Journal MVZ Córdoba
2022; may-august. 27(2):e2376
https://doi.org/10.21897/rmvz.2376

Original

Investigation of the effectiveness of various treatment methods in goats with cutaneous papillomatosis

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Received: April 2021; Accepted: December 2021; Published: May 2022.

ABSTRACT

Objective. This study investigated the recovery effects of levamisole, *Tarantula cubensis* extract, and levamisole + *T. cubensis* extract combination on goats with cutaneous papillomatosis. **Materials and methods.** Forty hair goats between 2 and 4 years of age in the lactation period, which were clinically diagnosed as having papillomatosis, were included in the study, and they were divided into four groups randomly, each having ten goats. No application was performed on goats in the control group. Levamisole (5 mg/kg, intramuscularly), *T. cubensis* extract (4 ml, subcutaneously), and both levamisole (5 mg/kg, intramuscularly) and *T. cubensis* extract (4 mL, subcutaneously) were administered to the goats in the levamisole group, *T. cubensis* extract group, and combined group, respectively, two times at weekly intervals. All animals in the groups were monitored at 15-day intervals for three months. **Results.** While no recovery was observed in the control group, regression was observed in only one animal's papilloma (10%). The recovery and regression rates were 40% and 40% in the levamisole group, 30% and 40% in the *T. cubensis* extract group, and 60% and 30% in the combined group, respectively. **Conclusions.** All three treatments applied in goats for treating cutaneous papillomatosis were found to be effective in different degrees. When compared between treatment groups, levamisole+*T. cubensis* extract combination was found to be a more effective treatment.

Keywords: Cutaneous papillomatosis; goat; levamisole; *Tarantula cubensis*; treatment (Source: CAB).

RESUMEN

Objetivo. Este estudio investigó los efectos de recuperación del levamisol, extracto de *Tarantula cubensis* y la combinación de levamisol + extracto de *T. cubensis* en las cabras con papilomatosis cutánea. **Materiales y métodos.** En el estudio se incluyeron cuarenta cabras de pelo entre 2 y 4 años de edad en período de lactancia, que fueron diagnosticadas clínicamente de papilomatosis, y se dividieron en cuatro grupos aleatoriamente, cada uno con diez cabras. No se realizó ninguna

How to cite (Vancouver).
Yildirim Y, Bozkurt G, Küçük A, Taşal İ. Investigation of the effectiveness of various treatment methods in goats with cutaneous papillomatosis. Rev MVZ Cordoba. 2022; 27(2):e2376. https://doi.org/10.21897/rmvz.2376

ISSNL: 0122-0268

aplicación en cabras en el grupo de control. Se administraron levamisol (5 mg / kg, por vía intramuscular), extracto de *T.cubensis* (4 ml, por vía subcutánea) y levamisol (5 mg / kg, por vía intramuscular) y extracto de *T.cubensis* (4 ml, por vía subcutánea) a las cabras en el grupo de levamisol, grupo de extracto de *T. cubensis* y grupo combinado, respectivamente, dos veces a intervalos semanales. Todos los animales de los grupos se controlaron a intervalos de 15 días durante tres meses. **Resultados**. Si bien no se observó recuperación en el grupo de control, se observó regresión en solo el papiloma de un animal (10%). Las tasas de recuperación y regresión fueron 40% y 40% en el grupo de levamisol, 30% y 40% en el grupo de extracto de *T. cubensis* y 60% y 30% en el grupo combinado, respectivamente. **Conclusión.** Se encontró que los tres tratamientos aplicados en las cabras para tratar la papilomatosis cutánea eran efectivos en diferentes grados. Cuando se comparó entre los grupos de tratamiento, se encontró que la combinación de extracto de levamisol + *T. cubensis* es un tratamiento más efectivo.

Palabras clave: Papilomatosis cutánea; cabra; levamisol; *Tarantula cubensis*; tratamiento (Fuente: CAB).

