ORIGINAL RESEARCH ARTICLE

Immunological effects of autohemovaccination and autogenous vaccination in cattle infected with cutaneous papillomatosis virus

Efectos inmunológicos de la autohemovacunación y la vacunación autógena en bovinos infectados con el virus de la papilomatosis cutánea

Efeitos imunológicos da auto-hemovacinação e vacinação autógena em bovinos infectados pelo vírus da papilomatose cutánea

Andrés Suárez-Usbeck[1*]; Pamela Vinueza[2,3]; Marilyn Mora[2]; Anahí Carrasco[1]; Tirsa Echeverría[1]; Andrea Vela[4].

[1] *Grupo de Investigación Ecuatoriano en Medicina y Sanidad Animal (GIEMSA, IIEMSA), Ecuador.*
[2] *Facultad de Ciencias Pecuarias, ESPOCH, Ecuador.*
[3] *Grupo de Investigación en Ciencias Veterinarias (GICV), ESPOCH, Ecuador.*
[4] *Universidad Técnica de Ambato, UTA, Ecuador.*

Received: February 27, 2024. Accepted: August 20, 2024.

***Corresponding author:** *Calle el Batán, Edificio Estrella X, Quito, Ecuador. Email: mvzandressuarez@gmail.com*

eISSN: 2256-2958 **Rev Colomb Cienc Pecu**
https://doi.org/10.17533/udea.rccp.e355957

To cite this article:

Suárez-Usbeck A, Vinueza P, Mora Marilyn, Carrasco A, Echeverría T, Vela A. Immunological effects of autohemovaccination and autogenous vaccination in cattle infected with cutaneous papillomatosis virus. Rev Colomb Cienc Pecu *Year, Vol, number, and pages pending.*
DOI: https://doi.org/10.17533/udea.rccp.e355957

Abstract

Background: Papillomavirus is known to induce the development of benign tumors in hosts that could progress to malignant cancers. Therapeutic strategies for treating cutaneous papilloma resulting from bovine papillomavirus (BPV) are particularly interesting. **Objective:** This study aimed to investigate the immunological effects of Autohemovaccination (AHV) and Autogenous Therapeutic Vaccine (ATV) in cattle in Ecuador. **Methods:** 150 animals with clinical symptoms of BPV were diagnosed using PCR and distributed among different groups. To create the vaccines, a total of 50 animals were used in AHV protocol n=25 with 20 mL and n=25 with 10 mL blood doses administered to them, in ATV protocol were used another 50 animals, n=25 animals with 10 mL and n=25 with 20 mL. The vaccines were stored at 4°C until used. Vaccines (AHV and ATV) were administered 4 times subcutaneous at 1 week times at 20 and 10 mL dosages, respectively. Hematological and immunological analyses involved the collection of blood samples both before and after each vaccination. The remaining subjects (n=50) will function as the positive control group. (C). **Results:** The findings demonstrated a significant increase in leukocyte count (14.5; 14; 15.4; 16 x 10^3/mm^3), lymphocyte percentage (55; 52; 58; 62%), and interleukin-6 levels (0.85; 0.80; 0.95; 0.97 ng/L) in both autogenous vaccination (ATV) at 10 and 20 mL doses and autohemovaccination (AHV) at 10 and 20 mL doses compared to the positive control (C) group (Leu: 13 x 10^3/mm^3; Lym: 50%; IL-6: 0.54 ng/L). Notably, neutrophil (33; 35; 44; 40%) and monocyte (5; 8; 12; 13%) percentages also increased in AHV and ATV. Encouragingly, within the initial month post-vaccination, both AHV and ATV exhibited signs of regression in cutaneous papilloma. **Conclusions:** In conclusion, bovine papillomatosis emerges as a condition with significant economic implications in the realm of dairy farming. This study underscores autogenous vaccination as a practical therapeutic approach, demonstrating its effectiveness in inducing lesion regression, notably by stimulating interleukin-6 (IL-6) and lymphocyte production in cattle infected with papillomavirus.

