

**Republic of Iraq  
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College of Veterinary Medicine**



**Study the clinical and pathological  
Changes that accompanied with Bovine  
Papillomatosis**

**A Graduation Project Submitted to the Department Council of  
the Internal and Preventive Medicine-College of Veterinary  
Medicine/ University of Al-Qadisiyah in a partial fulfillment  
of the requirements for the Degree of Bachelor of Science in  
Veterinary Medicine and Surgery.**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

فَتَعَلَى اللَّهِ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ  
إِلَيْكَ وَحْيُهُ، وَقُلْ رَبِّ زِدْنِي عِلْمًا ﴿١١٤﴾

صَدَقَ اللَّهُ الْعَظِيمُ

من سورة طه

# Certificate of Supervisor

I certify that the project entitled (Study the clinical and pathological changes accompanied with bovine papillomatosis ) was prepared by **Sajjad Majid Lateef** under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah.

Supervisor

**Lecturer Khalefa Ali Mansour**

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25 / 3 / 2018

## **Certificate of Department**

We certify that Sajjad Majid Lateef has finished his/her Graduation Project entitled (Study the clinical and pathological Changes that accompanied with **Bovine Papillomatosis** ) and candidate it for debating.

Instructor

**Dr. Muthanna H. Hussain**

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## Obstruct

Bovine papillomatosis are widespread disease mainly associated with benign, and malignant skin lesions (warts).which cause economic losses .The is caused by oncogenic virus (bovine papilloma virus BPV). Cattle warts effects the main breed (Cross breeds Holstein Friesian and Holstein )in Al-Qadisiyah province.

In present study ,twenty four were examined that shows clinically suspected as having cutaneous papilloma and papillomatosis from deferent area of Al-Qadisiyah province during August to December 2017.Four samples were submitted to histopathological examination ,the examination were done on infected cattle with age range from (16-28 months).

The clinical examination of affected showed that the lesions were located in deferent region of the body (neck, back, abdomen, thigh and around the anal). The lesion have a appear like cauliflower with horny hard papilloma or roof shapes with smooth exterior surface in size (1-8 cm)in diameter and variable in number from one to multiples warts .

The histopathological result shown fibripapilloma, projection of papillary of dermal and epidermal , and presence of koilocytes, swollen clear cytoplasm and/or perinuclear halo and pyknotic ,nucleus keratinocytes .On conclusion the bovine papillomatosis is distribution among grouped of breed of cattle and causing economic loss in cattle industry in Al-Qadisiyah province.

## 1-Introduction

Bovine papillomatosis consider benign proliferation or malignant tumors that affect young dairy and beef cattle like ,cutaneous fibropapilloma ,cancer of esophagus and urinary bladder ,benign fibroplasia(Xavier et al., 2005). )that producing economic significant such as loss of hide, low milk production and decreased growth of infected animal.( Xavier, *et al.*, 2005 )

The disease affects all ages and sexes of animals but the disease may affects the young animals to more than 2 years; however (Jelínek, and Tachezy, 2005)

Cattle warts caused by DNA virus that have circular ,double stranded of DNA genome ,exhibition to the squamous epithelial tissue and mucosal layer , (Munger and Howley, 2002 )

Papilloma virus classified under papillomaviridae, family that includes twenty-nine genera (Bernard, *et al.* 2010).Papilloma virus have four genera :Deltapapillomavirus, xipapillomavirus, epsilonpapillomavirus and dyoxipapillomavirus that contain thirteen genotyping and all of this genera that associated with BPV(Dagalp., 2017)

There are (13) genotyping of bovine papillomavirus (BPV type-1 to – BPV type 13) (Tomita et al., 2007; Hatama et al., 2008). The disease is commonly transmitted by direct contact with diseased animals and cutaneous abrasions is also spread the infection to other animal. The disease having its economic importance due to interfering with animal shows and sales, as generally BPV causes the animal to decrease or lose his condition particularly when the skin lesions infected with secondarily bacterial infection . Teat papilloma are interfering with teat milking process (Radostitis, *et al* 2007)

Lesion are appear mainly as mucocutaneous mass and are in distribution as large numbers due to highly proliferative growth infection. These skin warts

are often noticeable in the skin of the, tongue ,scalp, teats, oral cavity, penis, upper digestive tract and back of the animals (Munday JS 2014 ).

