Research in Veterinary Science 157 (2023) 17–25

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

The effect of multiple-dose ivermectin treatment on $CD4^+/CD8^+$ and the oxidative stress index in goats with udder viral papillomatosis

Gokhan Bozkurt [a,*], Feyyaz Kaya [b], Yakup Yildirim [c], Ramazan Yildiz [d], Orsan Gungor [a], Firat Dogan [e], Leyla Elif Ozgu Ayozger [f]

[a] Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, 15100 Burdur, Turkey
[b] Balikesir University, Faculty of Veterinary Medicine, Department of Internal Medicine, Balikesir, Turkey
[c] Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Virology, 15100 Burdur, Turkey
[d] Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Internal Medicine, 15100 Burdur, Turkey
[e] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Virology, 31060, Hatay, Turkey
[f] Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pathology, 15100 Burdur, Turkey

ARTICLE INFO

Keywords:
Capra hircus papillomavirus
Immune response
Ivermectin
mammary
PCR

ABSTRACT

This study aims to reveal the therapeutic effect of ivermectin against *Capra hircus* papillomavirus (ChPV-1) infection and on the $CD4^+/CD8^+$ (cluster of differentiation) and oxidative stress index (OSI). Twenty hair goats naturally infected with ChPV-1 were divided into two groups with equal numbers as the ivermectin group and the control groups. Ivermectin was administered subcutaneously at a dose of 0.2 mg/kg to the goats in the ivermectin group on days 0, 7, and 21. Blood samples were collected from the vena jugularis on days 0, 21, 45, and 90. The cluster of differentiation$4^+/CD8^+$ ratio was significantly higher in the ivermectin group than in the control group on the 90th day. Furthermore, the $CD8^+$ concentration was significantly decreased in the ivermectin group on the 90th day compared with the control group. Both total oxidant status (TOS) and OSI were found to be significantly higher in the control group on the 21st and 45th days than in the ivermectin group. On the 90th day, it was determined that the lesions in the ivermectin group improved significantly compared to those in the control group. Additionally, only in the ivermectin group was there a significant difference between the 90th day and the other days in terms of healing. As a result, it can be suggested that ivermectin has positive effects on the immune response and that its oxidative actions are of therapeutic value and do not harm the systemic oxidative status, as in untreated goats.

1. Introduction

Papillomaviruses (PVs) are a varied group of small, circular, non-enveloped, and double-stranded DNA viruses that infect a wide range of hosts. Papillomaviruses are oncogenic viruses that may cause both benign and malignant lesions by causing the proliferation of the stratified squamous epithelium of the skin and mucosa in different parts of the body. Most PV infections are asymptomatic without visible clinical signs (Saied et al., 2021). The clinical signs depend on various factors, such as environmental factors, virus oncogenicity, and the location of the infection (Antonsson and Hansson, 2002; Daudt et al., 2018). They are associated with both cutaneous and mucosal warts as well as neoplasia in different animal species, and viral infection is observed worldwide (Rector and Van Ranst, 2013; Zahra et al., 2019). Oral papillomas can cause various problems, such as dysphagia and limited movement ability, due to their physical location (Cook and Olson Jr., 1951). Other ruminant PV species beyond bovine papillomavirus (BPV) have received less attention. All ruminant PVs belong to the same genera as BPV, except ChPV-1 (*Capra hircus* papillomavirus), which was isolated from goats (Daudt et al., 2018).

There is a balance between reactive oxygen species (ROS) production and the antioxidant systems that are both enzymatic and non-enzymatic. Oxidative stress occurs when the oxidant-antioxidant balance is disturbed by the excessive production of ROS and/or deficiency of the

* Corresponding author at: Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Animal Hospital, Department of Obstetrics and Gynecology, 15100 Burdur, Turkey.
E-mail addresses: gbozkurt@mehmetakif.edu.tr (G. Bozkurt), feyyazkayaa@gmail.com (F. Kaya), yyildirim@mehmetakif.edu.tr (Y. Yildirim), ramazanyildiz@mehmetakif.edu.tr (R. Yildiz), orsangungor@mehmetakif.edu.tr (O. Gungor), firat9837@gmail.com (F. Dogan), layozger@mehmetakif.edu.tr (L.E.O. Ayozger).

https://doi.org/10.1016/j.rvsc.2023.02.006
Received 30 October 2022; Received in revised form 31 December 2022; Accepted 16 February 2023
Available online 22 February 2023

antioxidant system. Lymphocytes that have CD4 surface antigens are helper T cells that promote both cellular and humoral immune responses. Furthermore, lymphocytes that have CD8 surface antigens are suppressor/cytotoxic T cells. The ratio of lymphocytes ($CD4^+/CD8^+$) in circulating blood is an important criterion for evaluating the competency of cellular immunity (Flaminio et al., 1998). Generally, the cell-mediated immune response eliminates PV infections (O'Brien and Campo, 2002). Although many authors have reported that cellular immunity is critical to the regression of lesions and papillomas induced by PV, the precise immunological mechanisms that are necessary for rejecting papillomatosis are still poorly understood (Bassi et al., 2019).

Many researchers have used various treatments, such as surgery, laser surgery, radiation, and topical drugs, against PV infection without agreement on their efficacy (Cimtay et al., 2003; Hemmatzadeh et al., 2003; Kale et al., 2018; Murphy et al., 1999). However, Murphy et al. (1999) reported that cell-mediated response stimulation via injection of immunostimulatory agents provided promising results. The effects of immunostimulatory agents on the immune response, modulation of hematopoiesis, and augmented phagocytic activation of macrophages were reported (Cox, 1988). Ivermectin is one of the most well-known immunomodulatory agents (Sajid, 2004). Some researchers have reported that ivermectin may affect not only the neurophysiology of parasites but also the immune response of the host (Rao et al., 1987; Savanur et al., 1995). It is also well known that ivermectin causes some degree of oxidative stress and as such provides oxidative action against parasites, viruses, and cancers such as chronic myeloid leukemia K562 cells (Darwish and Eldakroury, 2020; Liu et al., 2016; Saied, 2021; Wang et al., 2018; Zhu et al., 2017).

Ivermectin has immunomodulatory properties associated with altered T lymphocyte functions, especially in T helper lymphocytes (Blackley and Rousseaux, 1991). Furthermore, ivermectin has antiviral activities against RNA viruses by binding importin alfa (Tu et al., 2020). Similarly, Lv et al. (2018) reported that in Pseudorabies virus infection, the drug inhibits viral entry into the nucleus in a dose-dependent manner. Thus, ivermectin significantly reduces viral DNA synthesis and inhibits virus production.

This study aimed to investigate the efficacy of multiple doses of ivermectin in goats infected with PV, as well as to determine its efficacy on $CD4^+$ and $CD8^+$ concentrations, the oxidative stress index (OSI), and healing status. In this study, we hypothesized that ivermectin may be effective in mammary papillomas caused by ChPV-1 infection, as in previous studies in bovines (Borku et al., 2007; Saied, 2021), and that the effect may be due to its therapeutic oxidative stress and immunomodulatory effect.

2. Material and methods

2.1. Ethical approval

The experimental procedures were approved by the Committee of Animal Experiments of Burdur Mehmet Akif Ersoy University. Approval number: 2020/78–651

2.2. Animals and management

Twenty naturally infected hair goats with udder papillomatosis during their lactation period were included in the study. Goats were milked by hand. All grazing goats were fed 900 g hay, 100 g corn silage, 200 g clover, 50 g barley, and 100 g concentrate feed per goat daily. This study was performed at Yakakoy/Burdur, which has a semi-Mediterranean climate (37_703460 N, 30_355977E; 1.138 m above sea level). There was no overcrowding in the flock, shelter, and building conditions, and they were grown in facilities that met the species' specific welfare standards. Starting and during the experimental process, all the goat's milk was examined using the California Mastitis Test (Schalm et al., 1971) regularly, and they were healthy. There was no disease related to postpartum period disorders, especially uterine infection, in goats. Additionally, it was determined that there was no parasitic load in the fecal samples taken before the study. No external parasites were found in the inspection (Soulsby, 1982).

2.3. Experimental design and collection of blood samples

The goats were randomly divided into two equal groups: experimental and control groups. There were almost equal papillomatosis lesions on the mammary halves of animals in both groups. The ivermectin group received 0.2 mg/kg ivermectin (Ivomec®, Boehringer Ingelheim) subcutaneously three times on days 0, 7, and 21. The control group received 0.2 ml/kg 0,9% NaCl subcutaneously from the interscapular region at the same time. In total, 8 ml of blood samples were collected from the vena jugularis into tubes with (EDTA) and without anticoagulants on days 0, 21, 45, and 90. All blood samples were collected before treatment. Examination of the lesions to determine healing was performed on days 21, 45, and 90. All goats were clinically examined at each blood sample collection, and none of the goats became ill during the experimental period.

