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Original

Repeated administration of autogenous vaccine decreases papillomatosis in female bovines

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ABSTRACT

Objective. The aim of the present study was to evaluate the control of bovine papillomatosis (BP) with an autogenous vaccine under tropical conditions in the State of Guerrero, Mexico. **Materials and methods.** For the experiment, 18 cows were used, divided in three treatment groups according to the ranch (six cows per group). Treatment 1 (T1-immunevaccine), treatment 2 (T2-histovaccine) and treatment 3 (T3-plasma therapy). **Results.** At the beginning of the study, a high incidence of papillomas was encountered among cows [(T1 (190 papilloma), T2 (95 papilloma) and T3 (100 papilloma)]. The administration of the autogenous vaccine produced a significant reduction (T1 and T3) in the papillomas number through the applications from 1 (147 and 66 papillomas) to 5 (10 and 9 papillomas); ($p<0.05$). There was also a significant difference among treatments according to the period of application (3 to 5) decreasing the number of lesions (period effect; $p<0.05$). In addition, an interaction treatment x period was observed, decreasing the number of papillomas in the fourth and fifth administrations (T1 and T3; $p<0.05$). **Conclusions.** The repeated administration of the autogenous vaccine in female bovines decreased the number of papillomas at the fourth and fifth application in treatments 1 and 3.

Keywords: Autovaccine; carcinoma; skin tags; treatment; papillomas (*Source: MeSH*).

RESUMEN

Objetivo. El presente estudio tuvo como objetivo evaluar el control de la papilomatosis bovina (PB) con vacuna autógena en condiciones del trópico de Guerrero. **Materiales y métodos.** En el experimento se usaron 18 hembras bovinas que fueron divididas en tres tratamientos de acuerdo al rancho (6 hembras/grupo). Tratamiento 1 (T1-inmunovacuna), tratamiento 2 (T2-histovacuna) y tratamiento 3 (T3-plasmaterapia). **Resultados.** Al inicio del estudio se encontró una alta incidencia de papilomas en las hembras bovinas [(T1 (190 papilomas), T2 (95 papilomas) y T3 (100 papilomas)]. Con la administración de la vacuna autógena disminuyó significativamente (T1 y T3) el número

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de papilomas a través de las aplicaciones de la 1 (147 y 66 papilomas) a la 5 (10 y 9 papilomas); ($p<0.05$). También existió una diferencia significativa entre tratamientos de acuerdo al periodo de aplicación de la vacuna (3 a la 5) disminuyendo el número de papilomas (efecto periodo; $p<0.05$). Por su parte, se observó una interacción tratamiento x periodo disminuyendo el número de papilomas en la cuarta y quinta administración (T1 y T3; $p<0.05$). **Conclusiones.** La administración repetida de la vacuna autógena en las hembras bovinas disminuyó el número de papilomas entre la cuarta y quinta aplicación en los tratamientos 1 y 3.

Palabras clave: Autovacuna; carcinoma; daño en la piel; tratamiento; verrugas (*Fuente: MeSH*).

INTRODUCTION

In Mexico, the tropical region has the highest potential for raising dual purpose cattle (1); which strongly contributes to improving nutrition in the poorer, more isolated regions of the country (2). The State of Guerrero stands among the main cattle producers, with 1.300.000 livestock, distributed in 42.000 production units. This livestock is distributed among the regions of Tierra Caliente, North, Costa Chica, Costa Grande and in the rest of the state (39, 18, 16, 16 y 11%: respectively) (2).

In the municipality of Cuajinicuilapa, in the Costa Chica region, diseases affecting cattle are important problems, such as: brucellosis, tuberculosis, pasteurellosis, leptospirosis, anaplasmosis, babesiosis, colibacillosis, salmonellosis and bovine papillomatosis (BP) (2). The BP is an infectious, contagious viral disease, which is characterized by the onset of tumors in skin and mucosa; of benign character and fibro-epithelial nature (3,4).

It has been proven that papilloma is caused by the bovine papilloma virus (DNA) types 1 to 6 (BPV 1 to 6) (5,6), which share antigenic components, but do not have an adequate cross immune reaction (7). Viruses types 1 and 2 affect different body parts such as head, neck, penis and vaginal mucus; type 3 affects only skin; type 4 affects the digestive tract; types 5 and 6 affect nipples in the form of rice-shaped and flat granules respectively (3,4). These tumor masses affect the skin, they look like a cauliflower with a rugged surface or covered with scabs with a color which varies from white to gray (8).

The BP condition strongly affects the animal's health and therefore, the farmers' economy. This viral disease is common among young cattle, although the condition is mild and tends to disappear (3). Animals with lesions caused by papillomas can suffer secondary bacterial infections or parasite infestations (myiasis by insect vectors) (4). In addition, cows with papillomas in nipples and udders can make milking difficult and cause mastitis (9). When fibrous papillomas are located in hooves or interdigital spaces, cushions and heels, these produce pain and can cause from limping to prostration, decreasing voluntary food intake and weight gain (10). The latter, alongside with the unattractive look of the hides, which hampers their marketing in the tannery and fur industry (3).

