4th Sci. Congr. of Egypt. Soc. For Anim. Manag. 25-28 Oct.,2010:140-156

CLINICO-EPIDEMIOLOGICAL AND THERAPEUTIC STUDIES ON BOVINE PAPILLOMATOSIS IN NORTHERN OASES

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ABSTRACT
Bovine papillomatosis is a viral disease of cattle characterized clinically by development of multiple benign tumours termed warts. The diagnosis of bovine papillomatosis was confirmed by clinical and pathological examinations of the warts. The prevalence of bovine papillomatosis in Northern Oases was recorded as 4.86%. The prevalence was higher in the females (2.99%) than males (1.87%).The prevalence was the highest in cattle less than one year old (2.99%). The infected cattle were examined for detection of external and internal parasites. The tick infestations were observed in 10 out of totally 13 infected cattle. The Fasciola eggs were also detected and counted in only 4 infected cattle while parasitic gastroenteritis (PGE) nematode eggs were detected and counted in only 5 infected cattle. The correlation between parasitic infestation and number of warts was statistically recorded. It was found that the correlation between number of warts and number of *Fasciola* eggs and number of parasitic gastroenteritis (PGE) nematode eggs was 0.6 and 0.89 respectively. Two therapeutic regimes were evaluated, regime-I and regime-II, all cattle treated were completely recovered in 15 to 115 days post-treatment. We concluded that the regime-I of treatment was better than regime-II depending on mean of days needed for healing and regression of warts that was 42 days for regime-I and 83 days for regime-II.

KEY WORDS: Bovine warts, prevalence, parasite, therapy, Northern Oases**.**

INTRODUCTION
Papilloma viruses are small (55 nm in diameter) non enveloped, icosahedral viruses, containing a double stranded, circular DNA genome about 8000 base pairs long. They are found throughout higher vertebrates, mostly mammals and birds, causing cutaneous and mucosal tumours (William, 2009). Bovine papillomatosis (warts) is a disease caused by host, site and lesion specific papillomaviruses. Bovine papillomavirus (BPV) has six serotypes hitherto **(Olson, 1990**). The

disease is usually spread by direct contact with infected animal where virus penetrates the skin via cutaneous abrasions. It gains its economic importance through interfering with animal sales and shows, and loss of condition in extensive cases especially when the lesions get infected secondarily with bacteria. Teat warts are also interfering with milking process **(Radostitis et al., 2007)**. These warts may spontaneously regress, occasionally persist, and, in presence of additional critical genetic or environmental factors, can progress to cancer **(Campo, 1987**). It is thought to be a multistep affair **(Koller and Olson, 1972 ; Lancaster and Olson, 1982**).

Papilloma virus infection developed as a result of the virus exposure to single or multiple lesions of the epithelium of the skin. The transformation and multiplication of papillomavirus infected basal cells, lead to wart formation, the most warts are benign and do not proliferate indefinitely causing cancer **(Shah and Howley, 1996).** Different methods have been used to treat bovine papillomas. Formalinized inactivated vaccine of bovine warts proved to be effective treatment and good prophylaxis against bovine papillomatosis **(Barthold et al., 1976; Hunt, 1984; Lesnik et al.,1999; Suveges and Schmidt, 2003)**. Intra-lesional immunotherapy by *Corynebacterium parvum* has also been reported by **Hall et al.(1994)**.

In this study, bovine papillomatosis was suspected in cattle in Northern Oases, 6th October governorate, the developed skin lesions were recorded along with age, sex of affected cattle, number and site of lesions, presence of external and internal parasites. The diagnosis was confirmed by pathological examinations. Therapeutic trials were done for the treatment of affected cattle with two different regimes.

MATERIAL AND METHODS

Clinical and epidemiological examination:

In northern oases, October 6th governorate, Egypt, 267 cattle were examined, general clinical examination was carried out and any skin lesions were recorded and described. Age and sex of infected cattle, sites and numbers of warts were recorded. Suspected infected cattle were visually examined for detection of external parasites and coprologically

examined for detection of internal parasites. The recorded data were statistically analyzed.

