattle, pigs, horses, mice, dogs and cats. In the study, iPPVO proved helpful in reducing clinical findings caused by some bacterial and viral infections such as equine herpes virus, feline herpes virus, canine adenovirus type 2, Pseudomonas aeruginosa, E. coli or Pasteurella multocida, Aujeszky disease virus, IBR and vesiculitis. This effect of iPPV was claimed to be due to the stimulation of early immune mechanisms such as innate macrophage cells and increased lymphocytes induced with interferon. In our study, increased IL 2, IL 6, IL 12 and IFN-γ and lymphocyte were defined in control-1 and experimental group animals may also help to increase effectivity of IBR vaccine due to iPPVO.

Arai et al. (1990) reported that IFN-γ and IL-10 expressions were induced in animals in the experimental group after iPPVO administration in rats. In another study, IFN-γ found to increase 15-fold 6, 12 hours after iPPVO administration in rats. It was also shown that injection of iPPVO at 24th and 48th hours increased IL-12 6-fold in mice (Anziliero, 2014). In this study, IL 2, IL 6, IL 12 and IFN-γ were also found to increased in iPPVO injected groups, control-1 and experimental group. In our study, IFN gamma increased approximately 3 times in the experimental group compared to the control-1 group and approximately 6 times compared to the control-2 group at the 2nd hour. IL 12 increased 2 times. These differences between studies may be due to differences in animal species and methods used.

A study in dogs reported a marked increase in monocytes, polymorphonuclear cells and phagocytotic activity along with peripheral blood leukocytes after stimulation with iPPVO (Schütze, 2009). In this study, similar hematologic findings were found that a significant increase determined in leukocytes count in the control 1 and experimental groups, which were applied iPPVO.

In a study, it was stated that some cytokine levels increased after iPPVO application in foals and that has been suggested to be beneficial in the prevention of Rhodococcus equi infections. (Dreismann, 2010). In another study, increases in blood cytokines levels were also detected in mice and rats riceived iPPVO (Anziliero et al., 2014). It also shown that iPPVO administration increases cyrokines productions and induces an immune response in horses (Horohov, 2008). In the present study, increases in cytokine levels (IL 2, IL 6, IL 12 and IFN-γ) were defined in the control-1 and experimental group, which might be induced iPPVO.

Virus-neutralizing antibodies are virus-specific antibodies that are responsible for killing the virus. The high level of these antibodies indicates that the virus-specific humoral protection is also strong. In a study on BHV-1, ELISA and serum neutralization (SN) test were performed in healthy cattle in the Konya region, and 19% positivity in ELISA and 13% in SN test were determined, respectively (Yanbakan, 2005). Although, this study shows that there may be seropositivity in healthy animals, a similar seropositivity was not determined in the control group 1 cattle used in our study. Studies have reported that the ELISA test is more sensitive than the virus neutralization test in determining seropositivity (Duman, 2013). In the present study, virus neutralizing antibody titers were determined. It was determined only in vaccinated cattle at a titer of 1/8 in 80% of the animals on the 7th day, at a titer of 1/4 in 90% of the animals on the 14th day, and at a titer of 1/16 on the 21st day in 80% of the cattle. On the other hand, in iPPVO and vaccinated cattle, on the 7th day, 1/8 titer was in 90% of the animals, on the 14th day, 1/32 titer in all animals, and on the 21st day, the virus neutralizing antibody titer was 1/64 titer in all animals (100%) was determined. These results show that virus neutralizing antibody titers increase over time both in the vaccine group and in the vaccine and iPPVO-administered groups. However, virus neutralizing antibody titers were found to be much higher in iPPVO and vaccinated cattle, and it was observed that these titers reached up to 1/64 titers in most of the animals on the 21st day. This results indicate that administration of iPPVO and IBR vaccine simultaneously increases virus neutralizing antibody titers in cattle.

In this study, it was determined that there was an increase in cytokine concentration with the immunomodulatory effect of iPPVO with the administration of iPPVO to cattle administered IBR vaccine. It was also noted that virus neutralizing antibody titers were significantly higher in cattle administered vaccine and iPPVO. In conclusion, iPPVO rises cytokine levels in IBR vaccinated cattle, showing the presence of immunomudulator effect of iPPVO. It was concluded that the administration of iPPVO with IBR vaccination may help to increase the effect of the vaccine. It is suggestive that administration of iPPVO and IBR vaccine simultaneously increases immun stimulation and strengthen immunoprotectivity in cattle.

