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Treatment of Bovine Papilloma

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Summary

In this study (35) cows suffering from bovine papilloma were subjected for three different types of treatments; The First group involved 15 animals treated with autogenous vaccine. The Second group involved 10 animals treated with prepared cell culture vaccine .While the third group involved 10 cows treated with virulent local Newcastle disease virus. In the First two groups the warts were surgically removed from cows showing lump lesions on skin of abdomen, neck and udder, and transferred aseptically to laboratory by using transport media.

Treatment of cows in the first group involved preparation of autogenous formalin (0.5%) in activated vaccine. Vaccination of these animals result in regression of the warts started after 2 or 3 weeks and complete disappearance of the warts after 30-60 days with a mean duration of 44.9 days.

Treatment of the second group involved preparation of papilloma cell culture inactivated vaccine 0.5% formalin from 3 papilloma cases. The response of these vaccinated cows result in regression of the warts lesions started after 2 or 3 weeks post the first vaccinal dose and complete disappearance of the warts after 30-60 days, with mean 43.8 days.

Treatment of the third group involved using of virulent NDV by subcutaneous injection and infiltration around the warts. Results showed successful regression of warts within a shorter period with mean of 30.1 days compared to treatment with autogenous and cell culture vaccine, with complete regression of the warts within 15-28 days.

علاج الأورام الحليمية البقرية

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الخلاصة

اجريت الدراسة على (35) بقرة يعانون من الأورام الحليمية عولجت بثلاثة أنواع مختلفة من العلاجات: المجموعة الأولى شملت (15) حيوان عولجت باللقاح الذاتي. والمجموعة الثانية شملت (10) حيوانات عولجت باللقاح المحظر من الزرع الخلوي والمجموعة الثالثة شملت (10) حيوانات عولجت بالعترة المحلية الضارية لفايروس النيوكاسل. في المجموعة الأولى من الأبقار التي تعاني من آفات ورمية على شكل كتل في منطقة البطن والرقبة والضرع ازيلت جراحيا ونقلت بواسطة وسط زرعي ناقل إلى المختبر. علاج الأبقار في المجموعة الأولى بواسطة اللقاح الذاتي المقتول المحضر من تلك الآفة الورمية ولاستجابة العلاجية في تلك الحيوانات الملقحة بدأت من 3-2 أسابيع من بداية العلاج ولوحظ إختفاء الآفة الورمية كليا من (30-60) يوم من بدء العلاج وبمعدل فترة استجابة 45 يوم.شمل علاج المجموعة الثانية تحضير لقاح الزرع الخلوي المقتول بنسبة 5% فورمالين من ثلاثة حالات عانت من الأورام الحليمية البقرية حيث استجابت الأبقار الملقحة بدئا من الأسبوع الأول والثاني من إعطاء أول جرعة علاجية وكان الاختفاء الكلي للورم من 30-60 يوم بمعدل استجابة علاجية 43.8. عولجت المجموعة الثالثة باستخدام العترة المحلية الضارية لفايروس النيوكاسل بواسطة الحقن تحت الجلد والارتشاح داخل الورم. أظهرت النتائج اختفاء تام للورم وبفترة قصيرة وبمعدل 30.1 مقارنة مع اللقاح الذاتي ولقاح الزرع الخلوي, ومع اختفاء تام للورم خلال 28-15 يوم.

Introduction

Bovine papillomatosis is a common viral disease of the skin, mostly of young cattle, manifested as benign tumours or warts, caused by bovine papillomavirus (BPV) (**1**). (BPV)-associated diseases are important in veterinary medicine and can be also considered as possible important models for the study of human papillomavirus (HPV).

Six types of BPV have been described (2), and recently, four other types were reported. BPV-1 causes teat and penile fibropapillomas; BPV-2 is associated with cutaneous warts,

alimentary fibropapillomas and urinary bladder tumors; BPV-3 causes cutaneous papillomas; BPV-4 is associated with pure epithelial papillomas of the upper gastrointestinal tract; BPV-5 induces fibropapillomas of the udder; BPV-6 causes papillomas of the teats; BPV-8 causes cutaneous papillomas; BPV-9/10 are associated with epithelial squamous papillomas of the udder (3).