Parasitic examination:

Ticks found on the cattle skin were identified macroscopically. *Internal parasites* were diagnosed by faecal examination**.** Faecal matter were collected by back racking from papillomatosis infected cattle and were examined by both concentration flotation and concentration sedimentation techniques according to Denham and Suswillo (1995). The Mc master technique for counting eggs of parasitic gastroenteritis (PGE) nematodes was also done (Dunn, 1969; Georgi, 1980; Whitlock,1948)**,** number of eggs per gram faeces was calculated by multiplying mean number of actually counted eggs per Mac master slide to 100. The Fasciola eggs were counted by using method of Happich and Boray (1969), the actually counted Fasciola eggs were considered eggs per gram faeces.

Pathological examination:

Histopathological and negative staining examinations of warts were done. Histopathological sections were carried out by fixing of excised warts of living animals in 10% neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 micron) and stained with hematoxyline and eosin according to Bancroft et. al., (1996).

Tissue samples were prepared for electron microscopy by the negative staining technique (Nenad et al., 2005), the pelleted viral particles were resuspended in distilled water and a drop of viral suspension was placed on a Petri dish. A Formvar-coated electron microscopy (EM) grid was placed Formvar side down on top of the virus drop for approximately 1-3 minutes. The grid was removed, blotted with filter paper and placed onto a drop of 2.0% phosphotungstic acid (PTA), pH 7.0, for one minute. The excess PTA was removed, and the EM grid was ready for viewing in the electron microscope unites of VACSERA company.

Treatment

The external or internal parasites infested cases were treated by injection of Ivermectin and oral dosing of triclabendazole. The papillomatosis infected cattle were treated with two different regimes and they were evaluated.

Anti-parasitic drugs were given for cattle proved to be infected, Ivermectin (Iveen®, ADWIA company, Egypt) was injected subcutaneous at a dose rate of 200mcg/kg bodyweight (1ml/50kg bodyweight) and triclabendazole was given orally at dose rate of 12 mg/kg bodyweight(Fascinex®, Novartis co.)

Surgical treatment of warts:

Sedation

Cattle was sedated with xylazine 2% solution (xylaject®, Adwia company, Egypt) at a dose rate of 0.1 mg per 1kg bodyweight by intramuscular injection. The animal was well restrained before surgical excision of warts by one of the following regimes:

Regime I*:* *Excision* of large sized warts was performed by sharp scalpel, and hemorrhage was controlled using *electrocautery*.

Regime II*:* *Curetting* to remove overgrowth of warts by a scalpel till blood oozes to allow the reintroduce of virus into blood (Autogenous vaccine).*Trichloro-acetic acid* was applied topically post-curetting to kill and remove the rest warts cells. *Levamizole* was injected subcutaneous at a dose rate of 1 ml/10 kg bodyweight as immune stimulant (Cam et al., 2007).

In both regimes, infected animal was injected with *Multivitamin*® (1 ml/10 kg bodyweight by intramuscular injection, NorBrook company,Egypt). A *Betadine*® spray (Bovidone iodine skin solution, Nile company for pharamaceuticals and chemical industries) was applied topically on the skin wounds to prevent secondary bacterial infection and myiasis.

Statistical analysis

All the obtained data werestatistically analyzed using SPSS (Version 16) program.

RESULTS

Clinical findings, epidemiological data, parasitic infestations, pathological findings, results of treatment including anti-parasitics and surgical treatment of warts using regime-1 or regime-2 and results of statistic analysis were illustrated as in tables-1,2 ,3,4 and 5. And figures 1,2,3,4,5 and 6.

Results of clinical examination

Out of 267clinically examined cattle only thirteen cases of bovine papillomatosis were recorded. The body temperature of infected cattle was normal (38-38.5°C). The appetite of infected cattle was normal. The body condition of heavily infected cattle was poor. The site of warts and the warts count per animal were recorded as shown in table 2. Generalized papillomatosis was observed in one case in which warts distributed on both sides of the body; and the larger sized warts were concentrated in the right side. The largest wart measured 13 cm in width and 20 cm in length, and extended from the base of the ear to reach the edge of the mandible. It was irregular, rough and sessile, with hyperkeratosis. The variable sized warts were observed in other 12 cattle at different sites of the body skin including Back , Chest and neck , Legs, Udder, Face and External genitalia.

Results of the epidemiological study

As presented in table (1), the prevalence of bovine papillomatosis in Northern Oases was recorded as 4.86%. The prevalence was higher in the females (2.99%) than males (1.87%). The prevalence was the highest in cattle less than one year old (2.99%). As illustrated in table (2),the number and percentage of papillomatosis lesions regarding site were recorded as generalized (1, 7.69%), Back (4, 30.76%), Chest and neck (3, 23.07%), legs (2, 15.38), udder (1, 7.69), face (1, 7.69) and external genitalia (1, 7.69%). Spearman rank correlation between number of warts and number of Fasciola eggs per gram faeces was 0.6 while between number of warts and number of PGE eggs per gram faeces was 0.89.