INTRODUCTION

Papillomaviruses have an icosahedral structure and hold a double-stranded DNA genome that has a length ranging between 6953 bp (*Chelonia mydas* papillomavirus type 1) and 8607 bp (canine papillomavirus type 1) (1,2,3). Agents initially identified as a genus within the Papoviridae family were later transformed into a family named Papillomaviridae (1). So far, more than 280 papillomavirus types have been identified and classified within 49 different genera by the International Committee on Taxonomy of Viruses (4). Virus types of these agents causing infection in animals are included within 32 different genera (5).

The first report on the clinical detection of papillomatosis in goats was published in 1954 (6). In one study, Capra hircus papillomavirus type 1 was identified from healthy goat skin (7). In another study, viruses were detected in two goats with multiple cutaneous and ocular neoplastic lesions (8). In a molecular study on a goat with cutaneous teat papillomatosis, a new type of papillomavirus, which is in close antigenic relationship with BPV-6 in the *Xipapillomavirus* genus, was isolated in Turkey (9). Although goat papillomatosis is considered to be strain and tissue specific, information about cross-infection between different types has also been reported in many studies (10,11). The virus, which can cause infection in all vertebrates, affects epithelial and mucosal tissues and creates lesions characterized by fibropapillomas, papillomas, and neoplasms (12,13,14).

The papillomavirus is transmitted between animals through many different methods. One of the transmission methods is direct contact (5,14). However, milking, ear tagging, feeding, and farming (halter, muzzle, etc.) equipment that is contaminated with infected animals also play a role in indirect transmission. In addition, some studies state that there may be transmission by arthropods and coitus (12,14). Factors such as malnutrition, hormonal imbalance, mutations, and long-term exposure to sunlight that cause immunosuppression in animals are also risk factors for the occurrence of infection (15).

Papillomaviruses have a high affinity for cutaneous and mucosal epithelia. As the causative agent cannot penetrate the skin, lesions generally appear in areas where skin integrity is damaged (abrasion and wound) (16). Cutaneous lesions might appear in various morphologies as a result of the infection. These are classified as filiform, peduncle, and atypical forms (17,18). The papillomas are usually found in the head, neck, udder and teat, abdomen, and digestive and genital canal mucosa (12,19). Cutaneous papillomatosis is the most common lesion caused by papillomaviruses in ruminants (11). Papillomas occurring in the udders of animals in dairy herds decrease milk production due to milking difficulties. In addition, injuries due to papillomatosis on the mammary skin can lead to secondary infections and mastitis (20,21).

Laboratory diagnosis is rarely needed as clinical signs are quite characteristic in papillomatosis (12). However, laboratory methods such as polymerase chain reaction (PCR), dot blot, Southern blot, immunohistochemistry, and electron microscopy are used in cases where clinical diagnosis is difficult (11,12,22).

Papillomaviruses cause economic losses to the dairy industry due to the occurrence of benign or malign tumors in the affected animals (11). There are many methods that can be used in the treatment of infected animals to prevent economic losses. Surgical methods (electrocautery, ligature, and cryotherapy) (23), homeopathy, immunomodulatory drug therapy, and antibiotherapy (24,25,26), chemical injections such as lithium antimony thiomalate (26), autovaccination, and autohemotherapy are some of these methods (23,27).

Levamisole and the *Tarantula cubensis* extract, which have been introduced in recent years and are effective in the treatment of many infections, are among the most important preparations. Levamisole is an anthelmintic commonly used in the treatment of parasitic infestations in farm animals (28). It is also known as a powerful immunomodulator and has been reported to be effective in the treatment of chronic diseases, tumoral formations, or inflammations by stimulating the cellular immunity of immunosuppressed animals (28,29). Also, *T. cubensis* extract has an immunostimulatory effect on the metabolism and can be used as a homeopathic method in the treatment of diseases (30,31). This extract has been used to treat mammary tumors in dogs, foot diseases of cattle, foot and mouth disease, papillomatosis, ecthyma (orf), abscess, and ulcers (32,33).