Keywords: *autogenous; autohemovaccine; bovine papillomavirus; cattle; IL-6; immunological effects; leucocyte; papillomavirus; PCR; vaccine.*

Resumen

Antecedentes: El Papillomavirus es conocido por inducir el desarrollo de tumores benignos en los hospedadores, los cuales podrían progresar a cánceres. Existen diversos enfoques terapéuticos para tratar la papilomatosis cutánea resultante del virus de la papilomatosis bovina (VPB), y la autohemovacuna (AHV) y la vacuna autógena (ATV) son métodos que se destacan entre todos ellos. **Objetivo:** El objetivo fue examinar los efectos inmunológicos de AHV y ATV en el ganado bovino en el Ecuador. **Métodos:** Se diagnosticaron 150 bovinos con síntomas de papilomatosis cutánea mediante PCR y se distribuyeron en diferentes grupos. Para crear las vacunas, se utilizaron un total de 50 animales en el protocolo AHV, n=25 con dosis de 20 mL y n=25 con dosis de 10 mL de sangre. En el protocolo ATV se utilizaron otros 50 animales, n=25 animales con 10 mL y n=25 con 20 mL. Las vacunas se almacenaron a 4°C hasta su uso. Las vacunas (AHV y ATV) se administraron 4 veces de forma subcutánea con intervalos de 1 semana en dosis de 20 y 10 mL, respectivamente a cada grupo tratamiento. Se realizaron análisis hematológicos e inmunológicos de muestras de sangre bovinas antes y después de cada vacunación. **Resultados:** Los resultados demostraron en ATV (10 y 20 mL) y AHV (10 y 20 mL) un aumento en leucocitos (14,5; 14; 15,4; 16 103/mm3), linfocitos (55; 52; 58; 62 %), e interleucina-6 (0,85; 0,80; 0,95; 0,97 ng/L), neutrófilos (33; 35; 44; 40%) y monocitos (5; 8; 12; 13 %) en comparación con C+ (Leu: 13 103/mm3; Lym: 50 %; IL-6: 0,54 ng/L; Neu: 34%; Mon: 6,5%). En las vacunas AHV y ATV, De manera alentadora, durante el primer mes después de la vacunación, se observaron signos clínicos que indican regresión en el papiloma cutáneo. **Conclusiones:** En conclusión, la papilomatosis bovina representa una condición de considerable importancia económica en la ganadería lechera. El estudio destaca la vacunación autógena como una intervención terapéutica práctica y efectiva, que promueve eficazmente la regresión de las lesiones, especialmente mediante la estimulación de la producción de IL-6 y linfocitos en bovinos infectados con papilomavirus.

Palabras clave: *autógeno; autohemovacuna; efectos inmunológicos; ganado; IL-6; leucocito; papilomavirus; PCR; vacuna; virus del papiloma bovino.*

Resumo

Antecedentes: Papilomavírus é conhecido por induzir o desenvolvimento de tumores benignos nos hospedeiros, que poderiam progredir para cânceres malignos. Existem diversas abordagens terapêuticas para tratar a papilomatose cutânea resultante do vírus da papilomatose bovina (VPB), e a auto-hemovacina (AHV) e a vacina autógena (ATV) destacam-se entre todas elas. **Objetivo:** O objetivo da pesquisa foi examinar os efeitos imunológicos da AHV e da ATV no gado bovino no Equador. **Métodos:** Foram diagnosticados 150 bovinos com sintomas de papilomatose cutânea por PCR e distribuídos em diferentes grupos. Para criar as vacinas, um total de 50 animais foi utilizado no protocolo AHV, n=25 com doses de 20 mL e n=25 com doses de 10 mL de sangue. No protocolo ATV foram utilizados outros 50 animais, n=25 animais com 10 mL e n=25 com 20 mL. As vacinas foram armazenadas a 4°C até o uso. As vacinas (AHV e ATV) foram administradas 4 vezes de forma subcutânea com intervalos de 1 semana em doses de 20 e 10 mL, respectivamente, para cada grupo de tratamento. Foram realizadas análises hematológicas e imunológicas coletando amostras de sangue antes e depois de cada vacinação. **Resultados:** Os resultados demonstraram em ATV (10 e 20 mL) e AHV (10 e 20 mL) um aumento notável em leucócitos (14,5; 14; 15,4; 16 103/mm3), linfócitos (55; 52; 58; 62 %), e interleucina-6 (0,85; 0,80; 0,95; 0,97 ng/L), neutrófilos (33; 35; 44; 40%) e monócitos (5; 8; 12; 13 %) em comparação com C+ (Leu: 13 103/mm3; Lym: 50 %; IL-6: 0,54 ng/L; Neu: 34%; Mon: 6,5%). Nas vacinas AHV e ATV, de maneira encorajadora, no primeiro mês após a vacinação, evidenciaram-se sintomas clínicos de regressão no papiloma cutâneo. **Conclusão:** Em conclusão, a papilomatose bovina representa uma condição de considerável importância econômica na pecuária leiteira. O estudo destaca a vacinação autógena como uma intervenção terapêutica prática e eficaz, que promove efetivamente a regressão das lesões, especialmente mediante a estimulação da produção de IL-6 e linfócitos em bovinos infectados com papilomavírus.