The papilloma virus belongs to family papillomaviridae produce in their hosts benign skin tumors (papillomas); which contain variable amount of infectious virus (1). Different methods have been used to treat bovine papillomas. A formalinized suspension of bovine warts with inactivated virus provides a vaccine for effective treatment and prophylaxis of bovine papillomatosis (4,5).

In Iraq, Al- Bana and Khazeil (6) prepared the bovine interferon and used it for treatment of bovine papilloma for the first time in country.

Our aim of this study was treatment of bovine papilloma by autogenous vaccine and cell culture vaccine prepared from established cell culture (7) and local virulent strain of NDV.

Materials and Methods

- **Collection of wart samples:**

Cauliflower like wart were collected from cow suffering from papilloma skin lesion on the udder, teat, abdomen and the back of affected animals.

Before surgical removal of the warts the animal were given of xylazine 2% (0.2 mg/kg.b.w), intramuscularly and lidocaine 2% as local anesthesia infiltrated around the lesion, after preparation of surgical site.

The warts were removed by surgical procedure. Pieces of the lesion were added to transport solution containing MEM medium supplemented with a mixture of Antibiotics (amikasin, streptomycin and amphotricin) in sterile containers.

Other small pieces were added to 10% formalin in plastic containers for histopathological examination.

- **Selection of animals with papilloma lesions:**

Thirty five cows were suffering from bovine papillomatosis in Al- Anbar province and the areas surrounding Baghdad from the North side of West brought by owners to Private Clinic during the period from November 2010 to May 2011, The age of animals ranged from (12-24) months Holstein breed, affected breed suffering from papillomatosis and the other cases occurred in dairy cows with the age ranged from 24- 48 months multiple papillomas (from 1 to 10 cm in diameter)on different parts of the body, these animals were divided into 3 groups first group included (15) cows treated with autogenous vaccine, second group included ten (10) animals treated with vaccine prepared from cell culture of bovine papilloma and third group included (10) cow treated with Newcastle disease virus.

- **Preparation and treatment with Autogenous vaccine:**

Following the method of (8).Fifteen animals with papilloma lesions were selected to be used in this study. The lesions were partially removed surgically and individually from each animals by aseptic surgical procedure.

The papilloma lesions were cut into small pieces by sterile scissors, washed thoroughly by sterile PBS and then ground by using sterile sand, and 10% suspension of the ground tissues was made by using sterile PBS. The suspension was centrifuged at 3000 rpm at 4 °C for 30 minuts to remove the large particles. Supernatant fluid was in sterile bottle, formaline (37- 40%) was added at final concentration of 0.5% to inactivate the virus, finally glycerine in equal volume was added to the prepared vaccine, and left for 24 hours at 4 °C.

To check sterility of vaccine, samples were inoculated in blood agar, nutrient agar and MacConkey agar at 37 °C for 48 hr and inoculated in sabarouds agar at 37 °C and 25 °C for 3-7 days.

If the prepared vaccine proved to be sterile: The same animals with papilloma lesions (15) were treated with their respective vaccine as follows:

1- The first dose was 10 ml given subcutaneously in shoulder regions.
2- The second dose was also 10 ml given subcutaneously in shoulder regions after 10 days from the first vaccine dose.

- **Preparation and treatment with cell culture vaccine:**

A. Preparation of cell culture vaccine:

The cell cultured medium of the prepared papilloma cell culture was used as source of this vaccine. Cell cultured medium was collected from the confluent primary papilloma cell culture flasks, followed by treatment with formaline at final concentration of 0.5% to inactivate the papilloma virus with continuous stirring at 37 °C for one hour, and then at 4°C for 24 hours. The next day equal parts of the formaline treated cell culture fluid mixed with glycerine and stored at 4°C until used for treatment.

B. Treatment by cell culture vaccine:

Small Animals with cutaneous papilloma were injected subcutaneously with 5-7 ml of the cell culture vaccine, second and third dose was given similarly often one week later.