CONCLUSION

In this study, it was determined that there was an increase in cytokine concentration with the immunomodulatory effect of iPPVO with the administration of iPPVO to cattle administered IBR vaccine. It was also noted that virus neutralizing antibody titers were significantly higher in cattle administered vaccine and iPPVO. In conclusion, iPPVO rises cytokine levels in IBR vaccinated cattle, showing the presence of immunomudulator effect of iPPVO. It was concluded that the administration of iPPVO with IBR vaccination may help to increase the effect of the vaccine. It is suggestive that administration of iPPVO and IBR vaccine simultaneously increases immun stimulation and strengthen immunoprotectivity in cattle.

DECLARATIONS

Ethics Approval

This study was approved by the Local Ethics Committee for Animal Care of the Burdur Mehmet Akif Ersoy University (12/12/2018- decision number: 470). Consent forms were signed by the animal owners.

Conflict of Interest

The authors declare that they have no compet of interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: NM, SE

Data collection and analysis: SE, NM

Drafting of the manuscript: NM, SE

Critical review: NM, SE

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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REFERENCES

Alkan, F., Bilge-Dagalp, S., Karapınar, Z., Timurkan, M.O., Coskun, N., & Burgu, I. (2018). Long-term study (2005–2010) on the vaccination with BoHV-1 glycoprotein E-deleted marker vaccine in selected two dairy herds in Turkey. Tropical Animal Health and Production, 50(2), 353-363. https://doi.org/10.1007/s11250-017-1440-3

Anziliero, D., Weiblen, R., Kreutz, L.C., Spilki, F., & Flores, E.F. (2014). Inactivated Parapoxvirus ovis induces a transient increase in the expression of proinflammatory, Th1-related, and autoregulatory cytokines in mice. Brazilian journal of medical and biological research. 47(2), 110- 118. https://doi.org/10.1590/1414-431X20133358

Arai, K.I., Lee, F., Miyajima, A., Miyatake, S., Arai, N., & Yokota, T. (1990). Cytokines: coordinators of immune and inflammatory responses. Annual review of biochemistry. 59, 783-836. https://doi.org/10.1146/annurev.bi.59.070190.004031

Avci, O., Bulut, O., & Dik I. (2016). Effects of inactive parapoxvirus ovis on cytokine levels in rats. Journal of Veterinary Medical Science. 78(1), 129-131. https://doi.org/10.1292/jvms.15-0231

Coşkun, D. (2017). Veteriner Destek Tedavi: Tarantula Cubensis Alkolik Ekstraktı, İnaktif Parapoxvirüs Ovis ve Corynebacterium Cutis Lizatı. Dicle Üniversitesi Veteriner Fakültesi Dergisi.10(1), 30-37.

Dinarello, C.A. (2000). Proinflammatory cytokines. Chest. 118(2), 503-508. https://doi.org/10.1378/chest.118.2.503.

Dreismann, G.M. (2010). Effects of the homeopathic preparation Engystol® ad us. vet. on stress induced parameters in weaning foals. Tieraerztliche Umschau, 65(12), 484-490.

Duman, R., Yavru, S., Bulut, O., & Avcı, O. (2013). Sığırlarda Bovine Herpesvirus-1 Enfeksiyonlarının Enzyme-Linked Immunosorbent Assay ve Mikronötralizasyon Testi ile Karşılaştırmalı Tespiti. Atatürk Üniversitesi Veteriner Bilimleri Dergisi. 8(2), 129-136.