This study aims to determine the effectiveness of levamisole, *T. cubensis* extract, and levamisole + *T. cubensis* extract combination in the treatment of cutaneous udder papillomatosis that was diagnosed clinically and by PCR in goats. In addition, the California Mastitis Test (CMT) score was also monitored for mammary health throughout the study period.

MATERIALS AND METHODS

Animal material. The animal material consisted of 40 hair goats with udder papillomatosis between 2 and 4 years of age on the 30 ±5th day of the lactation period. During the clinical examination, it was determined that most of the papillomas were located on udder halves, and there were also lesions in the neck (one goat), interscapular region (one goat), and abdominal and thoracic regions (two goats) in some animals. In this study, PCR, a well-known diagnostic method, was used to diagnose goat papillomavirus infection. Samples were taken from goats with multiple cutaneous tumor formations for the diagnosis of goat papillomavirus infection, which occurs commonly in herds. The samples were obtained after cleaning the area with water and soap and decontaminating it with 70% ethanol. Segments of warts were removed by a parallel incision in the surface of the skin using a disposable sterile scalpel and were kept in a sterile tube. PCR was applied according to the procedure. The sample was found to be goat papillomavirus nucleic acid positive.

All sampling was performed after the approval of the local ethics committee for animal studies. This research was conducted after the approval of Burdur Mehmet Akif Ersoy University Animal Testing Local Ethics Council (Approval Number: MAKÜ-HADYEK-2019/72–605).

Determination of groups and treatment methods. Goats with cutaneous udder papillomatosis were divided into four groups, each having ten goats: levamisole group, *T. cubensis* extract group, levamisole + *T. cubensis* extract combination group, and control group. Levamisole (Actipar®, Alke) was administered to goats in the levamisole group (5 mg/kg, intramuscularly) and *T. cubensis* extract (Theranekron®, Richter Pharma) was administered to goats in the *T. cubensis* extract group (4 ml, subcutaneously) two times at weekly intervals. Both levamisole and *T. cubensis* extract were administered to goats in the levamisole + *T. cubensis* extract combination group at the same dose and the same way as the other treatment groups. No application was performed to goats in the control group during the study.

Evaluation of regression and recovery. Udder halves with papillomatosis were checked for regression and recovery at each examination. The complete disappearance of papillomas in both udder lobes was described as recovery. In addition, the partial reduction of papillomas on the udder or separation from the tissue without bleeding when touched was described as regression. If there is partial recovery or regression in any udder halves, this is not described as recovery.

Clinical observation and California Mastitis Test (CMT). All groups were examined clinically at 15-day intervals for three months after treatment. Regression and recovery status of papillomatosis cases on the udder or teats and other areas was recorded.

Besides this, milk somatic cell count (SCC) scores were qualitatively monitored with CMT. The milk was taken from each udder halves. Before milk samples were collected, teats were cleaned with 70% alcohol. First, several milk samples were taken into strip cups, and milk was examined physically. Approximately 10 mL of the milk sample was taken into CMT cups, and it was mixed with the CMT solution at the same rate. The CMT score was evaluated by a single observer. Milk somatic cell scores were classified as 0, trace, +1, +2, and +3 (34).

Statistical evaluation. Chi-square statistical analysis was used during the statistical evaluation of regression and recovery between groups, and ANOVA (Minitab 16 package program) was used in evaluating the CMT score.

RESULTS

There was no recovery or regression in any group within the first 30 days posttreatment. Regression and recovery rates were detected, respectively, as 40% and 40% in the levamisole group (Fig. 1, B1 and B2), 40% and 30% in the *T. cubensis* extract group (Fig. 1, A1 and A2), 30% and 60% in the levamisole + *T. cubensis* extract combination group (Fig. 2, C1 and C2), and 10% and 0% in the control group at the end of the 90-day follow-up period (Fig. 2, D1 and D2). While the highest rate of regression was found in the levamisole group and *T. cubensis* extract group, the highest recovery rate was found in the levamisole + *T. cubensis* extract combination group. Recovery was not observed in the control group. There was no significant difference between the groups in terms of regression rates. There was a significant difference between the control group, combined group, and levamisole group in terms of recovery rates ($P<0.05$). Regression and recovery were mostly detected within 75–90 days ($P>0.05$) (Table 1).