Palavras-chave: *autógeno; auto-hemovacina; efeitos imunológicos; gado; IL-6; leucócito; papilomavírus; papilomavírus bovino; PCR; vacina.*

Introduction

Papillomaviruses (PVs) are DNA viruses that specifically infect epithelial cells and are characterized by the absence of an envelope. (Aksoy *et al.*, 2017). The PV family comprises five genera that affect cattle, namely Deltapapillomavirus, Xipapillomavirus, Epsilonpapillomavirus, Dyoxipapillomavirus, and Dyokappapapillomavirus. To date, 27 bovine papillomavirus (BPV) types have been identified with four of them present in Ecuador: BPV-1, BPV-2, BPV-6, and BPV-10 (Daudt *et al*., 2018; Méndez *et al*., 2021). It has been observed that lesions caused by BPV Types 1, 2, 13, and 14 (Deltapapillomavirus) pose a higher risk (Daudt *et al*., 2018).

The prevalence of BPV infections is global. Cutaneous papillomas manifest as typically benign tumors characterized by complicated etiology and pathogenesis, marked by epithelial proliferation. The various transmission pathways of BPV (direct and indirect contact) significantly contribute to the transmission of cutaneous papillomatosis, incorporating a myriad of risk factors (Atasever *et al.,* 2005; Dagalp *et al.,* 2017; Daudt *et al.,* 2018; Saied *et al*., 2021). Papilloma infections, particularly, tend to spread among animals with compromised immune systems (Alcigir *et al*., 2016; Alcigir and Timurkan, 2018). Various treatment approaches for BPV infection encompass antimony preparations, homeopathic drugs, ivermectin, and autogenous vaccination (Ranjan *et al*., 2013; Saied, 2021).

The objective of this study was to examine the immunological effects of autohemovaccine (AHV) and Autogenous Vaccine (ATV) in cattle infected with bovine papillomavirus (BPV) in Ecuador.

Material and Methods

All experiments were carried out in strict accordance with the principles of animal welfare and were approved by the Committee of Ethics in Animal Experimentation of the GIEMSA Welfare Department (protocol No. PI01/2023).

The experimental design involved 150 owned cattle exhibiting symptoms of cutaneous papilloma disease, diagnosed through Polymerase Chain Reaction (PCR) analysis described by Mendez *et al*. (2021) method. To extract DNA from the collected samples, 25 mg of tissue was processed using the DNeasy Blood & Tissue kit (Qiagen, Valencia, California, USA) following the established procedure. The extracted DNA was stored at -80°C until use.

The DNA samples were subjected to PCR using the primer pairs FAP-59/FAP-64 (Macrogen, Seoul, Republic of Korea) (FAP-59: 5'-TAACWGTIGGICAYCCWTATT-3'; FAP-64: 5'- CCWATATCWVHCATITCICCATC-3') and MY-09/MY-11 (IDT, San Diego, California, USA) (MY-09: 5'- CGTCCAAAAGGAAACTGAGC-3'; MY-11: 5'-GCACAGGGACATAACAATGG-3'). The primer pairs were designed to target the open reading frame of the major capsid protein L1 gene, which is highly conserved across all types of bovine and human papillomaviruses.