2.4. Hematological and biochemical analyses

Red blood cells (RBCs), packed cell volume (PCV), hemoglobin (HGB), white blood cells (WBCs), lymphocytes (LYMs), and granulocytes (GRAs) were measured for hematological analysis. This analysis was performed with the Abacus Junior Vet 5-Diatron hematology analyzer (Hungary).

Analysis of the $CD4^+$ concentration in the blood serum was assessed using a goat-specific $CD4^+$ ELISA kit (catalog number: MBS743702/MyBioSource, USA). The analysis of the $CD8^+$ concentration was performed via ELISA using a goat-specific ELISA kit (catalog number: MBS743702/MyBioSource, USA). The intensity of the color was measured spectrophotometrically at 450 nm in a microplate reader. Both $CD4^+$ and $CD8^+$ T lymphocyte analyses were performed using a Biotek ELx800 microplate spectrophotometer (USA).

Total antioxidant capacity (TAC) levels were measured using commercially available kits via the colorimetric method (catalog number: RL0017/Relassay, Turkey). This method is based on the bleaching of the characteristic color of a more stable 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation by antioxidants. The results were expressed as mmol Trolox equivalent/L (Erel, 2004).

Total oxidant status (TOS) levels were measured using commercially available kits (catalog number: GP81170/Relassay, Turkey). The ratio of TOS to TAC was accepted as the OSI. For the calculation, the resulting unit of TAC was converted to μmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/L)/TAC (μmol Trolox equivalent/L) (Erel, 2005; Yumru et al., 2009).

Biochemical analysis of the TAC and TOS was performed using a BS-400 automatic analyzer (China). Analyses of CD4+ and $CD8^+$ concentrations, TAC, and TOS were performed according to the manufacturer's protocol.

2.5. Collection of lesion samples

Tissue samples were collected from 20 hair goats with papilloma-like lesions on the udder before treatment and 45 and 90 days after treatment. During sample collection, 0.5 ml of 2% lidocaine was applied to the area around the papilloma-like lesion (Jangir et al., 2017), and tissue samples were scraped lightly with a scalpel and taken with living tissue. Papilloma sizes were between 0.5 and 2 cm on average. Two samples were brought to the diagnostic laboratory following the cold chain protocol. One tissue sample was fragmented with a scalpel in phosphate-buffered saline (PBS) containing antibiotics (streptomycin-20 mg/ml + penicillin-500 IU/ml) and stored at −80 °C until use in molecular

studies. One sample was stored in a 10% buffered formaldehyde solution for histopathological diagnosis.

2.6. Histopathological diagnosis

After macroscopic evaluation of the samples, they were fixed in 10% buffered formaldehyde. After their fixation, the tissues taken into the cassettes were subjected to the routine follow-up procedure in the tissue tracking device (Leica ASP300S, USA). Sections of 4–5 μm thickness taken from the paraffin-blocked tissues with the help of metal cloth mounts were examined under a light microscope by performing Harris Hematoxylin & Eosin staining (Merck, Germany) (Presnell et al., 1997).

2.7. Molecular diagnosis

DNA extraction from the papilloma samples was carried out according to Sambrook and Russell (2001). The following degenerate primer set MY09/MY11 (forward 5'-GCMCAG GGW CAT AAY A ATGG-3′; reverse '5'-CGTCCMARRGGA WAC TGA TC-3′) (Manos et al., 1989; Ogawa et al., 2004) was used for L1 gene amplification and detection of the presence of PV DNA. As a result of the PCR technique performed with the MY 09/11 consensus primer set, 450 (base pair) bp fragments were obtained. In addition, PV identification was achieved using a degenerate primer, and ChPV-1 type-specific primers were used for type determination. PCR was carried out according to Simeonea et al. (2008), and 237 bp (forward 5'-ACCCCAAAGCAAATTCAAATG-3′; reverse 5′-CTCAGCAACTATGTCTAAGC-3′) PCR products were visualized in a transilluminator after electrophoresis in a 1% agarose gel containing ethidium bromide and used voltage 90 for 1 h.

2.8. Statistical analyses

All statistical analyses were performed using the SPSS 25.0 software. Normality analysis of data was performed with the Shapiro–Wilk normality test. To examine the differences between groups belonging to the same sampling time, a *t*-test was used for parameters showing normal distribution; the Mann–Whitney *U* test was used for nonnormal distribution parameters. While examining the variation in biochemical-hematological parameters or ratios at different sampling times within the same group, the Kruskal–Wallis test, which is a nonparametric test, was used because of the low number of goats included in the groups. If a statistically significant difference was determined between the variables, the Mann–Whitney U test was used after the Kruskal–Wallis test (Yokus et al., 2004; Saied, 2021). The chi-square test was used both when evaluating the differences between sampling times in the same group and when evaluating the differences between groups at the same sampling time. The results of the analysis were accepted as significant if $P < 0.05$. The results were expressed as the mean ± standard error.

3. Results

3.1. Hematological and biochemical analyses

The hematological analysis results are described in Table 1.

For the $CD4^+$ concentration, a significant decrease was determined on the 90th day compared with the 0th ($P < 0.05$) and 21st ($P < 0.05$) days only within the control group. Furthermore, in $CD8^+$ concentration, a significant decrease was determined on the 90th day compared with the 0th ($P < 0.001$), 21st ($P < 0.001$), and 45th ($P < 0.001$) days only in the ivermectin group. In the $CD4^+/CD8^+$ ratio, a significant increase was determined on the 90th day compared with the 0th (P:0.001) and 21st (P:0.001) days only within the ivermectin group. Additionally, a significant difference ($P < 0.05$) was observed in the $CD4^+/CD8^+$ ratio in the ivermectin group compared to the control group on the 90th day. In TOS levels, a significant increase was determined on the 45th day compared with the 0th ($P < 0.001$) and 21st ($P < 0.01$) days within the control group. Additionally, a significant increase was determined between the 0th and 21st days ($p < 0.05$). However, a significant increase was observed in TOS levels in the control group compared with the ivermectin group on the 21st ($P < 0.05$) and 45th ($P < 0.01$) days. In TAC levels, a significant increase was observed on the 21st day compared to the other sampling times in the ivermectin group ($P < 0.01$). However, a significant decrease was observed on the 45th day compared with the 0th ($P < 0.05$) and 21st ($P < 0.01$) days within the control group. In OSI, significant increases ($P < 0.01$) were determined between the days only within the control group. In parallel to TOS levels, significant increases were observed in the OSI in the control group compared with the ivermectin group on the 21st ($P < 0.05$) and 45th ($P < 0.01$) days. The biochemical analysis results are described in Table 2.

Table 1
Hematological parameters.

Parameters	Sampling Day	Ivermectin Group	Control Group
RBC × 10^{12}/L	0	12.7 ± 0.4^{A}	11.9 ± 0.1^{aB}
	21	12.5 ± 0.4	11.9 ± 0.3^{a}
	45	12.6 ± 0.4	13.2 ± 0.4^{b}
	90	12.2 ± 0.4^{A}	14.2 ± 0.3^{bB}
PCV %	0	19.0 ± 0.4	19.0 ± 0.7^{a}
	21	18.9 ± 0.6	17.8 ± 0.2^{a}
	45	18.9 ± 0.5^{A}	21.3 ± 0.3^{bB}
	90	18.2 ± 0.7^{A}	22.2 ± 0.6^{bB}
HGB g/dl	0	6.6 ± 0.2	6.7 ± 0.3^{abc}
	21	6.5 ± 0.2	6.1 ± 0.2^{b}
	45	6.4 ± 0.2	6.9 ± 0.1^{ac}
	90	6.1 ± 0.2^{A}	7.3 ± 0.2^{acB}
WBC × 10^{9}/L	0	13.8 ± 1.3	13.3 ± 1.8
	21	13.6 ± 1.2	12.8 ± 1.3
	45	14.5 ± 1.3	13.2 ± 1.7
	90	11.6 ± 0.6	11.7 ± 0.9
LYM × 10^{9}/L	0	4.6 ± 0.6	4.9 ± 0.4
	21	5.1 ± 0.3	5.0 ± 0.4
	45	5.4 ± 0.5	4.5 ± 0.4
	90	4.2 ± 0.4	4.7 ± 0.2
GRA × 10^{9}/L	0	9.0 ± 0.7	9.1 ± 1.3
	21	8.3 ± 0.9	7.7 ± 0.9
	45	8.9 ± 0.8	8.6 ± 12
	90	7.8 ± 0.7	6.9 ± 0.9

RBC:Red Blood Cell, PCV:Packed Cell Volume, HGB:Hemoglobin, WBC:White Blood Cell, LYM: Lymphocyte, GRA: Granulocyte.
If the means within columns do not share the same superscripts such as "a,b,c" letters there is a statistically significant difference ($P < 0.05$). If the means within rows do not share the same superscript letters such as "A and B" there is a statistically significant difference ($P < 0.05$). There is no difference between days share the same letter.