On the other hand adult cows with cutaneous papilloma were administered 10 ml of the vaccine s/c, second and third dose were given similarly in 10 days apart (9).

- **Treatment with virulent local Newcastle disease virus (NDV).**
- **Isolation and identification of NDV.**

Clinically suspected NDV Samples included liver, lung and trachea were supplied by Dr. Emad J. Kammas, head of Pathology and Avian Diseases Department. Samples were directly processed by cutting into small pieces and ground by using sterile sand followed by adding cold sterile phosphate buffer saline (pH7.2) at concentration of 10%. The suspension was centrifuged at 3000 rpm for 30 min in cold centrifuge, the supernatant fluid collected and then treated with a high concentration of antibiotics (amikasine 5 mg/ml and streptomycin 10 mg/ml) for one hour at 25 °C. 0.1 ml of the prepared sample was inoculated in allantoic sac of ten days old chicken embryos (5 embryo for each sample). Five embryos were also inoculated by sterile PBS as a negative control and all embryos were incubated at 37 °C.

The embryonated eggs were examined twice daily for detection of any mortality. Any death after 24 hours post inoculation were put in a refrigerator for 24 hours. Allantoic fluid collected aseptically from these embryo, centrifuged (3000 rpm for 30 minute at 4°C). Then was dispensed into small tubes and stored at -20°C.

Results

- **Using of autogenous vaccine.**

Fifteen animals with bovine papillomatosis were treated with the autogenous vaccine, the response of these vaccinated animals was variable but mainly started after 2 or 3 weeks from giving the first dose of vaccine, and complete regression of the warts occurred after 30-60 days figure(1). Results of this treatment are summarized in (Table 1).

Table (1): Results of using Autogenous vaccine prepared from Bovine Papilloma (warts) after subcutaneous administration

No.	Age of animal	Sex	Site of wart lesion	Diameter of wart	Types of the virus	Response of treatment
1	18 months	Male	Face	2-3 cm.	BPV1	Start after 2 weeks and regression after 30 days
2	24 months	Female	Muzzle	1-3 cm.	BPV1	Start after 2 weeks and regression after 30 days
3	24 months	Female	Udder	3 cm.	BPV1	Started after 2 weeks and complete regression after 40 days
4	19 months	Male	Back	3 cm.	BPV2	Start after 2 weeks and regression after 40 days
5	36 months	Female	Face	3 cm.	BPV1	Start after 2 weeks and regression after 40 days
6	24 months	Female	Neck and back	2 cm	BPV1andBPV2	Start after 2 weeks and complete regression after 40 day
7	12 months	Male	Nose and muzzle	1-2 cm	BPV1andBPV2	Start after 2 weeks and complete regression after 40 day
8	24 months	Female	Nose and muzzle	2-3 cm	BPV1	Start after 3 weeks and regression after 42 day
9	20 months	Male	Back	8-10 cm	BPV2	Regression of the wart to small size after 3 weeks and disappear after 42 days
10	30 months	Female	Abdomen, thigh	3-4 cm.	BPV1andBPV2	Start at 3 weeks and complete regression after 50days
11	30 months	Female	Thigh	5 cm.	BPV1andBPV2	Start after 3 weeks and regression after 50 days
12	36 months	Female	Legs and extremities	2-5 cm.	BPV1andBPV2	Regression of the wart to small size after 3 weeks and disappear after 50 days
13	34 months	Female	Udder	4-5 cm.	BPV1	Regression of the wart to small size after 3 weeks and disappear after 60 days
14	36 months	Female	Neck and Shoulder	Multiple wart from (3-8)cm	BPV1andPBV2	Regression of the wart to small size after 3 weeks and disappear after 60 days
15	42 months	Female	Back	8 cm.	BPV2	Start after 3 weeks and regression after 60 days