This protocol was based on the study by Aydin *et al*., (2020) and Doğan *et al*., (2021). For AHV, a total of 50 cattle were utilized. Among them, n=25 animals received 20 mL blood doses, while another n=25 received 10 mL blood doses. Simultaneously, in ATV, another set of 50 cattle were included. Among these, n=25 cattle were administered 20 mL doses, and the remaining n=25 received 10 mL doses of autogenous vaccines; the control group (C) was n=50 animals with positive BPV and without vaccine doses.

The AHV involves collecting 10 and 20 mL of blood from each animal, treating it with 0.5% formalin to inactivate any present pathogens and maintain the stability of the suspension (Fernandes *et al*., 2022); The preparation of ATV vaccines involved 5g of papilloma tumor collected from the animals, were brought to the laboratory under a cold chain. The samples were prepared based on the protocol of Yildirim *et al*., (2023). The vaccines were then stored at 4°C until their use.

The administration of vaccines (AHV and ATV) occurred subcutaneously, with four doses given at one-week intervals. Two dosages were employed, 20 mL and 10 mL, representing the treatment groups.

Hematological and immunological analyses were carried out through the collection of 20 mL blood samples. Parameters examined included leukocyte count, lymphocyte count, interleukin-6 concentration, neutrophil count, and monocyte count. The data were presented as mean values with standard error. Statistical comparisons were performed between the treatment groups and the C group.

The results were analyzed using appropriate statistical methods, highlighting significant increases in leukocytes, lymphocytes, interleukin-6, neutrophils, and monocytes in both AHV and ATV groups to the C group. Additionally, observations of lesion regression in cutaneous papilloma were noted within the first-month post-vaccination in AHV and ATV groups.

Statistical analysis

All statistical analyses were conducted using SPSS v. 26. Categorical variables were examined through cross-tabulation, and percentages were compared using Pearson's $\chi 2$ test. Quantitative variables were presented as mean ± standard error (SE). ANOVA was utilized to compare the Control and Treatment groups, considering fixed effects.

Additionally, a non-parametric test (Mann–Whitney U test) was employed for comparing the Control and Treatment groups. Differences were deemed significant at $p < 0.05$.

Results

Figure 1 illustrates a portion of the PCR results, where analyses were conducted on 150 bovines distributed into one control group and four treatment groups. (AHV T1, n=25 ; AHV T2, n=25 ; ATV T1, n=25 and ATV T2, n=25, respective).

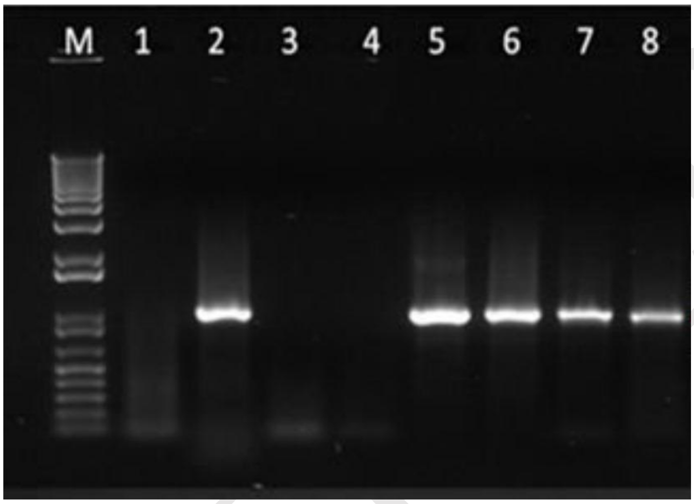

Figure 1. The electrophoresis results on a 1% agarose gel depict the detection of BPV by PCR. Lane M: Molecular weight marker 1kb plus DNA Ladder. Lane 1: negative control. Lane 2: positive control. Lanes 3-8 correspond to BPV samples.

Table 1 shows the hematological and immunological parameters analyzed included leukocyte count, lymphocyte count, interleukin-6 concentration, neutrophil count, and monocyte count.

Table 1. Hematological and immunological parameters of ATV and AHV vaccines.