3.2. Macroscopic examination

The cut surface of the tissue samples taken before the treatment was cauliflower-like, dark brown, and partially soft (Grade 1). In the post-treatment samples, it was noted that the tissue sample was drier and brittle, and its color changed from whitish cream to yellow (Grade 2). Grade 2 tissue samples had almost no cauliflower appearance and were smaller than grade 1 tissue samples. Macroscopic changes in grade 2 samples were also supported histopathologically, and this tissue was described as regressed tissue (Fig. 1).

3.3. Histopathological and molecular examinations

In the microscopic examination of the tissue sample taken before the treatment (Grade 1), the hyperkeratotic appearance of the stratum corneum layer was remarkable. Koilocytes in the stratum spinosum layer, which is the characteristic lesion of papillomavirus, and acanthosis (rete pegs) of the epidermis layer toward the dermis and histopathology were observed. Hydropic degeneration and a few mitotic

Table 2
Biochemical parameters.

Parameters (Unit)	Sampling Day	Ivermectin Group	Control Group
$CD4^+$ (ng/ml)	0	4.31 ± 0.66	3.39 ± 0.33^{a}
	21	4.11 ± 0.59	3.61 ± 0.43^{a}
	45	3.97 ± 0.50	2.84 ± 0.2^{ab}
	90	2.89 ± 0.30	2.46 ± 0.13^{b}
$CD8^+$ (ng/ml)	0	1.69 ± 0.36^{a}	1.19 ± 0.11
	21	1.77 ± 0.33^{a}	2.0 ± 0.42
	45	1.46 ± 0.25^{a}	1.37 ± 0.22
	90	0.61 ± 0.02^{b}	0.86 ± 0.12
$CD4^+/CD8^+$	0	2.64 ± 0.17^{a}	2.71 ± 0.05
	21	2.59 ± 0.25^{a}	2.40 ± 0.37
	45	2.91 ± 0.43^{ab}	2.87 ± 0.1
	90	4.16 ± 0.33^{bA}	2.67 ± 0.39^{B}
TOS (μmol/L)	0	11.64 ± 1.84	12.12 ± 0.8^{a}
	21	22.82 ± 4.32^{A}	30.08 ± 4.78^{bB}
	45	32.62 ± 10.30^{A}	56.20 ± 8.73^{cB}
TAC (mmol/L)	0	1.15 ± 0.02^{a}	1.26 ± 0.02^{a}
	21	1.49 ± 0.09^{b}	1.46 ± 0.09^{a}
	45	1.18 ± 0.02^{a}	1.13 ± 0.05^{b}
OSI	0	9.71 ± 1.62	9.78 ± 0.07^{a}
	21	14.63 ± 2.08^{A}	20.36 ± 2.93^{bB}
	45	26.87 ± 8.63^{A}	49.75 ± 7.90^{cB}

TOS: Total Oxidant Status, TAC: Total Antioxidant Capacity, OSI: Oxidative Stress Index.
If the means within columns do not share the same superscripts such as "a,b,c" letters there is a statistically significant difference ($P < 0.05$). If the means within rows do not share the same superscript letters such as "A and B" there is a statistically significant difference ($P < 0.05$). There is no difference between days share the same letter.

figures were found in the cells in the lower layers of the epidermis. It was observed that the keratinocytes in the sample were partly parakeratotic and partly orthokeratotic. Based on these data, the tissue sample was diagnosed as a papilloma (Fig. 2).

The presence of a thick stratum corneum layer was observed in regression samples (Grade 2). The presence of karyorrhectic nuclei and polymorphonuclear inflammatory cells rich in moderate to severe neutrophil leukocytes were observed between the hyperkeratotic layers of the samples. Additionally, a hyperkeratotic layer with polymorphonuclear inflammatory cells was detected on the surface of the stratum corneum (Fig. 3).

The results from the PCR indicated that ChPV-1 was molecularly detected in the pre-treatment udder papilloma samples according to the primers used. Fig. 4 shows the gel electrophoresis image of eight randomly selected samples from both groups. In all animals in both groups, ChPV-1 was detected in the samples taken before the treatment and in the regressed samples after treatment.

3.4. Healing and regression rates

In this study, healing refers to the complete clinical disappearance of the papilloma. While no significant difference was observed between the 21st and 45th days in terms of healing rates in the ivermectin group, a significant difference was observed between the 21st and 90th days ($P < 0.01$) and between the 45th and 90th days ($P < 0.05$). Additionally, no statistically significant results were found on all days in terms of healing rates in the control group. According to the results obtained on the 90th day, healing rates were found at 60% and 10%, regression rates at 40% and 30%, and no healing rates at 0% and 60% in the ivermectin group and control group, respectively (Table 3). A statistically significant difference was observed between the groups in terms of healing rates on the 90th day ($P < 0.05$). The goat udders before and after treatment on different days in the ivermectin and control groups are shown in Fig. 5.

4. Discussion

Recently, researchers have found much evidence about the efficacy of ivermectin on the immune system of laboratory animals and humans, in addition to its antiparasitic effects (Blackley and Rousseaux, 1991; Mudatsir et al., 2020; Savanur et al., 1996). Blackley and Rousseaux (1991) reported the enhancement effects of ivermectin on T lymphocyte count, antibody production, and the macrophage-dependent response of the immune system in mice. Mudatsir et al. (2020) concluded that ivermectin has antiviral properties by blocking importin alpha and beta-

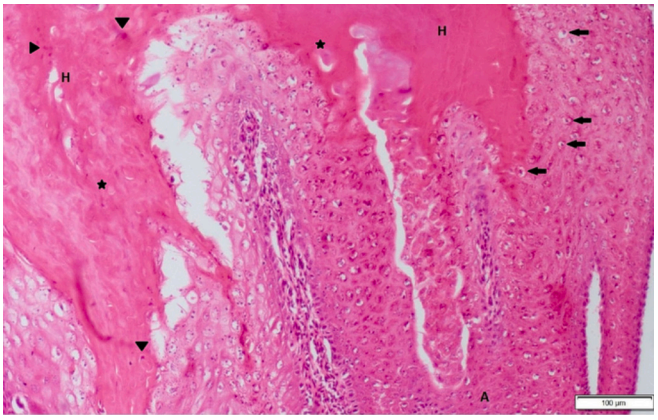

Fig. 2. Histopathological appearance of the papilloma and hyperkeratotic and acanthotic changes in the epithelium with koilocytes in the stratum spinosum layer which is the characteristic lesion of papillomavirus histopathology. H: Hyperkeratosis, A: Acanthosis, (→): Koilocyte, (▶): Parakeratotic hyperkeratosis cells and inflammatory cells nucleic residues, (*): Orthokeratotic hyperkeratosis cells. H&E, X10, Barr: 100 μm.

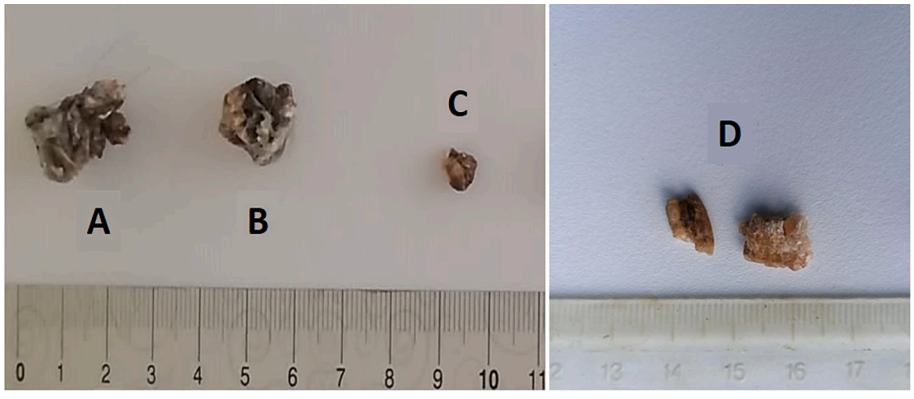

Fig. 1. Macroscopic appearance of papillomas and grading. **A,B:** Grade 1, **C, D:** Grade 2.