CMT scores were evaluated separately for each udder halves. There was no significant statistical difference between before and after treatment ($P>0.05$). While the CMT score decreased in the levamisole and combined group, it was partially increased in the *T. cubensis* and control group. During the study, no clinical mastitis was observed in goats.

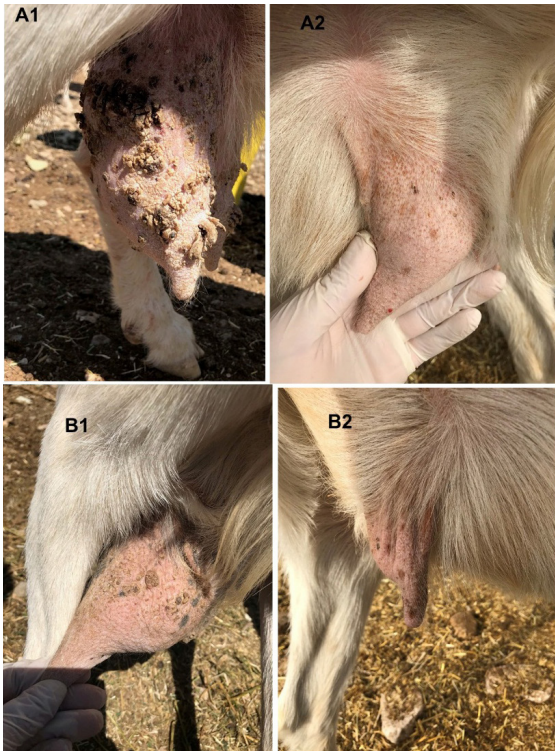

Figure 1. Pretreatment (A1) and posttreatment (A2) in the *T. cubensis* extract group. Pretreatment (B1) and posttreatment (B2) in the levamisole group.

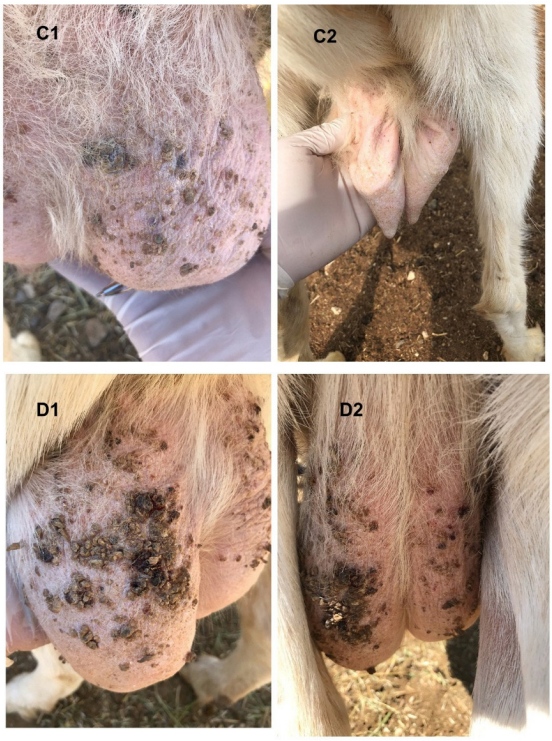

Figure 2. Pretreatment (C1) and posttreatment (C2) in the levamisole + *T. cubensis* extract combination group. Pretreatment (D1) and posttreatment (D2) in the control group. No recovery was observed in the control group (D2).