Variable	C	AHV T1 (10mL)	AHV T2 (20 mL)	ATV T1 (10 mL)	ATV T2 (20 mL)	p-value
	n=50	n=25	n=25	n=25	n=25	
Leukocyte (10^{3}/mL)	13	14.5*	14*	15.4*	16*	0.04
Lymphocyte (%)	50	55	52	58	62	0.08
interleukin-6 (ng/L)	0.54	0.85*	0.80*	0.95*	0.97*	0.02
Neutrophil (%)	34	33	35	44	40	0.15
Monocyte (%)	6.5	8*	8*	12*	13*	0.03

* Significant differences ($p < 0.05$).

The analysis of the results reveals statistically significant differences in several measured variables between the C group and the treatment groups (AHV T1, AHV T2, ATV T1, ATV T2). Specifically, the concentration of Leukocyte ($p=0.04$), Monocyte ($p=0.03$), and interleukin-6 ($p=0.02$) exhibits statistically significant differences with a significance level of 5%. These findings suggest that the administration of different volumes and types of treatment significantly impacts immune responses and interleukin-6 concentration compared to the C group.

On the other hand, although the differences in Lymphocyte (%) and Neutrophil (%) Variables did not achieve statistical significance at the 0.05 level. ($p=0.08$ and $p=0.15$, respectively), they may still indicate trends that could be relevant in a clinical context. These results emphasize the importance of considering multiple variables and their interaction when assessing the impact of various treatments on the immune system.

Discussion

Papillomavirus, a viral pathogen notorious for triggering benign tumors in mammals, has garnered extensive attention due to its implications for animal health and the livestock economy (Ugochukwu *et al*., 2019). Notably, BPV-induced cutaneous papillomatosis has driven investigations into effective therapeutic strategies, with a focus on AHV and ATV as promising approaches to manage the infection (Skeate *et al.,* 2016). This study contributes to investigating the immunological effects of AHV and ATV in cattle, particularly in the context of the Ecuadorian livestock, where dairy production holds significant importance.

The relevance of this study aligns with previous research emphasizing the efficacy of autogenous vaccines in controlling bovine papillomatosis (Skeate *et al.,* 2016). Studies like that of Tozato *et al.,* (2013) underscore the economic relevance of bovine papillomatosis in dairy production, emphasizing the urgency of developing therapies that not only alleviate symptoms but also promote the regression of cutaneous lesions.

Our findings, demonstrating significant increases in leukocytes, lymphocytes, interleukin-6, neutrophils, and monocytes in both the AHV and ATV groups ($P<0.05$) compared to the control group (C), provide novel insights into the immunological responses induced by these vaccines (Fernandes *et al*., 2022; Khattab *et al*., 2023). Consistent with existing literature, ATV was found to increase leukocyte counts, particularly lymphocyte and ratios. It was concluded that ATV promoted tumor tissue regression by elevating the levels of lymphocyte cells, which are pivotal for cellular immunity, and neutrophil cells, which are responsible for phagocytic activity (Aydin *et al*., 2020).

The experimental design, involving 150 cattle diagnosed with cutaneous papilloma through Polymerase Chain Reaction (PCR) analysis, adhered to ethical standards for animal welfare. Our study employed two dosages, 20 mL, and 10 mL, for AHV and ATV, demonstrating lesion regression within the first month post-vaccination (Lacey *et al*., 1999; Pathania *et al*., 2011; Salib and Farghali, 2011). These results corroborate with prior investigations of the implications of bovine papillomatosis disease and underline the importance of refining immunotherapeutic strategies for managing papillomavirus infections in cattle (Dal Pozzo and Thiry, 2014; Knight, 2015; Marć *et al.,* 2015). Overall, our study contributes valuable data to the field, enhancing our understanding of the immunological effects of AHV and ATV in the context of bovine papillomatosis.