In this respect, the establishment of specific safety regulations for BP control is not possible, due to the unpredictable nature of the virus. However, some actions can be taken to control the infection: avoiding direct contact among between healthy and infected animals, facility management, appropriate materials and equipment handling (11). Likewise, due to the presence of viral DNA in body fluids (blood, milk, urine and semen) artificial insemination programs should be implemented avoiding vertical transmission through semen bank analysis (12).

Finally, various treatments against BP have been suggested, however, the reported effectiveness has been very variable among treatments (11.1% up to 77.7%), with higher effectiveness among chemical agents (13). Some treatments include: Surgical intervention, vaccine against Newcastle disease virus, diazoaminodibenzamidine diaceturate, injecting concoctions made of bismuth and antimony, homeopathic treatments with Thuja, salicylic acid, fig tree latex, autohemotherapy and autogenous vaccines prepared from the infected animal's wart tissue (14,15). In addition, immune system stimulants such as levamisole and ivermectine have been successfully used in the treatment of BP (16).

Given the aforementioned information, the aim of this study was to evaluate the treatment of BP with an autogenous vaccine in the tropical conditions in the State of Guerrero.

MATERIALS AND METHODS

Area of study. The present study was done in the municipality of Cuajinicuilapa, Guerrero, Mexico; in the ranches El Cerrito, Las Lomas and El Charco Metepec, dedicated to growing-finishing commercial cattle (Swiss-American, Brahman and Beefmaster). The place is located in the sub-humid tropic at 6°28'18" N and 98°24'55" W, with maximum average temperatures and relative moisture in summer (33.6°C y 80%) and minimum in winter (21.2°C y 60%) (17).

Animals and treatments. For the experiment, 18 pubescent cows (Swiss-American x Brahman) aging 26.5±1.73 months, weighing 364±1.73 kg and body condition (BC) 3.5±0.6 units, fit for breeding were selected; the animals were divided in three treatments according to the ranch (6 animals/group). Treatment 1 (T1-immunevaccine; El Charco Metepec), treatment 2 (T2-histovaccine; Las Lomas) and treatment 3 (T3-plasma therapy; El Cerrito).

Sampling and therapy. A sample of blood and skin tissue was obtained from the 18 animals, in different anatomical regions (neck and tail) with the purpose of making an autogenous vaccine to control BP. For the T1-immunevaccine, 20 mL of blood from the right jugular vein was collected. Likewise, for the T2-histovaccine, papillomas were surgically removed from the cows, afterwards, they were weighed and mashed with 480 ml distilled water in a 366 g porcelain mortar; formaldehyde (0.1 mL/100 mL) was added to the mixture, let set for 24 h at 24°C, decanted, filtered and refrigerated at 8°C. On the other side, 10 ml of blood was collected from the coccygeal vein from individuals for T3-plasma therapy, centrifuged at 360 r.p.m. for 10 minutes, and 4 mL of plasma was recovered and placed in sterile jars at 8°C. Afterwards, the three groups were treated; on T1 the blood was subcutaneously applied after extraction, on the left side of the neck; for T2 and T3, 4 mL of the extraction was administered in the same way and same place. Each animal was given its own preparation as an autogenous vaccine. Additionally, during the study, five applications were performed to each cow at 7-day intervals between injections. Before the injection, the papillomas were counted and recorded.

Pre and post-experimental handling. All animals were subject to an exploratory clinical examination, physical condition, characteristics and location of papillomas were evaluated. Herd infestation degree, livestock density, age, live weight and BC were taken into account to select the subjects, (Table 1). The neck region was selected for sampling, as it was the anatomical area with the highest number of papillomas.

Table 1. Physiological characteristics of the animals selected for experimentation.

Treatments[1]	N[2]	Age[3]	LW (kg)[4]	BC[5]
T1	6	26.3 ± 1.6[a]	366 ± 58.7[a]	3.3 ± 0.5[a]
T2	6	27.0 ± 1.1[a]	377 ± 66.2[a]	3.7 ± 0.8[a]
T3	6	26.2 ± 2.5[a]	349 ± 76[a]	3.5 ± 0.5[a]

[1]Treatment 1, 2 y 3: T1, T2 y T3; [2]Animals: N; [3]Age in months; [4] Live weight in kilograms: LW (kg); [5] Body condition scale: BC – (1 al 5); Literal [a] between variables denotes that there were no significant differences ($p<0.05$).