Table 1. Regression and recovery of udder papillomatosis by working schedule

Groups	Status	Working Schedule (Days)						Total* (n)	Total (%)
		15	30	45	60	75	90		
Levamisole (n=10)	Regression	-	-	-	-	2	2	4	40
	Recovery	-	-	-	-	2	2	$4^{b,c}$	40
T. cubensis extract (n=10)	Regression	-	-	-	1	1	2	4	40
	Recovery	-	-	1	1	1	-	$3^{a,b,c}$	30
Levamisole + *T. cubensis* extract (n=10)	Regression	-	-	-	1	2	-	3	30
	Recovery	-	-	1	2	2	1	$6^{b,c}$	60
Control (n=10)	Regression	-	-	1	1	-	1	1	10
	Recovery	-	-	-	0	-	-	0^{a}	0

* Statistical evaluation was made between lines. Row values with different superscript letters are significantly different at $p<0.05$

DISCUSSION

Papillomaviruses are viral agents that cause proliferative benign tumor formations, neoplasia, and hyperplasia in cutaneous or visceral tissues (12,17). The virus, which has spread all around the world, also causes infections in mammals, birds, fishes, and reptiles (11). The papilloma agent causes chronic, contagious benign or malign lesions in animals such as goats, cattle, and sheep, which are used for milk and meat production. It results in large-scale economic losses (3,11).

Mastitis that may occur in goats during the study process was monitored with CMT (34). In our study, no clinical mastitis case was seen during clinical observations. This may be attributed to the fact that the goats used in the study did not have high milk yield, the absence of secondary bacterial infections was noted, and animals were young. Also, it can be explained that papillomas increased in the mammary cisterns rather than in teats, and offspring sucked their mother's breasts constantly.

Many methods are used in the treatment of papillomatosis in animals. Some of these methods are cell culture vaccination, autovaccination, immunomodulation, autohemotherapy, homeopathy, surgical interventions, and treatment with antibiotics (azithromycin) and chemical components. Activation of the immune system is essential in the treatment of papillomatosis. Cellular immunity plays a more active role than humoral immunity in response to papillomatosis (14). It has been determined that levamisole increases T lymphocyte and macrophage activities in immunosuppressed or healthy mammals (28). *T. cubensis* extract was tried for the treatment of teat tumors (32), oral papillomatosis in dogs (29,31), and cutaneous papillomatosis in cattle (24,33). For the treatment of cutaneous papillomatosis in goats (30), autohemotherapy was performed in two goats with papillomatosis on the teat and udder, and the treatment was found to be successful.

Çam et al (24) used *T. cubensis* extract for the treatment of cattle with cutaneous papillomatosis and applied it five times at 5-day intervals with a subcutaneous dose of 7.5 mL/day. Recovery was observed in 70% of the animals at the end of the study. Similarly, Paksoy et al (33) reported that *T. cubensis* extract was given to cows with teat papillomatosis two times at weekly intervals subcutaneously, and all animals recovered. Icen et al (31) applied *T. cubensis* extract to treat canine oral papillomatosis via subcutaneous injection at a dose of 2.5 mL/10 kg twice a week for three weeks. As a result of these treatments, 70% of the dogs with papillomatosis recovered. In another similar study, Tekelioğlu et al (29) applied *T. cubensis* extract in dogs with papillomatosis three times at 5-day intervals with a subcutaneous dose of 2 mL/10 kg and repeated the same dose in the first and third weeks following the last injection. At the end of the treatment, 30% of the dogs recovered.

In this study, recovery and regression rates of *T. cubensis* extract on cutaneous goat papillomatosis were investigated, and these effects were determined as 30% and 40%,

respectively (Table 1). This result supports the results of other research studies.

Another method used in papillomatosis treatment is levamisole. Çam et al (24) administered levamisole to 10 cattle with papillomatosis. Levamisole was applied for two days with a subcutaneous dose of 2.5 mL/kg/day at 5-day intervals, and this treatment was repeated for five weeks. At the end of a 3-month observation, a recovery rate of 90% was observed. Paksoy et al (33) injected levamisole into eight cattle with udder papillomas via intramuscular injection two times at weekly intervals with a dose of 5 mg/kg and observed a 50% recovery rate. Tekelioğlu et al. (29) performed the treatment with levamisole and azithromycin combination on six dogs with cutaneous papillomatosis. The combination of a 2-mg/kg dose of levamisole and a 10-mg/kg dose of azithromycin was administered orally once a day for ten days. A recovery rate of 33% was observed. In studies conducted by Paksoy et al (33), Tekelioğlu et al (29), and Çam et al (24), it has been reported that repeated doses and long-term injections increase the probability of success in the treatment of papillomatosis cases with levamisole.