In conclusion, this study delved into the immunological effects of AHV and ATV in cattle infected with BPV in the context of the Ecuadorian livestock industry. The investigation, grounded in the significant implications of papillomavirus for animal health, particularly in the dairy sector, addressed the pressing need for effective therapeutic interventions against bovine papillomatosis. The research, building upon previous studies on autogenous vaccines, demonstrated that AHV and ATV induced statistically significant increases in immune system. The results contribute novel insights into the immunological responses induced by AHV and ATV, providing valuable data for the refinement of therapeutic interventions against bovine papillomatosis and reinforcing the importance of considering multiple variables in assessing the impact of treatments on the immune system.

Declarations

Funding

This research received no external funding.

Acknowledgments

We thank Teresa Monteagudo for technical assistance with the Statistical analysis.

Conflicts of interest

The authors have read the journals policy and have the following competing interest: The authors have no competing interests.

Author contributions

Conceptualization, A.S.-U.; methodology, A.S.-U.; software, M.T.T.; validation, V.P., V.A. C.A. and M.T.T.; formal analysis, M.T.T..; investigation, A.S.-U; resources, A.S.-U; data curation, A.S.-U. and M.T.T.; writing original draft preparation, A.S.-U., V.P., V.A. C.A. and M.T.T; project administration, A.S.-U.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used.

References

Aksoy P, Gottschalk EY, Meneses PI. HPV entry into cells. Mutat Res Rev Mutat Res 2017; 772:13-22. doi: 10.1016/j.mrrev.2016.09.004

Alcigir ME, Atalay Vural S, Timurkan MO. Investigation of E2, E5 and E6 gene expression and DNA in situ fragmentation findings associated with progressive and regressive changes in benign neoplastic cutaneous lesions arising naturally from bovine papillomavirus-1 infection. Medycyna Weterynaryjna 2016; 72:549–557. https://doi.org/10.21521/mw.5562

Alcigir ME, Timurkan MO. The association between insulin- like growth factors I, II and bovine papillomavirus type-1 expressions in naturally occuring bovine fibropapilloma cases. Ankara Üniversitesi Veteriner Fakültesi Dergisi 2018; (65) 2:115–122. https://dergipark.org.tr/en/download/article-file/644923

Atasever A, Cam Y, Atalay O. Bir sığır sürüsünde deri papillomatosis olguları. Ankara Üniversitesi Veteriner Fakültesi Dergisi 2005; 52:197–200. https://teav.ankara.edu.tr/xmlui/bitstream/handle/20.500.12575/59133/1810.pdf?sequence=1

Aydin H, Gelen V, Şengül E, Yildirim S. Immunological effects of autogenous vaccine administration in cattle with cutaneous papillomatosis. Acta Vet Eurasia 2020; 46, 98-103. https://doi: 10.5152/actavet.2020.20002

Dagalp SB, Dogan F, Farzani TA, Salar S, Bastan A. The genetic diversity of bovine papillomaviruses (BPV) from different papillomatosis cases in dairy cows in Turkey. Arch Virol 2017; 162:1507–1518. https://doi.org/10.1007/s00705-017-3258-8

Dal Pozzo F, Thiry E. Antiviral chemotherapy in veterinary medicine: current applications and perspectives. Rev Sci Tech 2014; 33(3):791–797. https://doi.org/10.20506/rst.33.3.2318

Daudt C, Da Silva FR, Lunardi M, Alves CB, Weber MN, Cibulski SP, Canal CW. Papillomaviruses in ruminants: An update. Transbound Emerg Dis 2018; 65(5):1381–1395. https://doi.org/10.1111/tbed.12868

Doğan E, Erdağ D, Doğan AN, Kiliçle PA, Doğan A. Treatment of bovine skin papillomatosis with gasoline. Pak Vet J 2021; 41(1): 169-172. http://dx.doi.org/10.29261/pakvetj/2020.069

Fernandes KP, Alfieri AA, Darold GM, Boabaid FM, Agnol AM, Lunardi M. Case report: Mixed infection of bovine papillomaviruses associated with squamous papilloma of the upper alimentary tract in a dairy cow. Front Vet Sci 2022; 9:1020166. https://doi.org/10.3389/fvets.2022.1020166

Khattab MS, Ali AM, Osman AH, AbuBakr HO, Azouz RA, Ramadan ES, Farag HS. Bovine papillomatosis: A serological, hematobiochemical, ultrastructural and immunohistochemical investigation in cattle. Pakistan Vet J 2023; 43(2). https://doi.org/10.29261/pakvetj/2023.023