Feeding and housing. The experimental animals were confined in paddocks with the rest of the herd. The production system in the locations is semi-intensive, with day grazing and night confinement. The feeding consists of Guinea grass (*Panicum maximum*) and African Star grass (*Cynodon nlemfuensis*), supplemented with corn stubble and mineral salts during the dry season.

Statistical analysis. The data generated from the study was analyzed under a completely random design. A self-regressive covariance structure was done, where the treatment effect, period and interaction treatment per period were measured; all the analyses were carried out using the PROC MIXED, SAS procedure (18). The results are presented in average ± s.e.m. and percentages.

Ethical note. The experimental procedures used in this study complied with the norms for ethical use, care and welfare of research animals at national and international levels (19,20).

RESULTS

At the beginning of the study, a high incidence of papillomas was found in the cows, regardless of the treatment [(T1 (190 papillomas), T2 (95) and T3 (100) (Table 2)]. The application of the autogenous vaccine (T1 and T3) decreased the number of papillomas significantly through the applications (1 (147 and 66 papillomas) to 5 (10 and 9 papillomas); ($p<0.05$) (Figure 1).

There was also a significant difference (T1, T2 and T3) according to the vaccine's application period (3 to 5) with a decrease in the number of papillomas (period effect; $p<0.05$) (Figure 2). In addition, a decrease in the number of papillomas was observed at the fourth and fifth application on T1 and T3 (interaction treatment x period; $p<0.05$) (Figure 3). Finally, the information on the percentage decrease of papillomas through the application of the autogenous vaccine is presented on Table 2.

Table 2. Decrease in papillomatosis according to the repeated application of autogenous vaccine in bovine females.

ID	Ranch	Papillomas	Weeks				
			1	2	3	4	5
1203419433	CM	35	20 (57%)	12 (34%)	7 (20%)	-	-
1203419434	CM	8	7 (88%)	3 (38%)	-	-	-
1203419435	CM	86	76 (88%)	51 (59%)	36 (42%)	13 (15%)	8 (9%)
1203419436	CM	4	5 (125%)	5 (125%)	2 (50%)	1 (25%)	-
1203419437	CM	21	8 (38%)	3 (14%)	-	-	-
1203419438	CM	36	31 (86%)	13 (36%)	5 (14%)	3 (8%)	2 (6%)
1203419394	LM	2	-	-	-	-	-
1203419395	LM	6	5 (14%)	3 (8%)	3 (8%)	2 (6%)	2 (6%)
1203419396	LM	19	16 (84%)	12 (63%)	12 (63%)	10 (53%)	8 (42%)
1203419397	LM	26	21 (81%)	18 (69%)	18 (69%)	15 (58%)	12 (46%)
1203419398	LM	39	35 (90%)	34 (87%)	34 (87%)	31 (79%)	32 (82%)
1203419399	LM	3	-	-	-	-	-
1203419440	EC	15	10 (67%)	7 (47%)	5 (33%)	1 (7%)	-
1203419441	EC	4	3 (75%)	-	-	-	-
1203419442	EC	2	-	-	-	-	-
1203419443	EC	18	11 (61%)	6 (33%)	4 (22%)	-	-
1203419444	EC	58	42 (72%)	34 (59%)	25 (43%)	17 (29%)	9 (16%)
1203419445	EC	3	-	-	-	-	-

Charco Metepec: CM, Las lomas: LM y El Cerrito: CE; - indicate 0%.

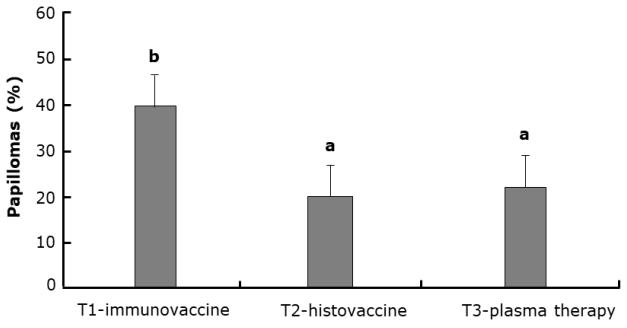

Figure 1. Percentage of papillomas in bovine females of the three autogenous vaccine treatments (T1-immunovaccine, T2-histovaccine and T3-plasma therapy). [a,b] Means with different literals between treatments differ statistically ($p<0.05$).

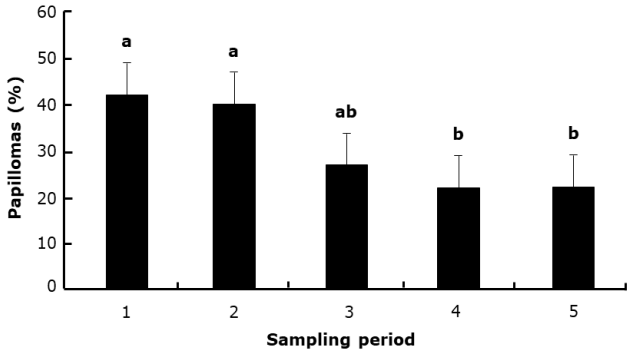

Figure 2. Effect of autogenous vaccine in relation to the sampling time. [a,b] Means with different literals between treatments differ statistically ($p<0.05$).