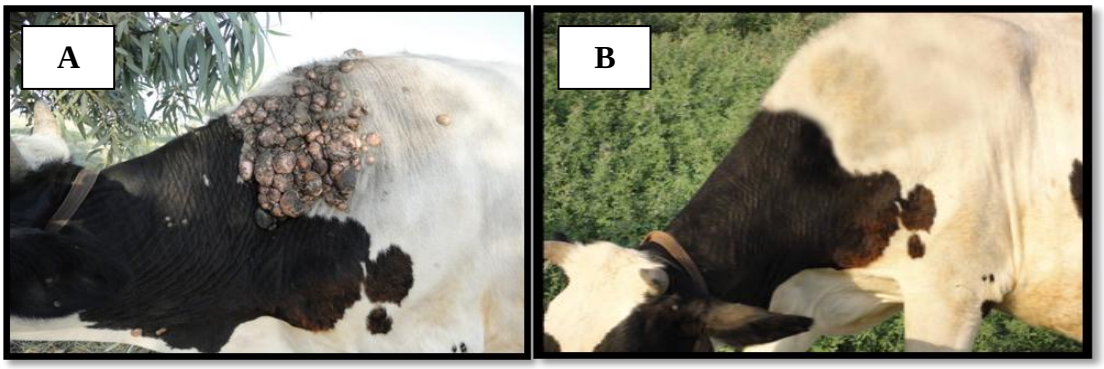

Fig. (1): (A) Shows severe papillomatosis on the front back of cow before treatment and (B) Shows the same cow with complete regression of the warts after 50 days of treatment with autogenous vaccine.

Using cell culture vaccine:

Ten animals with bovine papillomatosis were treated with cell culture vaccine which contained the cell culture medium of three papilloma cases or in other word, it is a heterogenous papilloma vaccine. The response in the vaccinated animals was also variable but mainly started after 2 or 3 weeks from the first dose and complete regressions of the warts was after 30-60 days figure (2), mean was 43.8 days, these are summarized in: (Table2).

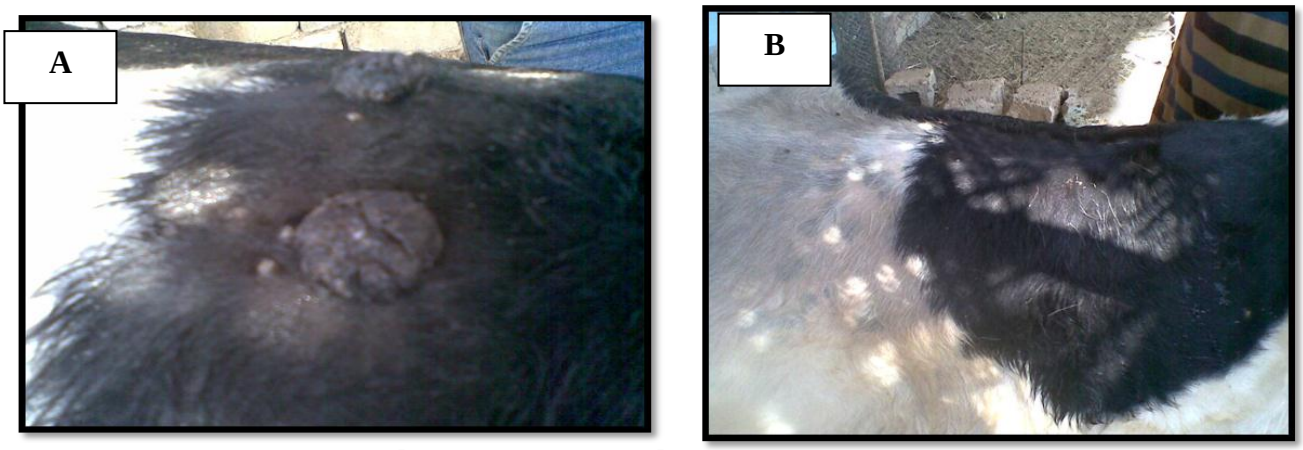

Fig. (2): (A) Showing cow with papillomatosis before treatment and (B) Showing cow with a complete regression of warts after 40 days of treatment with heterogeneous (cell culture) vaccine.