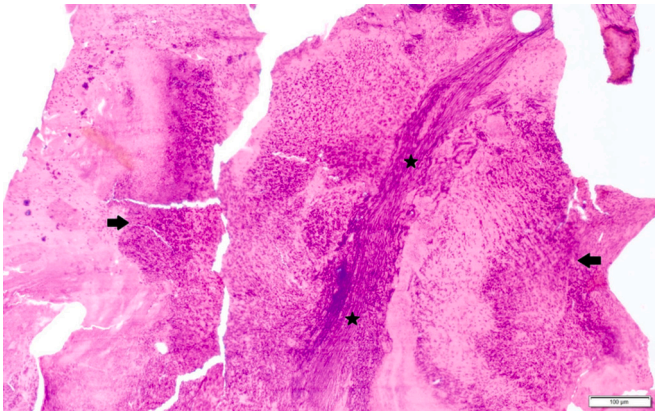

Fig. 3. Histopathological appearance of regressed papilloma.
(→): Hyperkeratosis of the stroma in the dermis and degenerated inflammatory cell infiltration in the hyperkeratotic area, (*): parakeratotic areas in the stratum corneum. H&E, X10, Barr: 100 µm.

1 inhibition. Nevertheless, researchers have reported several immunosuppressive effects of ivermectin in rabbits, rats, and lambs (Stankiewicz et al., 1995; Uhlir and Volf, 1992). Furthermore, Krishna and Klotz (1993) and Sajid et al. (2007) demonstrated that ivermectin has highly dose-dependent efficacy on the immune system and WBCs. However, Savanur et al. (1996) reported that ivermectin has a limited effect on the regulation of the immune system when administered at a therapeutic dose. Ogueji et al. (2019) performed an experimental study on juvenile *C. gariepinus*, which is highly sensitive to ivermectin, and they found that the levels of packed cell volume and red blood cells in the control group were higher than those in the experimental group. Furthermore, they reported that lymphocytes and neutrophils increased in the experimental group. In contrast, Akomas et al. (2011) determined an increase in hemoglobin and RBCs in ivermectin-treated goats compared with the control group. Additionally, they found significantly lower white blood cells in the experimental group. Saied (2021) reported significant increases in WBC, RBC, and PCV in cattle suffering from cutaneous papillomatosis treated with ivermectin compared with the untreated papillomatosis group. In contrast to the previous results, in our study, there were no significant differences determined between the groups in the hematological parameters. These differences in our results may be related to the dosage regimen of ivermectin and the inflammatory reaction induced by PV infection.

Generally, PV-related infections are eliminated by a cell-mediated immune response to viral antigens (O'Brien and Campo, 2002). Zhang et al. (2016) reported that the mean percentage of $CD8^+$ T lymphocytes that infiltrate into tumor cells tended to be higher in the human PV-positive group. In parallel, the mean $CD4^+/CD8^+$ ratio in tumors was also lower in the positive group. A lower number of $CD4^+$ T cells and a low $CD4^+/CD8^+$ T-cell ratio were demonstrated in peripheral blood by Levkutová et al. (1998) in BPV infections. Similarly, Bassi et al. (2019) reported a decreased $CD4^+/CD8^+$ ratio in the peripheral blood of cattle infected with BPV compared with the control group. Zafra et al. (2013) conducted an experimental study to evaluate peripheral blood lymphocyte subsets in goats infected with *F. hepatica*. There were no significant differences before the experimental infection between the control group and the other groups. However, they found a significant decrease in $CD4^+$ T lymphocytes in the infected groups compared with the control group after experimental infection. The reduction was observed only in $CD4^+$ T lymphocytes, not in $CD8^+$ T lymphocytes. Interestingly, Jolly et al. (1997) observed a decrease in both $CD4^+$ T lymphocytes and the $CD4^+/CD8^+$ T lymphocyte ratio accompanied by a slight increase in CD8+ T lymphocytes in goats infected with caprine

Table 3
Healing or regression of papillomas by groups and days.

Day	21st	45th	90th
Ivermectin Group	Regressed	Regressed	Healing
	Regressed	Regressed	Regressed
	No healing	Regressed	Regressed
	Regressed	Regressed	Healing
	Regressed	Regressed	Healing
	Regressed	Regressed	Healing
	Regressed	Regressed	Healing
	Regressed	Regressed	Regressed
	No healing	No healing	Regressed
	No healing	Regressed	Healing
Control Group	No healing	No healing	No healing
	No healing	No healing	No healing
	Regressed	Regressed	Healing
	Regressed	Regressed	Regressed
	Regressed	Regressed	Regressed
	No healing	No healing	No healing
	Regressed	Regressed	Regressed
	No healing	No healing	No healing
	No healing	No healing	No healing
	No healing	No healing	No healing

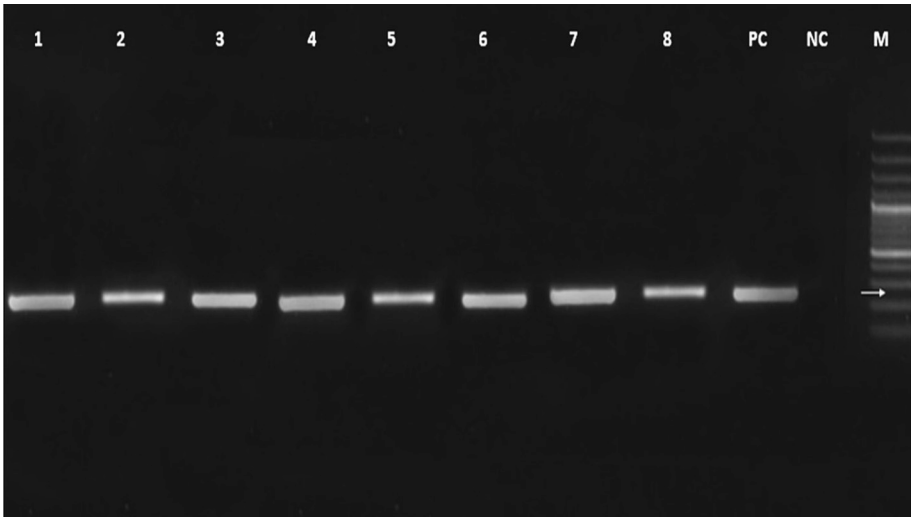

Fig. 4. Gel electrophoresis image of ChPV-1. **1–4:** Positive ChPV-1 samples in ivermectin group.
5–8: Positive ChPV-1 samples in control group (237 bp), **M:** Marker (100 bp), **NC:** Negative control, **PC:**Positive control, **(→):** 237 bp.