In our study, the recovery rate of goats applied with levamisole was determined as 40% (4/10). This rate was different from previous studies. It was concluded that the reason for this difference might be due to the fact that the research was conducted on different species, the stress factors (climate, lactation, lack of care and feeding, etc.) negatively affected the immune status of the animals used in the study, and goats had a very severe papillomatosis infection.

In this study, a combination of levamisole and *T. cubensis* extract was tried as a new method in the treatment of papillomatosis. In goats administered with this combination, a recovery rate of 30% (3/10) and a regression rate of 60% (6/10) were observed (Fig. 2). In the previous literature, no research has been found concerning the treatment of papillomatosis in which both levamisole and *T. cubensis* extract were used together. Therefore, this study can be considered a new method for the treatment of papillomatosis. However, repeating the research in different animal species and with a greater number of animals may provide more meaningful results.

In conclusion, The administration of levamisole, *T. cubensis* extract, and levamisole + *T. cubensis* extract combination to goats with papillomatosis provided different recovery rates. The combination dose of levamisole and *T. cubensis* extract was found to be a more effective treatment. More research should be carried out to determine the immunomodulatory, immunostimulatory, and hemopoietic effects of the treatments used in this study.

Conflict of interest

The authors disclosed that there are no conflicts of interest and all the authors have read and approved the manuscript before its submission.

REFERENCES

1. King AMQ, Adams MJ, Carsten EB, Lefkowitz EJ. Virus taxonomy: Classification and nomenclature of viruses. Ninth Report of the International Committee on Taxonomy of Viruses, Elsevier Inc. 2012.

2. Van Doorslaer K. Evolution of the Papillomaviridae. Virol. 2013; 445(1-2):11-20. https://doi.org/10.1016/j.virol.2013.05.012

3. Araldi RP, Assaf SMR, De Carvalho RF, Caldas MA, De Carvalho R, De Souza JM, Magnelli RF, Modolo DG, Roperto FP, Stocco RC, Beçak W. Papillomaviruses: a systematic review. Genet Mol Biol 2017; 40:1-21. https://doi.org/10.1590/1678-4685-gmb-2016-0128

4. Araldi RP, Melo TC, Neves AC, Spadacci-Morena DD, Magnelli RF, Modolo DG, De Sa Junior PL, Mazucchelli-de-Souza J, Carvalho RF, Beçak W, Stocca RC. Hyperproliferative action of bovine papillomavirus: genetic and histopathological aspects. Genet Mol Res. 2015; 14(4):12942-12954. https://doi.org/10.4238/2015.October.21.15

5. Rector A, Van Ranst M. Animal papillomaviruses. Virol. 2013; 445(1-2):213-223. https://doi.org/10.1016/j.virol.2013.05.007

6. Moulton JE. Cutaneous papillomas on the udders of milk goats. North American Veterinarian. 1954; 35:29–33.

7. Van Doorslaer K, Rector A, Vos P, Van Ranst M. Genetic characterization of the capra hircus papillomavirus: A novel close-to-root artiodactyl papillomavirus. Virus Res. 2006; 118(1-2):164–169. https://doi.org/10.1016/j.virusres.2005.12.007

8. Simeone P, Romanucci M, Rizzo C, Brandi R, Malatesta D, Di Guardo G, Bongiovanni L, Salda LD, Venuti A. Papillomaviruses in multiple tumours of twin goats. Open Vet. 2008; 2:33-36. https://doi.org/10.2174/1874318808002010033