Clinical examination and histopathology are Diagnostic methods include - as a differential, essentially and complementary to making diagnosis (Betiol *et al.*, 2012)

The Histopathological examination of the skin lesion is an essential procedure, subsequently it recognizes intraepithelial growths associated with oncogenic viruses, for instance those produced by BPV, constructing this a complementary to other methods of diagnosis ( Leto *et al.*, 2011). The pathological marks of the spinous layer contain cell hyperplasia (acanthosis) , papillomatosis, koilocytosis hyperkeratosis and parakeratosis (Turk *et al.*, 2005; Marins and Ferreira, 2011). Koilocytosis which is present in tissue that infected with BPV, this is not reflected a pathognomonic indicator. (Marins and Ferreira, 2011).

The goal of this study, to designate the describe the clinical and histopathological manifestation of bovine papillomatosis in cattle mainly type 1-2 that related with cutaneous papillomavirus.

## **2.Review**

### **2-1-History**

Papillomaviruses Researcher work started previously one hundred years ago which approved through several stages , the cell-free transmission of canine warts are established by original work in England (McFadyean and Hobday, 1898 ) .

In Brazil the source of infectious bovine papillomae was originally established by (Magelhaes.,1920) .

(Olson and Crook, 1951) were explained the giant papillomas development of these BPV types normally by these studies , whose exhibited the mode of infection by the BPV viruses to other host causing equine sarcoids. These aggressive local tumours are also distinguished in domestic horses under the natural conditions . On the other hand , the molecular analyses by( Lancaster and Olson , 1978) recommended that initiated of infection by equine sarcoids by transmission of bovine papillomavirus (BPV) to the horses by experimental initiation .

In 1959 this group reported other signal surveillance , exactly the induction of bladder tumours in cattle by BPV infection (Olson et al .,1959). Lastly , the transforming action of BPV provisions were described in bovine and murine cells by (black et al. , 1963) and( Thoras et al ., 1963) this work was the first time that tissue culture studies were used in papillomavirus research and they extremely influenced development in following years .

The renaissance in papillomavirus research and the proposal of this technology to the BPV system categorized part of the BPV genome as the essentnials responsible for transformation in tissue cultures , through improvement of molecular biology and DNA-cloning methods in the 1970s (Lowy el at ., 1980). In 1982 , BPV-1 was the first genotype of papillomavirus to be wholly sequenced (Chen et al ., 1982)

The consideration in studies of BPV is mostly based on the ease which some of the greatest prevalent BPV types (BPV-1 and -2 ) can be used in tissue culture studies , the study the machanisms of persistence of the viral genome , in addition the patterns of expression of the viral genes . Furthermore , increased the number of studies on these types of virus lead to usage of BPV DNA in vehicle vectors and the episcopal determination of this DNA greatly . The influence of research on BPV to this field was mostly



through the analysis of BPV that induced cell transformation ,the structural and efficient characterizations of individual viral genes and gene products and the division of the viral genome . The data developed mainly helped early studies on HPV infections. Oesophageal carcinomas have additional to this interest , that is created from BPV-4- Positive (Campo., 1987).

## **2-2-Pathogenesis**

Papillomas (warts) are induced in the skin and mucosal epithelia at specific sites (De Villiers et al., 2004) and differ in their tissue specificity and the associated disease (McMurray et al., 2001).

The highly tissue-specific papillomaviruses can be divided into two groups: one group is primarily found in cutaneous epithelia (skin), in which there is thickening of the epidermis, and the other group is predominantly present in mucosal epithelia, involving the oral pharynx, esophagus, or genital tract (Howley and Lowy, 2001).

Bovine papillomavirus, as is known for other mammal-infecting papillomaviruses , is believed to selectively infect epithelial tissue , completing its biological cycle in the upper layers of the stratified epithelium , with expression of oncogenic proteins , as well the L1 and L2 capsid-forming proteins , until now (Campo., 2003).

The virus infects the basal keratinocytes, replicating its genome in the differentiating spinous and granular layers causing the excessive growth that is characteristic of wart formation. Expression of the late structural proteins of the virus is limited to differentiated cells of the squamous layer where the new virus particles are encapsulated and shed into the environment as the cells die (Radostits *et al.* , 2007 ).