Knight CG. The association between equine Papillomavirus type 2 and equine Squamous cell carcinomas: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Veterinary Science at Massey University, Palmerston North, New Zealand. Doctoral dissertation, Massey University; 2015. http://hdl.handle.net/10179/9863

Lacey CJ, Thompson HS, Monteiro EF, O'neill T, Davies ML, Holding FP, Roberts JS. Phase IIa safety and immunogenicity of a therapeutic vaccine, TA-GW, in persons with genital warts. J Infect Dis 1999; 179(3):612–618. https://doi.org/10.1086/314616

Marć MA, Domínguez-Álvarez E, Gamazo C. Nucleic acid vaccination strategies against infectious diseases. Expert Opin Drug Del 2015; 12(12):1851–1865. https://doi.org/10.1517/17425247.2015.1077559

Méndez I, Muñoz FA, González M, Martínez AY, Hernández LE. Características histopatológicas y detección de Papilomavirus en la fibropapilomatosis bovina en el estado de San Luis Potosí, México. Rev Mex Cienc Pecu 2021; 12(1):286–297. https://doi.org/10.22319/rmcp.v12i1.5999

Pathania S, Kumar P, Devi LG, Kumar D, Dhama K, Somvanshi R. Preliminary assessment of binary ethylenimine inactivated and saponized cutaneous warts (BPV-2) therapeutic vaccine for enzootic bovine haematuria in hill cows. Vaccine 2011; 29(43):7296–7302. https://doi.org/10.1016/j.vaccine.2011.07.065

Ranjan R, Ghumman SP, Bhath GR, Singh RS. Efficacy of autogenous vaccine and auto-hemotherapy in bovine cutaneous papillomatosis. Intas Polivet 2013; 14:411–415. Online ISSN: 2249–8796. https://www.entomoljournal.com/archives/2020/vol8issue5/PartL/8-5-280-291.pdf

Saied AA, Metwally AA, Mohamed HM, Haridy MA. The contribution of bovines to human health against viral infections. Environ. Sci. Pollut 2021; 28(34), 46999-47023. https://doi.org/10.1007/s11356-021-14941-z

Saied AA. Regression of bovine cutaneous papillomas via ivermectin-induced immunostimulant and oxidative stress. J. Adv. Vet. Anim 2021; 8(3), 370. https://doi.org/10.5455/javar.2021.h525

Salib FA, Farghali HA. Clinical, epidemiological and therapeutic studies on Bovine Papillomatosis in Northern Oases, Egypt in 2008. Vet World 2011; 4(2):53–59. http://dx.doi.org/10.5455/vetworld.2011.53-59

Skeate JG, Woodham AW, Einstein MH, Da Silva DM, Kast WM. Current therapeutic vaccination and immunotherapy strategies for HPV-related diseases. Hum Vaccines Immunother 2016; 12(6):1418–1429. https://doi.org/10.1080/21645515.2015.1136039

Tozato CC, Lunardi M, Alfieri AF, Otonel RAA, Di Santis GW, de Alcântara BK, Headley SA, Alfieri AA. Teat papillomatosis associated with bovine papillomavirus types 6, 7, 9, and 10 in dairy cattle from Brazil. Braz J Microbiol 2013; 44 (3). https://doi.org/10.1590/S1517-83822013005000057

Ugochukwu IC, Aneke CI, Idoko IS, Sani A, Amoche AJ, Mshiela WP, Ede RE, Ibrahim ND, Njoku CI, Sackey AK. Bovine papilloma: aetiology, pathology, immunology, disease status, diagnosis, control, prevention and treatment: a review. Comp Clin Pathol 2019; 28, 737–745. https://doi.org/10.1007/s00580-018-2785-3

Yildirim Y, Küçük A, Kale M, Hasircioğlu S, Atli K, Saltik HS. Evaluating the Efficiency of Various Treatment Methods in Cattle Cutaneous Papillomatosis. Vet It 2023; 59(4). https://doi.org/10.12834/VetIt.3138.21450.2