9. Dogan F, Dorttas SD, Dağalp SB, Ataseven VS, Alkan F. A teat papillomatosis case in a Damascus goat (Shami goat) in Hatay province, Turkey: a new putative papillomavirus? Arch Virol. 2018; 163(6):1635–1642. https://doi.org/10.1007/s00705-018-3781-2

10. Lunardi M, Alfieri AA, Otonel RAA, De Alcantara BK, Rodrigues WB, De Miranda AB, and Alfieri AF. Genetic characterization of a novel bovine papillomavirus member of the Deltapapillomavirus genus. Vet Microbiol. 2013; 162(1):207–213. https://doi.org/10.1016/j.vetmic.2012.08.030

11. Daudt C, Da Silva FRC, Lunardi M, Alves CBDT, Weber MN, Cibulski SP, Alfleri AF, Alfleri AA, Canal CW. Papillomaviruses in Ruminants: An update. Transbound Emerg Dis. 2018; 65(5):1381-1395. https://doi.org/10.1111/tbed.12868

12. Maclachlan NJ, Dubovi EJ. Papillomaviridae and Polyomaviridae. In: Fenner's Veterinary Virology. 5th ed, Academic Press, London. 2016. https://doi.org/10.1016/B978-0-12-800946-8.00011-8

13. Tan MT, Yıldırım Y, Sözmen M, Dağalp SB, Yılmaz V, Kırmızıgül AH, and Gökçe E. A histopathological, immunohistochemical and molecular study of cutaneous bovine papillomatosis. Kafkas Univ Vet Fak Derg. 2012; 18:739-744. https://doi.org/10.9775/kvfd.2012.5341

14. Ugochukwu ICI, Aneke CI, Idoko IS, Sani NA, Amoche AJ, Mshiela WP, Ede RE, İbrahim NDG, Njoku CIO, Sackey AKB. Bovine papilloma: aetiology, pathology, immunology, disease status, diagnosis, control, prevention and treatment: a review. Comp Clin Path. 2019; 28:737–745. https://doi.org/10.1007/s00580-018-2785-3

15. Hamad MA, Al-Shammari AM, Odisho SM, Yaseen NY. Molecular and phylogenetic analysis of bovine papillomavirus type 1: first report in Iraqi cattle. Adv Virol. 2016; 1–7. https://doi.org/10.1155/2016/2143024

16. Nasir L, Brandt S. Papillomavirus associated diseases of the horse. Vet Microbiol. 2013; 167(1-2):159–167. https://doi.org/10.1016/j.vetmic.2013.08.003

17. Da Silva FRC, Daudt C, Streck AF, Weber MN, Filho RVL, Driemeier D, Canal CW. Genetic characterization of Amazonian bovine papillomavirus reveals the existence of four new putative types. Virus Gen. 2015; 51(1):77–84. https://doi.org/10.1007/s11262-015-1220-y

18. Lunardi M, Tozato CC, Alfieri AF, De Alcantara BK, Vilas-Boas LA, Otonel RAA, Headley SA, Alfieri AA. Genetic diversity of bovine papillomavirus types, including two putative new types, in teat warts from dairy cattle herds. Arch Virol. 2016; 161(6):1569–1577. https://doi.org/10.1007/s00705-016-2820-0

19. Roperto S, Russo V, Leonardi L, Martano M, Corrado F, Riccardi MG, Roperto F. Bovine papillomavirus type 13 expression in the urothelial bladder tumours of cattle. Transbound Emerg Dis. 2016; 63(6):628–634. https://doi.org/10.1111/tbed.12322

20. Sharma S, Mahajan V, Gupta DK, Singh RS, Bansal BK. An epidemiological and pathological study of teat papillomas in caprines. Intas Polivet. 2015; 16:119-120. https://www.karger.com/INT

21. Zhu W, Yuan D, Norimine J, Gao N, Fan S, Du Y, Li F, Yang M, Hu S, Dong J. Teat papillomatosis in dairy herds: First detection of bovine papillomavirus type 10 in China. J Vet Med Sci. 2019; 81(7):1063–1066. https://doi.org/10.1292/jvms.18-0449