Results of parasitic examination

of 13 papillomavirus infected cattle, 10 infected with ticks (76.92%),4 with fasciola (30.76%) and 5 with PGE (38.46%).

Results of pathology of bovine warts

Histopathologically, there was marked parakeratotic hyperkeratosis with long, thick, hair-like cornified surface projections and papillate epidermal hyperplasia, with patchy areas of erosion, ulceration, and neutrophil infiltration. The underlying dermal papillae had a moderate infiltration of neutrophils, eosinophils, and fewer lymphocytes (Prince Edward, 1994).
Bovine papilloma viruses were identified in the examined tissue samples of skin warts as recorded by Shah and Howley (1996). The virus was small and non-enveloped. The diameter of virus measured approximately 60 nm and it was composed of capsomeres arranged in icosahedral symmetry of the capside.

Results of treatment

All cattle treated were completely recovered in 15 to 115 days post-treatment. The mean period needed for healing and regression of warts was 42 days for regime-I and 83 days for regime-II.

DISCUSSION

Although bovine papillomatosis is a self-limiting disease, the warts in our study need long time to regress and animal to recover. The diagnosis of bovine papillomatosis was confirmed by presence of variable sized cutaneous warts and the histopathology findings. Economical impact of the disease is clearly observed in loss of animal condition, secondary bacterial infection, skin myiasis, interfering with lactation process and lastly reduction of animal price and sometimes hinders the sale.

Out of 13 bovine papillomatosis infected cattle, 5 male and 8 female cattle, were infected. The role of sex in the infection may return to the female cattle usually under stress factors such as gestation, lactation and progression in age. On other hand, male cattle are usually directed to fattening and meat production and are mostly slaughtered at age of 2 years or less of 13 infected cattle, 8 infected cattle were less 1 year of age , 3 infected cattle were less 2 years of age , 2 infected cattle were over 2 years of age. It clear the young ages are more susceptible to the infection than the adult as described by Otter and Leonard(2003)**,** who recorded an outbreak of fibropapillomas in calves. It is thought to be due to ill-

developed immune system, alkaline pH of the skin of young ages; that may facilitate virus infection and also young ages are more susceptible to parasitic infestation and exposure to stress factors.

Ten cases out of 13 papillomatosis infected cattle suffered ticks infestation with a percentage of 76.92 %, so we believe ticks play a role in papillomatosis infection. It is thought that tick has two inducing role for bovine papillomatosis, firstly piercing skin causes skin route for the virus to enter and infect basal keratinocytes, replicating its genome in the differentiating spinous and granular layers causing excessive growth rate forming warts (Radostitis et al., 2007). Secondly, its immune suppressive role, which facilitate virus infection to form warts (Lesnik et al., 1999; Jitka et al.,2004). The tick suck a large volume of host blood where it inserts its hypostome into the skin and secretes a cement from the salivary glands to hold the hypostome in place. Ticks is damaging skin barrier while feeding on host blood; secretes saliva to pierce skin and prevent clotting of blood , tick saliva has immune suppressive effect as recorded by Jitka et al.(2004)**,** they confirmed that Th2 cytokines; IL-6 and IL- 10 were down regulated by salivary gland extract of *Ixodes ricinus.*

The immune suppression enhances papilloma virus infection (Lesnik et al., 1999; Brady et al., 1999; Koski and Scott, 2003). Both PGE nematodes and Fascioliasis play a role as immune suppressive so that they facilitate virus infection. Five out of 13 papillomatosis infected cattle were suffering parasitic gastroenteritis PGE nematodes that have an immune suppressive effect as recorded by (Koski and Scott, 2003), they stated that deficiencies of iron, molybdenum, copper, and zinc, had been associated with higher worm burdens consequently affected immune response. Four out of 13 papillomatosis infected cattle were suffering from Fasciola that have an immune suppressive effect as reported by Brady et al.(1999), they mentioned that Th1 response to *B. pertussis* antigens was markedly suppressed and the bacterial infection was exacerbated following infection with *F. hepatica*. As in table (2), The immune suppressive effect of both parasites was clear where the correlation between number of warts and number of fasciola eggs per gram faeces was 0.6 and the correlation between number of warts and number of PGE eggs per gram faeces was 0.89.