Frey, H.R., & Liess, B. (1971). Vermehrungskinetik und verwendbarkeit eines stark zytopatogenen VDMD-Virusstammes für diagnostische untersuchungen mit der Mikrotiter-Methode. Zentralblatt für Veterinärmedizin Reihe B. 18(1), 61-71. https://doi.org/10.1111/j.1439-0450.1971.tb00343.x

Friebe, A., Siegling, A., Friederichs, S., Volk, H.D., & Weber, O. (2004). Immunomodulatory effects of inactivated parapoxvirus ovis (ORF virus) on human peripheral immune cells: induction of cytokine secretion in monocytes and Th1-like cells. Journal of Virology. 78(17), 9400–9411. https://doi.org/10.1128/jvi.78.17.9400-9411.2004

Horohov, D.W., Breathnach, C.C., Sturgill, T.L., Rashid, C., Stiltner, J.L., Strong, D., Nieman, N., & Holland, R.E. (2008). In vitro and in vivo modulation of the equine immune response by parapoxvirus ovis. Equine Veterinary Journal 40, 468-472. https://doi.org/10.2746/042516408X322111

Jones, C., & Chowdhury, S. (2010). Bovine herpes virus I (BHV-1) is an important cofactor in the bovine respiratory disease complex. The Veterinary Clinics of North America: Food Animal Practice. 26(2), 303-321. https://doi.org/10.1016/j.

cvfa.2010.04.007

Kaeber, G. (1964). In Diagnostic Procedures For Virus And Rickettsial Disease. Public Health Association. 3, 48-50.

Kook, I., Henley, C., Meyer, F., Hoffmann, F.G., & Jones, C. (2015). Bovine herpesvirus 1 productive infection and immediate early transcription unit 1 promoter are stimulated by the synthetic corticosteroid dexamethasone. Virology, 484, 377-385. https://doi.org/10.1016/j.virol.2015.06.010

Kyriakis, S.C., Tzika, E.D., Lyras, D.N., Tsinas, A.C., Saoulidis, K., & Sarris, K. (1998). Effect of an Inactivated Parapoxvirus Based Immunomodulator (Baypamun) on Post Weaning Diarrhoea Syndrome and Wasting Pig Syndrome of Piglets. Research in Veterinary Science. 64(3), 187-190. https://doi.org/10.1016/S0034-5288(98)90122-9

Ons, E., Van Brussel, L., Lane, S., King, V., Cullinane, A., Kenna, R., Lyons, P., Hammond, T.A., Salt, J., & Raue R. (2014). Efficacy of a Parapoxvirus ovis-based immunomodulator against equine herpesvirus type 1 and Streptococcus equi equi infections in horses. Veterinary Microbiology. 173(3-4), 232-240. https://doi.org/10.1016/j.vetmic.2014.07.015

Raaperi, K., Bougeard, S., Aleksejev, A., Orro, T., & Viltrop, A. (2012). Association of herd BRSV and BHV-1 seroprevalence with respiratory disease and reproductive performance in adult dairy cattle. Acta Veterinaria Scandinavica. 54, 4. https://doi.org/10.1186/1751-0147-54-4

Schütze, N., Raue, R., Buttner, M., & Alber, G. (2009): Inactivated parapoxvirus ovis activates canine blood phagocytes and T lymphocytes. Veterinary Microbiology. 137 (3-4): 260-267. https://doi.org/10.1016/j.vetmic.2009.01.035

Weber, O., Mercer, A.A., Friebe, A., Knolle, P., & Volk, H.D. (2013). Therapeutic immunomodulation using a virus-the potential of inactivated orf virus. European Society of Clinical Microbiolog. 32, 451□ 460. https://doi.org/10.1007/s10096-012-1780-x

Weber, O., Siegling, A., Friebe, A., Limmer, A., Schlapp, T., Knolle, P., Mercer, A, Schaller, H., & Volk, H.D. (2003). Inactivated parapoxvirus ovis (Orf Virus) has antiviral activity against hepatitis B virus and herpes simplex virus. The Journal of general virology. 84(7), 1843-52. https://doi.org/10.1099/vir.0.19138-0

Winkler, M.T., Doster, A., & Jones, C. (2000). Persistence and reactivation of bovine herpesvirus 1 in the tonsils of latently infected calves. Journal of virology. 74, 5337-5346. https://doi.org/10.1128/jvi.74.11.5337-5346.2000

Yanbakan S. (2005). A comparative evaluation of bovine herpesvirus–1 infection by enzyme linked immunosorbent assay and serum neutralization test in Konya province. Hayvancılık Araştırma Dergisi. 15(1), 5-8.