22. Munday JS. Bovine and human papillomaviruses: A comparative review. Vet Pathol. 2014; 51(6):1063–1075. https://doi.org/10.1177/0300985814537837

23. Ranjan R, Ghumman SPS, Bhatt GR, Singh RS. Efficacy of autogenous vaccine and auto-hemotherapy in bovine cutaneous papillomatosis. Intas Polivet. 2013; 14:411-414. https://www.thefreelibrary.com/Efficacy+of+autogenous+vaccine+and+auto-hemotherapy+in+bovine...-a0375288607

24. Çam Y, Kibar M, Atasever A, Atalay Ö, Beyaz L. Efficacy of levamisole and tarantula cubensis venom for the treatmentof bovine cutaneous papillomatosis. Vet Rec. 2007; 160(14):486-488. https://doi.org/10.1136/vr.160.14.486

25. Zafar MA, Hasan M, Riaz A, Shamim A, Iqbal F, Yousaf A. Comparative therapeutic evaluation of partial excision of lesions and administering autogenous vaccine along with immune-modulators for the treatment of bovine papillomatosis. Vet Scie Res Review. 2015; 1:6-9. http://dx.doi.org/10.17582/journal.vsrr/2015.1.1.6.9

26. Vijayasarathi MK, Shebaa A, Venkatesan M. Therapeutic management of bovine calf papillomatosis. Indian Vet. 2018; 95(09):60-61. http://ivj.org.in/users/members/viewa

27. Kale M, Saltık HS, Hasırcıoğlu S, Yıldırım Y, Yavru S, Mamak N, Atlı K. Treatment of bovine papillomavirus-induced teat warts in a cow by using podophyllin magistral formula and autologous vaccine applications together. Indian J Anim Res. 2019; 53:832-836. https://doi.org/10.18805/ijar.B-911

28. Renoux G. The general immunopharmocology of levamisole. Drugs. 1980; 20(2):89-99. https://doi.org/10.2165/00003495-198020020-00001

29. Tekelioğlu BK, Parkan C, Yılmaz A, Turan N, Gürel A, Berber K, et al. Canine papillomatosis: Clinical outcome after oral isotherapy, subcutaneous tarantula cubensis venom and oral levamisole-azithromycin treatment. Bulg J Vet Med. 2017; 20(Suppl. 1):248-253. http://uni-sz.bg/truni6/wp-content/uploads/vmf/file/43%20Tekelioglu+.pdf

30. Dhule SJ. Auto-Haemotherapy for management of caprine papillomatosis. Intas Polivet. 2013; 14(2):425-426. https://www.cabdirect.org/cabdirect/FullTextPDF/2014/20143138960.pdf

31. İçen H, Sekin S, Simsek A, Kochan A, Tunik S. The efficacy of tarantula cubensis extract (theranekron) in treatment of canine oral papillomatosis. AJAVA. 2011; 6(7):744-749. https://scialert.net/abstract/?doi=ajava.2011.744.749

32. Gültiken N, Vural RM. The effect of tarantula cubensis extract applied in pre and postoperative period of canine mammary tumours. JIVS. 2007; 2:13-23. https://dergipark.org.tr/tr/download/article-file/268754

33. Paksoy Z, Gulesci N, Kandemir FM, Dinçel GD. Effectiveness of levamisole and tarantula cubensis extract in the treatment of teat papillomatosis of cows. Indian J Anim Res. 2015; 49(5):704-708. https://doi.org/10.18805/ijar.5586

34. McDougall S, Murdough P, Pankey W, Delaney C, Barlow J, Scruton D. Relationships among somatic cell count, California mastitis test, impedance and bacteriological status of milk in goats and sheep in early lactation. Small Ruminant Res. 2001; 40(3):245–254. https://doi.org/10.1016/S0921-4488(01)00185-7