The tumor contains epithelial and connective tissues and can be a papilloma or a fibropapilloma , depending on the relative proportions of epithelial and connective tissue present ; papillomas contain little connective tissue , and fibropapillomas are mostly fibrous tissue ,with very little epithelial tissue , papillomas are the result of basal cell hyperplasia without viral antigen production . fibropapillomas are uncommon in horses , but are the common lesion in cattle , sheep and wild ruminants , Latent infection in the skin and lymphocytes has been demonstrated in cattle (Radostits *et al.*,2007).

## **2-3- papillomavirus**

Papillomavirus is a member of family papillomavirus, whereas in the prior times papilloma virus were together with the polyomaviruses under the family papovaviridae, (De Villiers *et al.*,2004).

Bovine papillomavirus are presently well categorized and classified into, three separate genera – ,Epsilon papillomaviruses , Xipapillomaviruses and Deltapapillomaviruses every one related with epithelia lesions of exact histological nature (Borzacchiello and Roperto , 2008).

The BPVtype-1 and type -2 are ordered as Delta papillomaviruses (Campo *et al.*, 1992). Typically, these types(1-2) make the p produced fibropapillomas, that linked with the sub epithelial fibroblasts (Jelinek and Tachezy , 2005).

Bovine papillomavirus type1-2 are only , producing the equine sarcoid (Nasir and campo , 2008 ), Currently , the genome of a novel ( Delta BPV-13) was completely sequenced (lunardi *et al.*.,2013).

These type of viruses may considered completely epitheliotropic, making the formation of "true, papilloma " devoid of the association of fibroblasts (Jarrett *et al.*, 1984; Zhu *et al.*,2012).

BPVs-5 and -8 induce both true papillomas and fibropapillomas in being classified into a third genus ,(Epsilonpapillomavirus) (Tomite *et al.*, 2007) . This virus was recorded as causes and isolated from( acutaneous papilloma)skin lesion and from healthy skin of teat samples (Ogawa *et al.* , 2007 ) .

BPV can induce fibropapillomas and papillomas in the mucous lesions and skin (Nassir and campo , 2008 ) , which may can degenerate or change to malignant lesions , some BPV types1-2 are complicated in the urinary bladder and upper part of digestive tract (Silva *et al.*, 2011)

## **2-4- Clinical signs**

Cattle warts were horny papillae and cauliflower-like with or roof shaped , a smooth outer external (1-5 cm) in diameter variable in number from few to six. Usually affected locates were neck, back ,near the eyes, abdomen , and legs. BPV have been reported in buffaloes individual a few sporadic infected cases but in cattle is a well-known disease (Sood *et al.*,2006)

The infected tissue was composed of hyperplastic epidermis layer supported by thin, inconspicuous, dermal layer stalks (Turk *et al.*, 2005).

The cattle warts shows cauliflower-like shaped and were diagnosed as papilloma and fibropapilloma/.The lesions were characterized by keratohyalin and presence of koilocytes, inclusion bodies and granules tissue (Singh *et al.*, 2009).

## **2-5Diagnosis**

### **2-5-1 Clinically**

### **2-5-2 Electron microscopy**

The examination of tumor tissue by Electron microscopy by the negative discoloration technique revealed virions that very related to BPV. The characteristic papillomavirus, virions are composed of (capsomeres) which arranged in icosahedral symmetry, of the capsid. sporadic virions that were set up in the hyperkeratotic of the surface layer. While, large groups were formed in the deep layers. Together coreless particles and solitary destroyed with electron dense core, space were existent in the aggregates (Turk *et al.*.,2005).

### **2-5-3 Histopathological examination**

The most histopathological alterations for papillomatosis is characterized by ,hyperkeratosis . This lesion occur due to proliferation of the squamous epithelium layer , that produced by infection with papilloma virus. The term of acanthosis is useful to a proliferation of the cell of the (malpighian) layer whether( neoplastic) or otherwise whilst( hyperkeratosis) means thickening of the ,stratum corneum (Pangty *et al.* , 2010 )

Histologically the mass tumors were regarded as by fibroblastic proliferation by means of overlying (acanthosis hyperkeratosis) and (parakeratosis) cells .The tumor cells, displayed an infiltrative growth at the interface with regular tissue increasing the dermis and this is surrounding by moderate (epithelial hyperplasia layer (Marin and Travassos, 2011). The Epidermis layer are characterized by papillary projection with elongated rate of epidermis layer. Inflammatory cells were an be noted in this area.