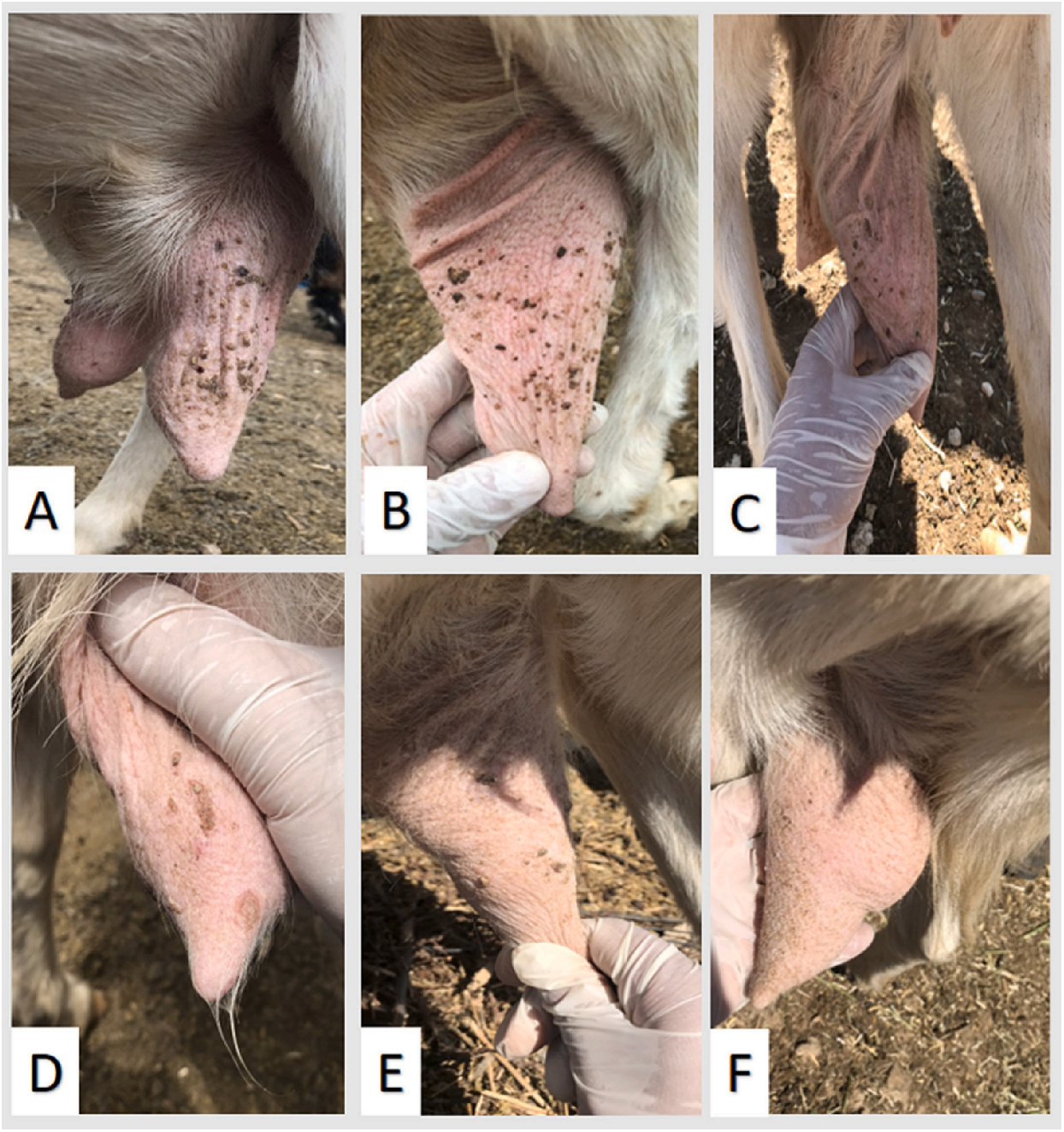

Fig. 5. Papillomatosis in goat udder halves, regression and healing. Presence of papillomatosis (0th day) in the ivermectin group (A) and control group (B), Regressed papillomatosis (45th day) in the ivermectin group (C—D), Regressed papillomatosis (45th day) in the control group (E), Healed papillomatosis (90th day) in the ivermectin group (F).

arthritis encephalitis virus compared with uninfected goats. In the present study, looked at $CD4^+$ and $CD8^+$ concentrations, no differences were observed between the groups. However, a significant reduction was determined in $CD4^+$ concentration on the 90th day compared with the first and 21st days in the control group. In contrast, in the ivermectin group, a significant decrease was determined in $CD8^+$ concentration on the 90th day compared to the other sampling times, while $CD4^+$ T lymphocyte levels were unchanged. The reduction in $CD4^+$ T concentration in our control group may be related to the effects of the infection (Levkutová et al., 1998; Zafra et al., 2013). Bassi et al. (2019) reported that PV stimulates a pro-inflammatory reaction with the contribution of $CD8^+$ T cells. Furthermore, they showed the involvement of $CD8^+$ T cells in the immune response and suggested that $CD8^+$ T cells enhance the ineffective pro-inflammatory-immunoregulatory responses that may contribute to the persistence of the lesions. In the present study, we observed a prominent decrease in $CD8^+$ concentration in the ivermectin group, unlike the control group. This decrease may result from the anti-inflammatory and immunoregulatory effects of ivermectin (Arevalo et al., 2021; Blackley and Rousseaux, 1991; Sajid, 2004). Although Santin et al. (2000) reported many contrasting results about IL-10 and $CD8^+$ T lymphocyte interactions from previous studies, they also showed that IL-10 increases the production and cytotoxic effects of human papillomavirus-specific $CD8^+$ T lymphocytes. Furthermore, Sia et al. (2020) determined that ivermectin cream stimulates IL-10 release in wounds. Zhang et al. (2016) showed increased $CD8^+$ T lymphocyte infiltration into PV-positive tumors compared with uninfected tumors.

In summary, increased IL-10 levels due to ivermectin treatment may have stimulated $CD8^+$ T lymphocytes and increased their migration to tumor cells. Therefore, ivermectin may have caused a significant decrease in peripheral blood $CD8^+$ concentration levels in the ivermectin group compared with the control group.

Acquired immune deficiency syndrome (AIDS) is one of the major infectious diseases in humans, and many studies have been performed on its pathophysiology and treatment thus far. The $CD4^+/CD8^+$ ratio is commonly used in AIDS studies to make a prognostic evaluation (Clifford et al., 2009; Lang et al., 2010; Leung et al., 2013). However, the effectiveness of the ratio is shown not only in AIDS studies but also in non-AIDS studies (Clifford et al., 2009; Lang et al., 2010; Syrjala et al., 1991). In humans, generally, the $CD4^+/CD8^+$ ratio declines with age and is related to cumulative inflammation and mortality (Czesnikiewicz-Guzik et al., 2008; Deeks, 2011). Although the healthy $CD4^+/CD8^+$ ratio is defined poorly, ratios between 1.5 and 2.5 are generally considered normal (Amadori et al., 1995). In parallel with humans, the healthy $CD4^+/CD8^+$ ratio ranges between 1.25 and 1.69 in goats (Caro et al., 1998; Jolly et al., 1997; Navarro et al., 1995). In our study, we observed that the $CD4^+/CD8^+$ ratio ranged between 2.59 and 4.16 and 2.40 and 2.87 in the ivermectin group and the control group, respectively. It can be suggested that variable and relatively high $CD4^+/CD8^+$ ratios were observed in both groups. This condition may be related to the inflammation caused by PV infection. However, the ratio on the 90th day was significantly higher in the ivermectin group than in the control group. Furthermore, although no significant difference was observed in the ratio of the control group during the experiment, a statistically significant increase was determined in the ratio of the ivermectin group on the 90th day compared with the first two sampling times. Except for ChPV-1, all the PVs of ruminants are in the same genera. Antigenic differences in the infectious agent may be responsible for the different results of the $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratios compared with the results of previous studies performed in other ruminant species. However, we thought that differences in species can be very important in immune system-related studies. Different experimental conditions, such as management, feeding, and medical interventions, are also as important as differences in species.

Borku et al. (2007) reported a high healing rate ranging from 77% to 88% in the treatment groups as a result of their study on the effectiveness of ivermectin treatment in bovine cutaneous PV infection. Furthermore, they reported no regression in the control group. Similarly, Saied (2021) reported that cattle with cutaneous papillomatosis after treatment with ivermectin showed full healing in the treatment group, while there was no improvement in the control group. Additionally, Aslan and Oruc (2010) reported complete healing in a horse with cutaneous papillomatosis after ivermectin treatment. In this study, comparing the healing status of the groups, the ivermectin group's healing status was significantly higher than that of the control group on the 90th day. In parallel, there was a significant difference between the 90th day and the other days only within the ivermectin group. Our healing rate results are compatible with previous reports. It is important to note that both the $CD4^+/CD8^+$ ratio and the healing status of the groups were significantly different on the 90th day between the groups. Both the ratio and the healing status are compatible, and the difference in healing can be caused by the various effects of ivermectin, which were stated before. It can be concluded that the results of this study are compatible with previous reports.