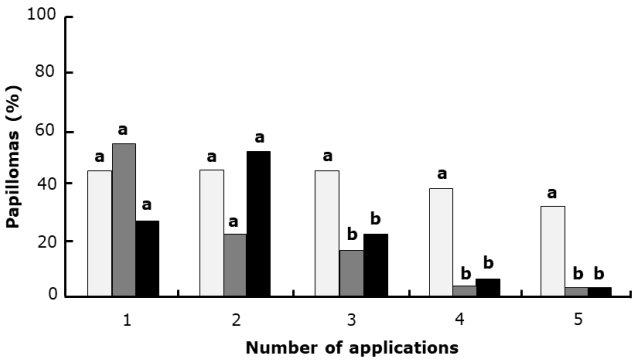

Figure 3. Effect of the application on the percentage of papillomas present after the application of autogenous vaccine (T1, T2 and T3). [a,b]Means with different literals between treatments differ statistically ($p<0.05$).

DISCUSSION

The data presented on this study indicate that the repeated application of an autogenous vaccine decreased significantly the number of papillomas on the cows on T1 and T3 between the fourth and fifth applications. There was also an interaction between treatment x period on T1 and T3. Previous works have described the epidemiology of papillomatosis and the different species that are prone to the viral disease (cattle, equine, swine, among others); however, cutaneous papillomas are caused by specific viral types (4,6,12). In fact, on cattle, the BP is more frequent and intense, and each anatomical region of the animal has a specific viral type (BPV1 to 6) (5,9). In the current study, the selected anatomical region was the neck table, as it was the most affected by papillomas; also, only female bovines were selected due to the high population density of female cattle in the growing-finishing ranches. In fact, Violet et al (21) evaluated in their study, age, sex and location of papillomas and found a higher percentage of lesions on females (77%) than in males (43%).

In the present study, three treatments were used on young cows (26.2±2.5 months); which showed a significant response decreasing the papillomas on T1 and T3. In this respect, Aricapa et al. (13) worked with cows between 12 and 20 months of age, using a self-vaccine treatment, and the results were similar to the current work's. In contrast, Özyurtlu and Aslan (22) found that blood therapy is not effective since the lesions do not disappear completely. In this regard, some studies mention finding viral DNA in blood, which means this method is a transmission means of the BP, as well as other diseases (4,13). On another hand, plasma therapy can be used successfully since its specific, and this treatment does not pose the risk of transmission to other animals of the same species (22).

It has been reported that the prevalence of BP in cattle herds is around 10%, although it can be very variable due to the virus characteristics and different risk factors (inherent to the animal, age, parasites and immune-suppressors) (23,24). The data from the present study confirm this information since only 10% of animals from the three herds presented the viral disease. Contrary to these results, in the state of Yucatan, Mexico, the prevalence found was of 20% (25). The latter leads to assume this poses no important economic implications for cattle growers; however, this is not the case as there are other factors involved.

According to the literature, papillomatosis has been reported as the second most important skin neoplasia in bovines, after scale cell carcinoma (26). The latter is due to the high morbidity of BP, making it a serious oncological disease for cattle farmers because it is difficult to avoid affecting other animals from the herd, consequently, it is a factor which leads to important economic losses in cattle farming, resulting from arrested development, low corporal condition, decreased milk production and important losses due to early discarding of animals (25). In addition, it causes losses to the farmers in the hide market due to the poor looks of the hides which makes it difficult to market as they cannot be used by the leather industry (3); as well as a deterioration in the animal's health since the BP features tropism to cells of other important organs (esophagus papilloma, bladder carcinogenesis among others) which can lead to the animal's death (25-29).

Altogether, the results of the present study highlights that the effectiveness of the vaccine-based treatment depend both from internal and external factors of the animal: the first ones at immune level, age, secondary diseases level; and the second ones involve climate handling, feeding and the dose of each treatment. Although the autogenous vaccine can be used with good results, it is recommended to use it individually to avoid contagion of this and other diseases related to body fluids (30). The latter is mentioned, because in the present study the vaccine was prepared for each animal with its own tissue, to avoid contagion.

In conclusion, the repeated administration of the autogenous vaccine on cows decreased the number of papillomas between the fourth and fifth application of treatments 1 and 3. This information is encouraging for cattle farmers on tropical regions as they represent sustainable alternatives to treating animals infected with BP.

Conflict of interests

The authors of the present study declare that there is no conflict of interests with the publishing of this manuscript.

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