Table (2): Results of using Cell culture vaccine (heterogeneous) prepared from Bovine Papilloma(warts) after sub cutaneous injection

No.	Age of animal	Sex	Site of wart	Diameter of wart	Type of the virus	Response of treatment
1	14 months	Male	Face and muzzle	1-2 cm	BPV1	Start after 2 weeks and regression after 30 days
2	21 months	Female	Legs and extremities	2-3 cm	BPV1and BPV2	Regression of the wart to small size after 2 weeks and disappear after 40days
3	18 months	Male	Neck and face	1-3 cm	BPV1	Start after 2 weeks and regression after 40 days
4	17 months	Female	Back	4-5 cm	BPV1and BPV2	Start after 2 weeks and regression after 40 days
5	16 months	Female	Upper and lower eye lid also in muzzle	2-4 cm	BPV1and BPV2	Regression of the wart to small size after 3weeks and disappear after 42days
6	30 months	Female	On the ear	2 Cm	Not tested	Regression of the wart to small size after 3weeks and disappear after42days
7	24 months	Male	Upper the right eyes	3 cm	BPV1	Regression of the wart to small size after 3weeks and disappear after 42 days
8	19 months	Female	Neck and lower jaw	2-3 cm	Not tested	Start after 3 weeks and regression after 42 days
9	27 months	Female	Shoulder and extremities	3-4 cm	BPV1and BPV2	Regression of the wart to small size after 3weeks and disappear after 60 days
10	36 months	Female	Udder	2-4 cm	BPV1	Regression of the wart to small size after 3 weeks days and disappear after 60 day

Using of NDV for treatment of bovine papilloma:

A. Results of isolation and propagation of virulent NDV:

First passage of ND suspected lesions (liver, spleen and brain) resulted in death of all inoculated chicken embryos in 48 hours post inoculation (PI) with severe hemorrhages on the dead embryos. In contrast to control embryos inoculated with PBS remained a live for 96 hours PI. Allantoic fluid harvested from dead embryos was positive for agglutination of chicken RBCs (0.5%) and the titer was 2^8/0.025 ml.

Second passage of the heamagglutinating virus in chicken embryo result in death of infected embryos in 48 hours .Result of titration of the second passage was 10^9 ELD50/0.1 and $\mathbf{2^8}$ heamagglutinating units (HA)/0.025 ml.

B. Virus Identification:

Reference Monoclonal NDV antiserum inhibited heamaglutination of chicken RBCs induced by the isolated virus with titer of 128.

C. Treatment of bovine papilloma cases:

Ten animals treated with a local virulent isolate of Newcastle disease virus showed a successful regression of warts within a shorter period (30.1 days) compared with treatment by autogenous vaccine (44.9 days) or heterogeneous cell culture vaccine (43.8 days).

Details about the response of these treated animals were variable, but mainly started after 1 and 3 weeks from starting treatment with virulent NDV virus by injection and infiltration inside the warts and complete regression after (15-60) days of treatment (figure3), and table (3).