Lancaster and Olson this term used to described by his type of papilloma in cattle with acanthosis , with keratohyaline granules and koilocytes, (Jelinek and Tachezy, 2005; Tomita *et al.*, 2007) but for the first time infection an endophytic type of papilloma ,has been designated and further study is needed as such lesions were observed in cattle (Pangty *et al.*,2010)

## **2-5-4 Detection of virus by PCR**

## **2- 6- Treatment**

The cattle warts are treatment is not commonly required and most the warts regress spontaneously. Surgical removal of the tumor is possible but may lead to recurrence of the lesions . formaldehyde may be used to disinfection with of stands, paling posts and other environmental, virus reservoirs can be prevent the transmission.

## **2-7-Prevention**

There is Vaccines against bovina papilloma virus (types 1- 2 and 4 )have been established by M. Saveria Campo and others.

Prophylactic vaccination (i.g, vaccination of cattle wart-free animals to prevent occur the infection) with whole virus (e.g. formalin killed papilloma tissue suspension),, virus-like particles (L1 L2)the L1 protein or (for BPV-4), L2 protein advises long-lasting protection against, challenge with the same BPV types , but is commonly ineffectual against existing cattle warts. Protection revels to be refereed via type-specific neutralizing antibodies. The vaccination of young calves as first as 4–6 weeks might be needed to prevent the infection. ( Brandt *et al.*, 2011)

Vaccination (i.g. vaccination of animals with existing warts) with BPV type-4 or BPV type-2 /L2 induces first progression of warts. Wart rejection involvement a cell-mediated, immune- response, with infiltration of the site by great numbers of macrophages and lymphocytes. These vaccine coordination have helped as models for the effective development of (prophylactic vaccines) against the human papillomavirusin all types associated with cervical and other cancers.

### **3-1-Clinical examination and Samples collections**

Clinical inspection of twenty four of animals (water buffaloes and cattle) that suffering from skin tumors in different part of the body (Neck, around the anal, near the eyes , shoulders and abdomen ) in different area of AL-Qadisiyah province .

papilloma biopsies that were collected from cow chronically affected with cutaneous papillomatosis. by incision tissue pieces about 2-5 cm<sup>3</sup> by surgical blade and sterile artery forceps and transported into sterile container with 10% formalin to the laboratory as soon as possible.

### **3-2-Histopath procedure**

Preparation of the samples to histopathological sectioning according to Al- Attar et al. (1982) and Noory (1989) methods as follows:

- Warts samples were fixed by using of formalin (10% ) for (48) hrs.
- Then Samples that were washed in distilled water for( three hrs).this steps come out to remove the formalin residue .
- After that the samples were pass in to a arranged series of increasing ethanol concentration (70%, 80%, 90%, 95% and 100)% for about (2 )hrs. for all concentration.
- Then the tissue samples that were clean by xylene for 3 times to( 1.5 hr).
- All samples were fixed by liquefied paraffin in (56 C<sup>o</sup>) for( 2 times).
- After embedded the samples were divided by rotary microtome (to 5  $\mu$  m thinness )and brought to water bath (50C<sup>o</sup>) and placed it on slides which decorated withmayor albumin.
- The new Blocks were deparaffinized by laying in oven (60 C<sup>o</sup>) for 5 hrs. then put them in xylene for (one hour).
- Then they were dried from xylene, washed with water and put the slides in harris -hematoxylene stain for 15 minutes and washed with flow water.

## **4-Result**

### **4-1-Clinical observation results:**

The clinical diagnosis were depend on clinical inspection of infected animals .Twenty four of animal that suspected having cutaneous papillomatosis ,cutaneous papilloma that distribution on different part of the body of infected animals with range (18-36) months of age .those animals Clinically examined , the temperature were normal ( $38.5^{\circ}\text{C}$ )

The site of lesion were distributed on both or one side and appear like cauliflower with horny hard papilloma or roof shapes with smooth exterior surface in size (1-8 cm)in diameter and variable in number from one to multiples warts .The common sites were forehead ,neck, shoulder ,back, ear ,around eyes ,abdomen ,legs and nose(fig. 1,2).