The two therapeutic regimes were evaluated for treatment of bovine papillomatosis, regime-I and regime-II. In regime-I, The surgical excision of large warts was done using a scalpel and bleeding was controlled by electrocautery. The metastatic virus particles may circulate in the blood and act as auto-vaccine. The second regime including curetting the warts aimed to reintroduce the virus to circulation which was considered as autogenous vaccination, topical application of trichloro-acetic acid was done and the injection of immune stimulant (Levamizole) were used for treatment of animals. the regression of warts and time elapsed till recovery of infected animals were taken in consideration. The regression of warts and healing were observed 15-115 days after the beginning of treatment. Regime-I of treatment was better than regime-II depending on mean days required for regression of warts and healing, for regime-I was (42) days while for regime-II was (83) days.

Treatment of bovine papillomatosis with autogenous vaccine produced by grinding and suspending wart indicate variable results. In regime-I, the blood circulating metastatic virus particles stimulate the immune system. In regime-II, curetting of warts aimed to reintroduce papilloma virus into blood (autogenous vaccination) and application of trichloroacetic acid aimed to kill warts cells, papilloma virus, skin bacteria and stop light bleeding. The rate of success in both regimes I and II was 100% that is agree with that was reported that the treatment with autogenous vaccine showed 93.5% success with no difference in the used vaccine after 105 days of vaccination. Autogenous vaccine prepared from sterile homogenized wart and was injected twice; it was proved to prevent new cases and to treat sick animals (Suveges and Schmidt, 2003). Our results disagreed with that recorded by Smith (1990), who found that the treatment with autogenous wart vaccine was failed. Commercial vaccines for cattle rarely seem to effectively promoted regression of existing warts or to prevent malignant progression, although they might be capable of preventing the development of new lesions if the same strain is involved (Smith, 1990; Campo, 1991; Scott and Anderson, 1992).

The role of Levamizole as immune stimulant is mandatory in many infections as reported by Cam et al.(2007), they evaluated it for treatment of bovine cutaneous papillomatosis. Amery and Butterworth (1983)

found that Levamizole had good effect as an immunomodulator for blood disorders, renal failure, vasculitis and photosensitivity, inspite of that, the immune stimulating effect of levamizole against bovine papillomatosis was non-obvious where the levamizole may promote the general immune response but it could not help in eliminating the warts in rapid manner. That was disagree with the past studies of the role of immunomodulator against bovine papillomatosis. As shown in several *in vivo* studies, the stimulation of endogenous, non-antigen related defense mechanisms by parapoxvirus-based immunomodulators opens up new possibilities for the control and treatment of infectious diseases in domestic animals (Strube et al., 1989; Ziebell et al.,1997; Castrucci et al., 1998; Kyriakis et al., 1998; Glitz, 2002). The Inactivated parapox ovis viruses had a complex genetic structure and thereby they were considered as non-specific strong immunomodulator, which induced host immune reaction. There was evidence that such immune reactions resulted in more than elimination of the virus (Fachinger et al., 2000).

The efficacy of bovine papillomatosis treatment with the autogenous vaccine and a parammunity inducer was observed to be useful for the earlier regression of papillomas in the early stage of disease (growing stage of warts). It was believed that a parammunity inducer also shows a beneficial effect in additional treatment of bovine papillomatosis (Nenad et al., 2005)**.** That was disagree with our conclusion, the surgical excision of warts is better than curetting and autogenous vaccine.

Table-1: Epidemiological data of papillomavirus infected cattle

Papillomatosis	**Infected cattle**		**Non infected cattle**	**Total**
	Number	**Prevalence (%)**	**Number**	
Male cattle	5	1.87	108	**113**
Female cattle	8	2.99	146	**154**
Cattle less than 1 year old	8	2.99	76	**84**
Cattle less than 2 years old	3	1.12	57	**60**
Cattle from 2 to 5 years old	1	0.37	63	**64**
Cattle above 5 years old	1	0.37	58	**59**
Total	**13***	**4.86**	**254**	**267**

Table-2: Site and number of warts and the accompanied parasitic infestations.