Oxidants and antioxidants are generally in balance, and disruption of the balance results in oxidative stress (Baydar et al., 2018; Brzezinska-Slebodzinska et al., 1994). Oxidative stress can also stimulate apoptosis, and generally, it is considered to participate in $CD4^+$ T-cell apoptosis in AIDS (Repetto et al., 1996). In this study, the $CD4^+$ concentration consistently decreased during the experimental process in both groups. A significant decrease in $CD4^+$ concentration on the 90th day compared to the first two sampling times in the control group is critical. In parallel with these results, there was a significant increase in TOS levels in the control group during the experiment. In contrast to the control group, no significant increase was observed in TOS levels in the ivermectin group during the experiment. However, TOS levels were significantly higher in the control group than in the ivermectin group on the 21st and 45th days. Karapehlivan et al. (2013) investigated OSI in healthy goats in the early and late lactation periods. At the end of their study, they determined OSI values of 2.53 ± 0.32 and 1.46 ± 0.25 in the early and late lactation periods, respectively. In the present study, OSI values were found to be prominently higher than their results, and the effects of the infection may be responsible for the increased OSI values. Although there was a statistically significant difference in the TAC level between the day 21st and days 0th and 45th in the treatment group, no difference was observed between days 0th and 45th. In the control group, a significant difference was observed only on the 45th day and the other days. In the control group, a significant difference was observed only on the 45th day. We hypothesize that these changes, such as the $CD4^+/CD8^+$ ratio and OSI, were probably caused by the fact that ivermectin indirectly supports immunomodulatory and antioxidant mechanisms. Muller et al. (2015) reported that in humans, parallel to aging, while oxidative stress increases, the $CD4^+/CD8^+$ ratio inverts. Webb et al. (2008) reported that antioxidant supplementation increases the $CD4^+/CD8^+$ ratio in cats infected with feline immunodeficiency virus. Czaja (2007) showed that cytokines and ROS that are produced by human papillomavirus are critical in the pathology of the disease. In parallel, Nayki et al. (2017) reported an increased OSI in humans infected with human papillomavirus compared with healthy controls. Various antitumoral effects of ivermectin, such as apoptosis, autophagy, pyroptosis, and inhibitory effects on tumor angiogenesis, are well known (Tang et al., 2021). Zhang et al. (2016) reported that ivermectin significantly inhibits proliferation and induces apoptosis in multiple renal cell carcinoma cell lines. Furthermore, ivermectin was significantly less ineffective in normal kidney cells than in tumoral cells. Mechanistically, ivermectin stimulates mitochondrial dysfunction by reducing mitochondrial membrane potential, ATP production, and mitochondrial respiration. They reported that data show the preferential toxicity of ivermectin to tumoral cells, and this effect may be related to the increased mitochondrial mass in tumoral cells. It can be argued that the significant differences in OSI, TAC, and TOS levels obtained in this study, both in group sampling times and between groups, are consistent with the results of previous studies. Moreover, we believe that the healing status, the antitumoral activity of ivermectin, and OSI results are in line with each other.

McBride and Striker (2017) reported that a low or inverted $CD4^+/CD8^+$ ratio is considered a signal of chronic inflammation and immune senescence in addition to altered immune function. Similarly, Kalinkovich et al. (1998) reported that a decreased $CD4^+/CD8^+$ ratio was associated with chronic helminth infection. Bassi et al. (2019) reported that papillomavirus mainly triggers $CD8^+$ T cells with a pro-inflammatory profile, and this unbalanced immune response may contribute to the persistence of papillomavirus-related lesions. Kaleci and Koyuturk (2020) reported that the cell proliferation index in wound healing significantly decreased when oxidative stress increased. On the other hand, with a reduction in oxidative stress after resveratrol administration, the cells showed improved proliferation and higher migration rates. In our study, the CD4+/CD8+ ratio and OSI results of the ivermectin group were generally higher than those of the control group. We hypothesize that ivermectin treatment prevented a high increase in oxidative stress via immunomodulatory effects, and the laboratory findings of the groups are compatible with their healing status. It can be suggested that reduced oxidative stress and an increased $CD4^+/CD8^+$ ratio may contribute to the healing of papillomavirus-related lesions.

The low number of goats included in the study, the lack of a healthy control group, and the lack of immunohistochemical evaluation of the lesions and measurement of the level of important interleukins such as IL-10 are some deficiencies in the present study. On the other hand,

collecting blood samples from goats at four different times, evaluating the $CD4^+/CD8^+$ ratio, and having a molecular diagnosis of PV using PCR can be considered strengths of this study. To our knowledge, this is the first study to investigate the efficacy of ivermectin in the treatment of ChPV-1-associated tumors, as well as the relationship between $CD4^+$, $CD8^+$, TOS, and TAC levels and ivermectin in ChPV-1 infection.

5. Conclusions

Previous studies that were performed on the therapeutic efficacy mechanisms of ivermectin and this study were considered together, and it can be suggested that using $CD4^+/CD8^+$ and OSI can be beneficial to evaluate the healing of the papillomas caused by ChPV-1. In conclusion, it can be suggested that ivermectin is beneficial in goats infected with ChPV-1.

Declaration of Competing Interest

None.

Acknowledgements

We commemorate the people who died in the earthquake on February 6, 2023, whose epicenter was Maraş, with respect and mercy, and we dedicate this publication to them.

References

Akomas, S.C., Obijuru, O.C., Herbert, U., 2011. Hematologic and serologic changes following ivermectin treatment in mange infested west african dwarf goats. Adv. Environ. Biol. 5, 2557–2560.

Amadori, A., Zamarchi, R., De Silvestro, G., Forza, G., Cavatton, G., Danieli, G.A., Clementi, M., Chieco-Bianchi, L., 1995. Genetic control of the CD4/CD8 T-cell ratio in humans. Nat. Med. 1, 1279–1283. https://doi.org/10.1038/nm1295-1279.

Antonsson, A., Hansson, B.G., 2002. Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. J. Virol. 76, 12537–12542. https://doi.org/10.1128/jvi.76.24.12537-12542.2002.

Arevalo, A.P., Pagotto, R., Porfido, J.L., Daghero, H., Segovia, M., Yamasaki, K., Varela, B., Hill, M., Verdes, J.M., Vega, M.D., Bollati-Fogolin, M., Crispo, M., 2021. Ivermectin reduces in vivo coronavirus infection in a mouse experimental model. Sci. Rep. 11, 71312. https://doi.org/10.1038/s41598-021-86679-0.

Aslan, O., Oruc, E., 2010. Is it possible to treat equine papillomatosis with ivermectin given orally? Kafkas Univ. Vet. Fak. Derg. 16, 1065–1068. https://doi.org/10.9775/kvfd.2010.1884.

Bassi, P.B., Araujo, F.F., Garcia, G.C., Costa e Silva, M.F., Bittar, E.R., Bertonha, C.M., Martins-Filho, O.A., Araujo, M.S.S., Bittar, J.F., 2019. Haematological and immunophenotypic evaluation of peripheral blood cells of cattle naturally infected with bovine papillomavirus. Vet. J. 224, 112–115. https://doi.org/10.1016/j.tvjl.2018.12.004.

Baydar, E., Yuce, A., Metin, G., Kizil, O., 2018. The antioxidant status and biochemical parameters in kid goats naturally infected with peste des petits ruminants virus. Acta Sci. Vet. 46, 1527. https://doi.org/10.22456/1679-9216.81812.

Blackley, B.R., Rousseaux, C.G., 1991. Effect of ivermectin on the immune response in mice. J. Vet. Res. 52, 593–595.

Borku, M.K., Atalay, O., Kibar, M., Cam, Y., Atasever, A., 2007. Ivermectin is an effective treatment for bovine cutaneous papillomatosis. Res. Vet. Sci. 83 (3), 360–363. https://doi.org/10.1016/j.rvsc.2007.01.016.

Brzezinska-Slebodzinska, E., Miller, J.K., Quigley, J.D., Moore, J.R., Madsen, F.C., 1994. Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. J. Dairy Sci. 77, 3087–3095. https://doi.org/10.3168/jds.S0022-0302(94)77251-9.

Caro, M.R., Gallego, M.C., Buendia, A.J., Navarro, E., 1998. Postnatal evolution of lymphocyte subpopulations in peripheral blood and lymphoid organs in the goat. Res. Vet. Sci. 65, 145–148. https://doi.org/10.1016/S0034-5288(98)90166-7.

Cimtay, I., Biricik, H.S., Keskin, O., Baba, Z.F., 2003. Comparative studies on the efficacy of different vaccination methods in the treatment of bovine papillomatosis. Turk. J. Vet. Anim. Sci. 27, 929–933.

Clifford, G.M., Rickenbach, M., Lise, M., Dal Maso, L., Battegay, M., Bohlius, J., El Amari, E.B., Karrer, U., Jundt, G., Bordoni, A., Ess, S., Franchesci, S., 2009. Hodgkin lymphoma in the Swiss HIV cohort study. Blood. 113, 5737–5742. https://doi.org/10.1182/blood-2009-02-204172.

Cook, R.H., Olson Jr., C., 1951. Experimental transmission of cutaneous papilloma of the horse. Am. J. Pathol. 27, 1087–1097.