Clinical evaluation shown varied lesions, with tumors some occasionally ulcerated, circumscribed and others have rough morphology, with dark and gray in color, some warts adhered to the skin with presence of hair, and flat, another pedunculated form with cauliflower aspect and intermediate neoformations that assumed characteristics in the sessile and pedunculated lesions ( fig.3 ).



Figure (1) Multiple warts spread in both side and appear like cauliflower with horny hard papilloma or roof shapes with smooth surface in size (1-8 cm)



Figure (2) Lesion (papilloma) that were found near the eye .



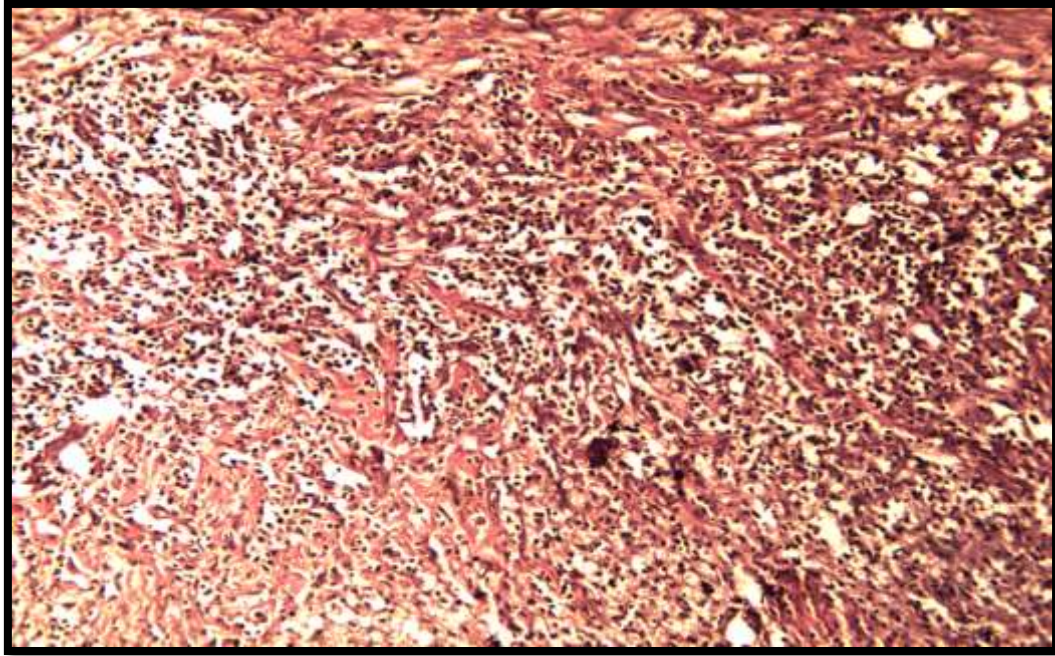
Figure (3) Tumors, circumscribed and others have rough morphology, some warts adhered to the skin with presence of hair, and flat, another pedunculated.