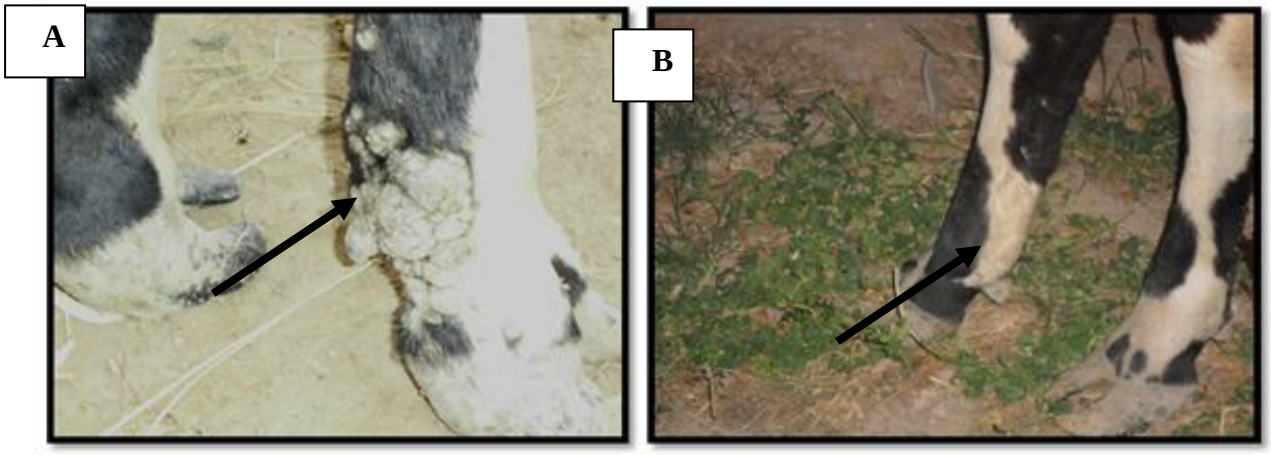

Fig. (3) (A): Cow with sever papillomatosis before treatment and (B) the same cow with a complete regression of warts after 25 days of treatment with local virulent NDV.

Table (3): Newcastle Disease virus for Treatment of Bovine Papilloma Virus by Infiltration injection

No.	Age of animal	Sex	Site of wart	Diameter of wart	Type of the virus	Response of treatment
1	14 months	Male	neck	3 cm	BPV1	Started after 1 week and complete regression after15 days
2	20 months	Female	Muzzle and submandibular	2-3cm	BPV1andBPV2	Started after 1 week and complete regression after 25 days
3	28 months	Female	Left legs	2 cm	Not tested	Started after 1 week and complete regression after 25 days
4	24 months	Female	Legs and extremities	2-5 cm	BPV1andBPV2	Started after 10 days and complete regression after 28 days
5	24 months	Male	Beside the horn	3cm	Not tested	Started after 1 week and complete regression after 28 days
6	36 months	Female	Left legs	1-2cm	Not tested	Started after 10 days and complete regression after 30 days
7	36 months	Female	Skin, abdomen	3.5 cm	BPV1andBPV2	Started after 10 days and complete regression after 30 days
8	36 months	Female	Shoulder region	3-4 cm	BPV2	Started after 10 days and complete regression after 30 days
9	48 months	Female	back	5-8cm	BPV1andBPV2	Started after3 weeks and complete regression after 60 days
10	48 months	Female	back	10 cm.	BPV1andBPV2	Started after3 weeks and complete regression after 60 days

The HI test of six cows treated with NDV showing no antibodies titer against NDV in the sera of these cows before one hour of treatment, while at 7th day of injection of virulent NDV the mean of HI antibodies titer became (64) and elevated in 21 days to (128) table (4).

Table (4): Response of animals affected by bovine papilloma to treatment with NDV

No.	Age of animal*	Sex	HI titer before treatment	HI titer after 7 days of treatment	HI titer after 21 days of treatment
1	20 months	Female	Zero	64	128
2	28 months	Female	Zero	64	128
3	24 months	Female	Zero	128	256
4	24 months	Male	Zero	64	128
5	48 months	Female	Zero	32	64
6	48 months	Female	Zero	32	64
	mean	-	Zero	64	128

HI= heamagglutination Inhibition test.

***These animals were treated by NDV intra wart injection.**

Discussion

The results of using the autogenous vaccine in the first groups, are in agreement with (10, 8, 4, 5), as they showed that using formalinized inactivated virus suspension of bovine warts provided an effective treatment and prophylaxis of bovine papillomatosis. Also in agreement with (11), who reported that the use of autogenous vaccine prepared from warts of affected age animals in younger, showed better signs of improvement after the second injection, as the growth became brittle and began to drop away after 30 days of the first injection (Table1) and (Fig. 1 A and B).

The good results obtained by the prepared cell culture vaccine is not surprising became in our coming for us as our investigation by using polymerase chain reaction (PCR) showed that the cell culture prepared vaccine contain two genotypes of bovine papilloma virus which include BPV1 and BPV2. The final results are in agreement with (12), who showed that the

vaccine which contained two types of papilloma virus genotypes (BPV-2 as for BPV-1), based on the L1 protein of BPV-2 elicits neutralizing antibodies and confers protection from papillomavirus infection. In addition to that (13) and (14) suggested that bovine papilloma vaccine should contain both types of viruses (BPV-1 and BPV-2) and encouraging results were obtained with this bivalent vaccine.