Cox, W.I., 1988. Examining the immunologic and haematopoietic properties of an immunostimulant. Vet. Med. Small. Anim. Clin. 6, 424–428.

Czaja, M.J., 2007. Cell signaling in oxidative stress-induced liver injury. Semin. Liver Dis. 28, 378–389. https://doi.org/10.1055/S-2007-991514.

Czesnikiewicz-Guzik, M., Lee, W.W., Cui, D., Hiruma, Y., Lamar, D.L., Yang, Z.Z., Ouslander, J.G., Weyand, C.M., Goronzy, J.J., 2008. T cell subset-specific susceptibility to aging. Clin. Immunol. 127, 107–118. https://doi.org/10.1016/j.clim.2007.12.002.

Darwish, A.A., Eldakroury, M.F., 2020. The effect of ivermectin injection on some clinicopathological parameters in camels naturally infested with scabies. Adv. Anim. Vet. Sci 8, 34–40.

Daudt, C., Da Silva, F.R., Lunardi, M., Alves, C.B.D.T., Weber, M.N., Cibulski, S.P., Alfieri, A.F., Alfieri, A.A., Canal, C.W., 2018. Papillomaviruses in ruminants: an update. Transbound. Emerg. Dis. 65, 1381–1395. https://doi.org/10.1111/tbed.12868.

Deeks, S.G., 2011. HIV infection, inflammation, immunosenescence, and aging. Annu. Rev. Med. 62, 141–155. https://doi.org/10.1146/annurev-med-042909-093756.

Erel, O., 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem. 37, 277–285. https://doi.org/10.1016/j.clinbiochem.2003.11.015.

Erel, O., 2005. A new automated colorimetric method for measuring total oxidant status. Clin. Biochem. 38 https://doi.org/10.1016/j.clinbiochem.2005.08.008, 1103–1011.

Flaminio, M.J.B.F., Rush, B.R., Cox, J.H., Moore, W.E., 1998. CD4+ and CD8+ T-lymphocytopenia in a filly with pneumocystis carinii pneumonia. Aust. Vet. J. 76, 399–402. https://doi.org/10.1111/j.1751-0813.1998.tb12387.x.

Hemmatzadeh, F., Fatemi, A., Amini, F., 2003. Therapeutic effects of fig tree latex on bovine papillomatosis. J. Vet. Med. B Infect. Dis Vet. Public Health 50, 473–476. https://doi.org/10.1046/j.1439-0450.2003.00702.x.

Jangir, B.L., Bind, R.B., Kumar, P., Somvanshi, R., 2017. Pathological studies and detection of different bovine papilloma virus types in buffalo cutaneous warts. Turk. J. Vet. Anim. Sci. 41, 306–311. https://doi.org/10.3906/vet-1605-17.

Jolly, P.E., Gangopadhyay, A., Chen, S., Reddy, P.G., Weiss, H.L., Sapp, W.J., 1997. Changes in the leukocyte phenotype profile of goats infected with the caprine arthritis encephalitis virus. Vet. Immunol. Immunopathol. 56, 97–106. https://doi.org/10.1016/s0165-2427(96)05726-1.

Kale, M., Saltik, H.S., Hasircioglu, S., Yildirim, Y., Yavru, S., Mamak, N., Atli, K., 2018. Treatment of bovine papillomavirus-induced teat warts in a cow by using Podophyllin magistral formula and autologous vaccine applications together. Indian J. Anim. Res. 53, 832–836. https://doi.org/10.18805/ijar.B-911.

Kaleci, B., Koyuturk, M., 2020. Efficacy of resveratrol in the wound healing process by reducing oxidative stress and promoting fibroblast cell proliferation and migration. Dermatol. Ther. 33, e14357 https://doi.org/10.1111/dth.14357.

Kalinkovich, A., Weisman, Z., Greenberg, Z., Nahmias, J., Eitan, S., Stein, M., Bentwich, Z., 1998. Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. Clin. Exp. Immunol. 114, 414–421. https://doi.org/10.1046/j.1365-2249.1998.00736.x.

Karapehlivan, M., Kaya, I., Sag, A., Akin, S., Ozcan, A., 2013. Effects of early and late lactation period on plasma oxidant/antioxidant balance of goats. Kafkas Univ. Vet. Fak. Derg. 19, 529–533. https://doi.org/10.9775/kvfd.2012.8315.

Krishna, D.R., Klotz, U., 1993. Determination of ivermectin in human plasma by high performance liquid chromatography. Arzneimittelforschung. 43, 609–611.

Lang, S., Mary-Krause, M., Cotte, L., Gilquin, J., Partisani, M., Simon, A., Boccara, F., Costagliola, D., 2010. Impact of individual antiretroviral drugs on the risk of myocardial infarction in human immunodeficiency virus-infected patients: a case-control study nested within the French hospital database on HIV ANRS cohort CO4. Arch. Intern. Med. 170, 1228–1238. https://doi.org/10.1001/archinternmed.2010.197.

Leung, V., Gillis, J., Raboud, J., Cooper, C., Hogg, R.S., Loutfy, M.R., Machouf, N., Montaner, J.S.G., Rourke, S.B., Tsoukas, C., Klein, M.B., 2013. Predictors of CD4: CD8 ratio normalization and its effect on health outcomes in the era of combination antiretroviral therapy. PLoS One 8, e77665. https://doi.org/10.1371/journal.pone.0077665.

Levkutová, M., Revajová, V., Levkut, M., Leng, L., 1998. Subpopulations of lymphocytes in cattle naturally infected with papillomavirus. Acta Vet. Hung. 46, 13–18.

Liu, Y., Fang, S., Sun, Q., Liu, B., 2016. Anthelmintic drug ivermectin inhibits angiogenesis, growth and survival of glioblastoma through inducing mitochondrial dysfunction and oxidative stress. Biochem. Biophys. Res. Commun. 480, 415–421. https://doi.org/10.1016/j.bbrc.2016.10.064.

Lv, C., Liu, W., Wang, B., Dang, R., Qiu, L., Ren, J., Yan, C., Yang, Z., Wang, X., 2018. Ivermectin inhibits DNA polymerase UL42 of pseudorabies virus entrance into the nucleus and proliferation of the virus in vitro and vivo. Antivir. Res. 159, 55–62. https://doi.org/10.1016/j.antiviral.2018.09.010.

Manos, M.M., Ting, Y., Wright, D.K., Lewis, A.J., Broker, T.R., Wolinsky, S.M., 1989. The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cell 7, 209–214.

McBride, J.A., Striker, R., 2017. Imbalance in the game of T cells: what can the CD4/CD8 T-cell ratio tell us about HIV and health? PLoS Pathog. 13 (11), e1006624 https://doi.org/10.1371/journal.ppat.1006624.

Mudatsir, M., Yufika, A., Nainu, F., Frediansyah, A., Megawati, D., Pranata, A., Mahdani, W., Ichsan, I., Kuldeep, D., Harapan, H., 2020. Antiviral activity of ivermectin against sars-cov-2: an old-fashioned dog with a new trick- a literature review. Sci. Pharm. 88, 36. https://doi.org/10.3390/scipharm88030036.

Muller, G.M., Gottlieb, M.G.V., Correa, B.L., Filho, I.G., Moresco, R.N., Bauer, M.E., 2015. The inverted CD4:CD8 ratio is associated with gender-related changes in oxidative stress during aging. Cell. Immunol. 296, 149–154. https://doi.org/10.1016/j.cellimm.2015.05.006.

Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C., Studdert, M.J., 1999. Veterinary Virology, third ed. Academic Press, London.

Navarro, J.A., Caro, M.R., Seva, J., Rosillo, M.C., Gomez, M.A., Gallego, M., 1995. Study of lymphocyte subpopulations in peripheral blood and secondary lymphoid organs in

the goat using monoclonal antibodies to surface markers of bovine lymphocytes. Vet. Immunol. Immunopathol. 51, 147–156. https://doi.org/10.1016/0165-2427(95)05497-9.

Nayki, C., Gunay, M., Kulhan, M., Nayki, U., Cankaya, M., Kulhan, N.G., 2017. Serum levels of soluble interleukin-2 receptor in association with oxidative stress index in patients with different types of HPV. Ginekol. Pol. 88, 355–359. https://doi.org/10.5603/GP.a2017.0067.

O'Brien, P.M., Campo, M.S., 2002. Evasion of host immunity directed by papillomavirus-encoded proteins. Virus Res. 88, 103–117. https://doi.org/10.1016/s0168-1702(02)00123-5.