Animal serial number	animal		Warts		Parasitic infestation		
					Internal		External
	Sex	Age (years)	Site	Number	Fasciola (EPG)*	PGE (EPG)*	Ticks
1	Male	Less than 1	Generalized	113	+(7)	-	+
2	Female	Less than 1	Back	9	-	+ (500)	+
3	Male	Less than 1	Chest and neck	17	+(2)	-	+
4	Female	Less than 2	Legs	11	-	+(700)	+
5	Female	Over 5	Udder	1	+(3)	-	-
6	Female	Less than 1	Face	2	-	-	+
7	Male	Less than 1	External genitalia	2	+(13)	-	+
8	Female	Less than 1	Back	23	-	+(900)	+
9	Female	From 2 to 5	Chest and neck	3	-	+(500)	-
10	Male	Less than 2	Chest and neck	17	-	-	+
11	Female	Less than 2	Back	1	-	+(500)	-
12	Male	Less than 1	Back	15	-	-	+
13	Female	Less than 1	Legs	4	-	-	+

***EPG: eggs per gram faeces.**

Table-3: Comparison of the counts of warts, PGE eggs and fasciola eggs.

Counts	Number of examined animals	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
Warts	13	112	1	113	16.77	8.275	29.836
PGE eggs	5	400	500	900	620.00	80.000	178.885
Fasciola eggs	4	11	2	13	6.25	2.496	4.992

Table-4: Results of treatment of internal and external parasites and warts.

Animal serial number	Treatment					
	Anti-parasitic		Treatment of warts			
			Regime I		Regime II	
	Tri-clabendazole	Ivermectin	Animals treated	Days needed for healing and regression of warts	Animals treated	Days needed for healing and regression of warts
1	+	+	+	93	-	-
2	+	+	+	57	-	-
3	+	+	+	68	-	-
4	+	+	-	-	+	75
5	+	-	+	21	-	-
6	-	+	+	18	-	-
7	+	+	+	15	-	-
8	+	+	-	-	+	115
9	+	-	-	-	+	62
10	-	+	-	-	+	71
11	+	-	-	-	+	63
12	-	+	-	-	+	109
13	-	+	+	19	-	-

Table-5: Comparison of Regime-I and II for treatment of warts.

Treatment	Number of treated cattle	Days needed for healing and regression of warts				
		Minimum	Maximum	Sum	Mean	Std.Deviation
Regime-I	7	15	93	291	41.57	31.026
Regime-II	6	62	115	495	82.50	23.441

Figure-1: A calf showing multiple large sized warts distributed all over the skin before treatment (right side).

Figure-2: The same calf showing smaller sized warts on the left side of skin.

Figure-3: The excised large sized warts.

Figure-4: The Calf after excision of large sized warts.

Figure-5: Fasciola egg (X100).

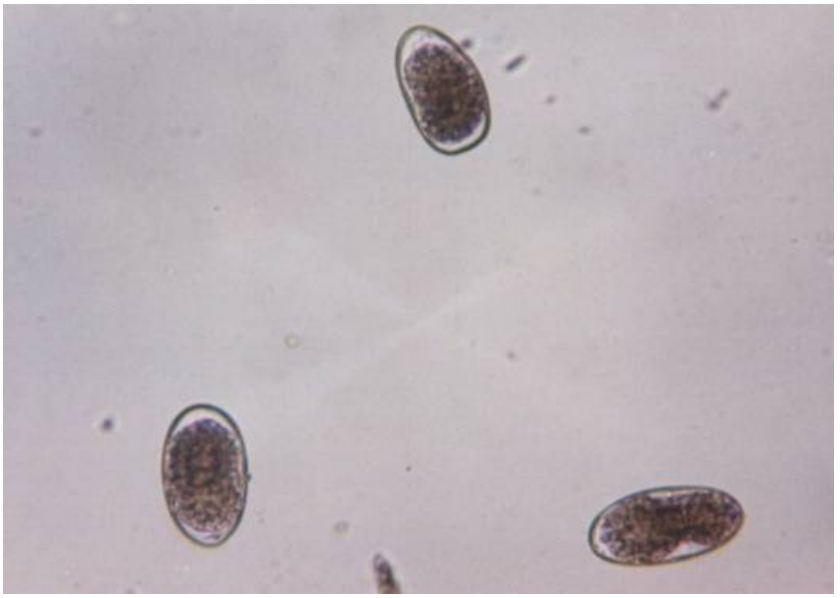

Figure-6: Parasitic gastroenteritis nematodes eggs (X40).

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