The results of using local virulent NDV is in agreement with (15) who demonstrated that NDV vaccine showed antineoplastic activity in treatment of bovine papilloma led to stimulation of antibody response and limited increase in TNF-α activity and may enhance clinical recovery in bovine papillomatosis. Our results showed that using of NDV gave a better response within a shorter period (30.1days) compared with treatment by autogenous vaccine (45 days) or heterogeneous cell culture vaccine (43.8 days).

References

1. Zuckerman, A. G.; Banatvala, G. and Pattison, J. R. (2000). Principle and Practice of Clinical Virology, 4th Ed., P: 607.
2. Murphy, E. A.; Gibbs, E. P.; Horzinek, M. C. and Studdert, M. J. (2007). Veterinary Virology, 3rd Ed., P: 336.
3. Borzacchiello, G. and Roperto, F. (2008). Bovine papillomaviruses, papillomas and cancer in cattle. Vet. Res., 39: 45.
4. Lesnik, F.; Bires, J.; Suli, J.; Posivak, J.; Mattova, J.; Svrcek, S.; Sevcikova, Z.; Kvokacka, V.; Gaspar, V.; Levkut, M. and Buleca, J. (1999). Autovaccination and metabolic profiles at bovine papillo-matosis. Slovak Vet. J., 24: 290-294.
5. Suveges, T. and Schmidt, J. (2003). Newer data on the occurrence in Hungary of losses caused by and ways of control of bovine papillomatosis. Magyar Allatorvosok Labya,125: 83-87.
6. Al-Banna, A. S. and Khazeil, A. Z. (2000). Production and application of interferon for treatment of papilloma virus. M.Sc. Thesis, College of Veterinary Medicine , University of Baghdad.
7. Hamad, M. A..; Al-Banna, A.S.; Nahi, Y.(2011). Cell culture established from warts of Bovine Papilloma. Al-Anbar Vet. J., 4(Suppl.1): 77-81.
8. Hunt, E. (1984). Fibropapillomatosis and papillomatosis. Vet. Clin. North Am. Large Anim. Pract., 6: 163-167.
9. Prasad, C.B., Singh, M.P. and Deokiouliyar, U.K. (1980): A Note on Successful Treatment of Generalised Cutaneous Papillomatosis with Autogenous Vaccine in Cross-Bred Cattle. Indian Vet. J., 57 (11), 950-952.
10. Barthold, S. W.; Olson, C. and Larson, L. (1976). Precipitin response of cattle to commercial wart vaccine. Am. J. Vet. Res., 37: 449-451.
11. Pangty, K.; Singh, S. H.; Awadh, B. P. and Ramesh, S. (2010). Preliminary binary ethylenimine (BEI) inactivated bovine papillomavirus (BPV) vaccine trial against cutaneous warts in bull calves: a pathological assessment. Braz. J. Vet. Pathol., 3(2): 105-110.
12. Modis, Y., Trus, B. L. and Harrison, S. C. (2002). Atomic model of the papillomavirus capsid. EMBO J., 21: 4754-4762.
13. Van Dyk, E.; Oosthuizen, M. C.; Bosman, A. M.; Nel, P. J.; Zimmerman, D. and Venter, E. H. (2009). Detection of bovine papillomavirus DNA in sarcoid affected and healthy free-roaming zebra (*Equus zebra*) populations in South Africa. J. Virol. Meth., 158: 141-151.
14. Pangty, K. (2009). Molecular diagnosis of bovine papillomatosis/ enzootic bovine haematuria and assessment of BPV hamster tumour model for immunoprophylaxis / antitumour therapeutics. M.Sc. Thesis, Vet. Pathology. Deemed University, IVRI, Izatnagar, UP.
15. Avki, S.; Turutoglu, H.; Simsek, A. and Unsal, A. (2004). Clinical and immunological effects of Newcastle disease virus vaccine on bovine papillomatosis. Vet. Immunol. Immunopathol., 98 (1-2): 9-16.