Ogawa, T., Tomita, Y., Okada, M., Schinozaki, K., Kubonoya, H., Kaiho, I., Shirisawa, H., 2004. Broad-spectrum detection of papillomaviruses in bovine teat papillomas and healthy teat skin. J. Gen. Virol. 85, 2191–2197. https://doi.org/10.1099/vir.0.80086-0.

Ogueji, E.O., Nwani, C.D., Mbah, C.E., Nweke, F.N., 2019. Acute hematological toxicity of ivermectin to juvenile Clarias gariepinus. Toxicol. Environ. Chem. 101, 3–6. https://doi.org/10.1080/02772248.2019.1691554.

Presnell, J.K., Schreibman, M.P., Gretchen, L., 1997. Humason's Animal Tissue Technigues, fifth ed. Johns Hopkins University Press, Maryland.

Rao, U.R., Chandrashekar, R., Subrahmanyam, D., 1987. Effect of ivermectin on serum dependent cellular interactions to dipetalonema viteae microfilariae. Trop. Med. Parasitol. 38, 123–127.

Rector, A., Van Ranst, M., 2013. Animal papillomaviruses. Virology 445, 213–223. https://doi.org/10.1016/j.virol.2013.05.007.

Repetto, M., Reides, C., Gomez, M.L., Costa, M., Griemberg, G., Llesuy, S., 1996. Oxidative stress in blood of HIV patients. Clin. Chim. Acta 255, 107–117. https://doi.org/10.1016/0009-8981(96)06394-2.

Saied, A.A., 2021. Regression of bovine cutaneous papillomas via ivermectin-induced immunostimulant and oxidative stress. J. Adv. Vet Anim Res. 8, 370–377. https://doi.org/10.5455/javar.2021.h525.

Saied, A.A., Metwally, A.A., Mohamed, H., Haridy, M.A., 2021. The contribution of bovines to human health against viral infections. Environ. Sci. Pollut. Res. 28, 46999–47023. https://doi.org/10.1007/s11356-021-14941-z.

Sajid, M.S., 2004. Studies on the Immunomodulatory Effect of Ivermectin in Rabbits. M. Sc. Thesis. Department of Veterinary Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.

Sajid, M.S., Iqbal, Z., Muhammad, G., Sandhu, M.A., Khan, M.N., Saqib, M., Iqbal, M.U., 2007. Effect of ivermectin on the cellular and humoral immune responses of rabbits. Life Sci. 80, 1966–1970. https://doi.org/10.1016/j.lfs.2007.02.025.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual, fourth ed. Cold Spring Harbor Laboratory, New York.

Santin, A.D., Hermonat, P.L., Ravaggi, A., Bellone, S., Pecorelli, S., Roman, J.J., Param, G.P., Cannon, M., 2000. Interleukin-10 increases Th1 cytokine production and cytotoxic potential in human papillomavirus-specific CD8+ cytotoxic T lymphocytes. Virol. 74, 4729–4737. https://doi.org/10.1128/jvi.74.10.4729-4737.2000.

Savanur, N.H., Honnegowda, Shastri, K.N.V., Krishnappa, G., Narayana, K.K., 1995. Effect of ivermectin on immune response in rabbits inoculated with Salmonella antigen. Indian Vet. J. 72, 221–223.

Savanur, N.H., Honnegowda, G., Krishnappa, Shastri, Narayana, K.K., 1996. Effect ofivermectin on lymphocyte status in rabbits. Indian Vet. J. 73, 501–503.

Schalm, O.W., Carrol, E.J., Jain, N.C., 1971. Bovine Mastitis a Symposium. Physical and Chemical tests for Detection of Mastitis. Lea and Febiger, Philadelphia.

Sia, D.K., Mensah, K.W., Opoku-Agyemang, T., Folitse, R.D., Darko, D.O., 2020. Mechanisms of ivermectin-induced wound healing. BMC Vet. Res. 16, 397. https://doi.org/10.1186/s12917-020-02612-z.

Simeonea, P., Romanuccib, M., Rizzoa, C., Brandia, R., Malatestab, D., Di Guardob, G., Bongiovannib, L., Salda, L.D., Venuti, A., 2008. Papillomaviruses in multiple tumours of twin goats. Open. Vet. Sci. J. 2, 33–36. https://doi.org/10.2174/1874318800802010033.

Soulsby, E.J.L., 1982. Helminths, Arthropods and Protozoa of Domesticated Animals, seventh ed. Bailliere Tindall, London.

Stankiewicz, M., Cabaj, W., Jonas, W.E., Moore, L.G., Millar, K., Ng Chie, W., 1995. Influence of ivermectin on cellular and humoral immune responses of lambs. Vet. Immunol. Immunopathol. 44, 347–358. https://doi.org/10.1016/0165-2427(94)05308-f.

Syrjala, H., Surcel, H.M., Ilonen, J., 1991. Low CD4/CD8 T lymphocyte ratio in acute myocardial infarction. Clin. Exp. Immunol. 83, 326–328. https://doi.org/10.1111/j.1365-2249.1991.tb05636.x.

Tang, M., Hu, X., Wang, Y., Yao, X., Zhang, W., Yu, C., Cheng, F., Li, J., Fang, Q., 2021. Ivermectin, a potential anticancer drug derived from an antiparasitic drug. Pharmacol. Res. 162, 105207 https://doi.org/10.1016/j.phrs.2020.105207.

Tu, Y.F., Chien, C.S., Yarmishyn, A.A., Lin, Y.Y., Luo, Y.H., Lin, Y.T., Lai, W.Y., Yang, D. M., Chou, S.J., Yang, Y.P., 2020. A review os SARS-CoV-2 and the ongoing clinical trials. Int. J. Mol. Sci. 21, 2657. https://doi.org/10.3390/ijms21072657.

Uhlir, J., Volf, P., 1992. Ivermectin: its effect on the immune system of rabbits and rats infested with ectoparasites. Vet. Immunol. Immunopathol. 34, 325–336. https://doi.org/10.1016/0165-2427(92)90173-n.

Wang, J., Xu, Y., Wan, H., Hu, J., 2018. Antibiotic ivermectin selectively induces apoptosis in chronic myeloid leukemia through inducing mitochondrial dysfunction and oxidative stress. Biochem. Biophys. Res. Commun. 497, 241–247. https://doi.org/10.1016/j.bbrc.2018.02.063.

Webb, C.B., Lehman, T.L., McCord, K.W., 2008. Effects of an oral superoxide dismutase enzyme supplementation on indices of oxidative stress proviral load, and CD4:CD8 ratios in asymptomatic FIV-infected cats. J. Feline. Med. Surg. 10, 423–430. https://doi.org/10.1016/j.jfms.2008.01.008.

Yokus, B., Cakir, D.U., Kurt, D., 2004. Effects of seasonal and physiological variations on the serum major and trace element levels in sheep. Biol. Trace Elem. Res. 101 (3), 241–255. https://doi.org/10.1385/BTER:101:3:241.

Yumru, M., Savas, H.A., Kalenderoglu, A., Bulut, M., Celik, H., Erel, O., 2009. Oxidative imbalance in bipolar disorder subtypes: a comparative study. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33, 1070–1074. https://doi.org/10.1016/j.pnpbp.2009.06.005.

Zafra, R., Perez, J., Buffoni, L., Martinez-Moreno, F.J., Acosta, I., Mozos, E., Martinez-Moreno, A., 2013. Peripheral blood lymphocyte subsets in Fasciola hepatica infected and immunised goats. Vet. Immunol. Immunopathol. 155, 135–138. https://doi.org/10.1016/j.vetimm.2013.06.006.

Zahra, S., Ahmed, H., Korritum, A., Elkhenany, H., Khalifa, H., 2019. Equine sarcoids: distribution sites, common types, and diagnosis. AJVS. 60, 123–128. https://doi.org/10.5455/ajvs.19531.

Zhang, D., Tang, W.J., Tang, D., Zhou, J., Chou, L., Tao, L., Lu, L.M., 2016. The ratio of CD4/CD8 T-cells in human papillomavirus-positive laryngeal squamous cell carcinoma accounts for improved outcome. Acta Otolaryngol. 136, 826–833. https://doi.org/10.3109/00016489.2016.1164341.

Zhu, M., Li, Y., Zhou, Z., 2017. Antibiotic ivermectin preferentially targets renal cancer through inducing mitochondrial dysfunction and oxidative damage. Biochem. Biophys. Res. Commun. 492, 373–378. https://doi.org/10.1016/j.bbrc.2017.08.097.