#### **4-2-Histopathological results:**

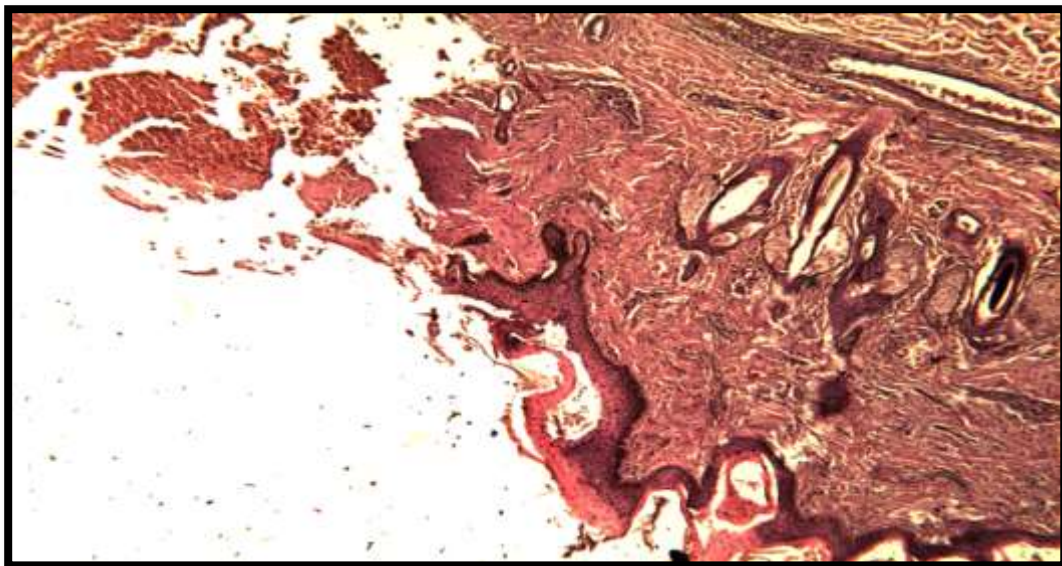
Histopathological changes of the affected skin (epidermis, dermis) layers showed, characteristic pathological changes as Severe infiltration of inflammatory cell, Presence of abundant collagen, marked, hyperkeratosis, thickening and hyperplasia of epidermis. (Figure, 6) Higher magnification, there is congestion of blood vessels with high infiltration of inflammatory cells mainly macrophages and proliferation of fibroblasts, also there is ballooning swelling, vacuolation of stratum spinosum layer with downward hyperplasia of stratum basal and infiltration of inflammatory cell in the dermis. (Figure 5, 7). Furthermore, there is marked ballooning degeneration of epidermal cell with eosinophilic intracytoplasmic inclusion bodies were also shown to appear. (Figure 5, 7). Also The Histopathological changes of epidermis layer, were similar to result in other studies that showed vacuolation and ballooning and swelling of the stratum layer and spinocytes layer with downward (hyperplasia) of stratum layer. (Figure, 8).



On conclusion the bovine papillomatosis (cattle warts )are economic disease spread among cross breed cattle in different field in AL-Qadisiyah provinces , cattle warts , have clinical and histopathological signs that used in diagnosis of disease.

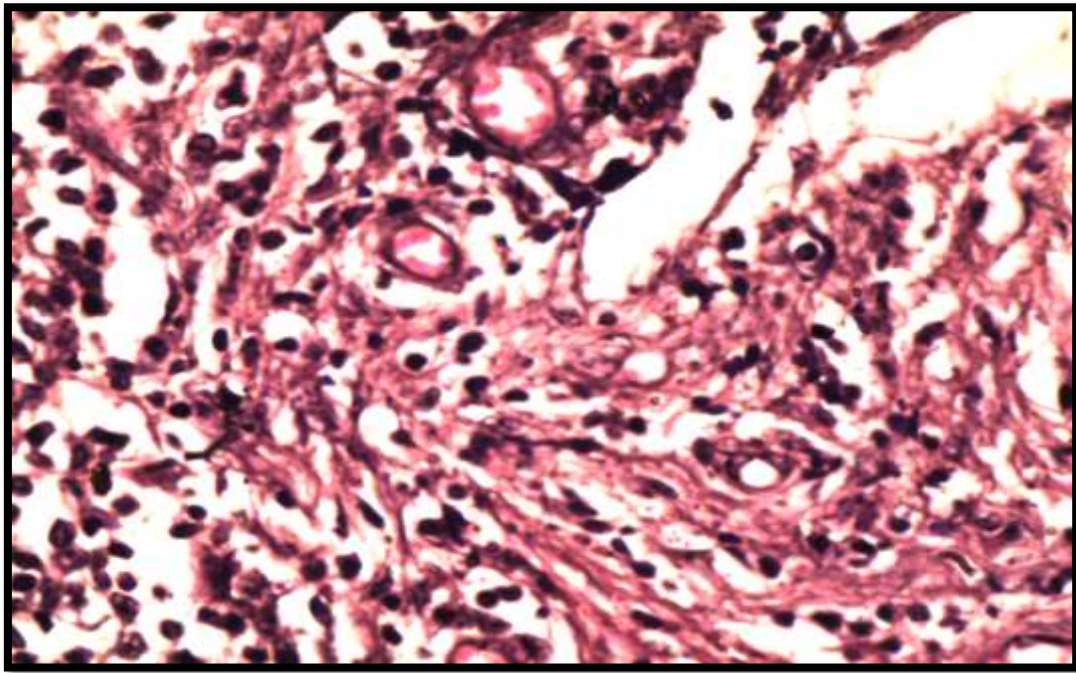


**Figure 5: Severe infiltration of inflammatory cell , along the dermis with Presence of abundant collagen**

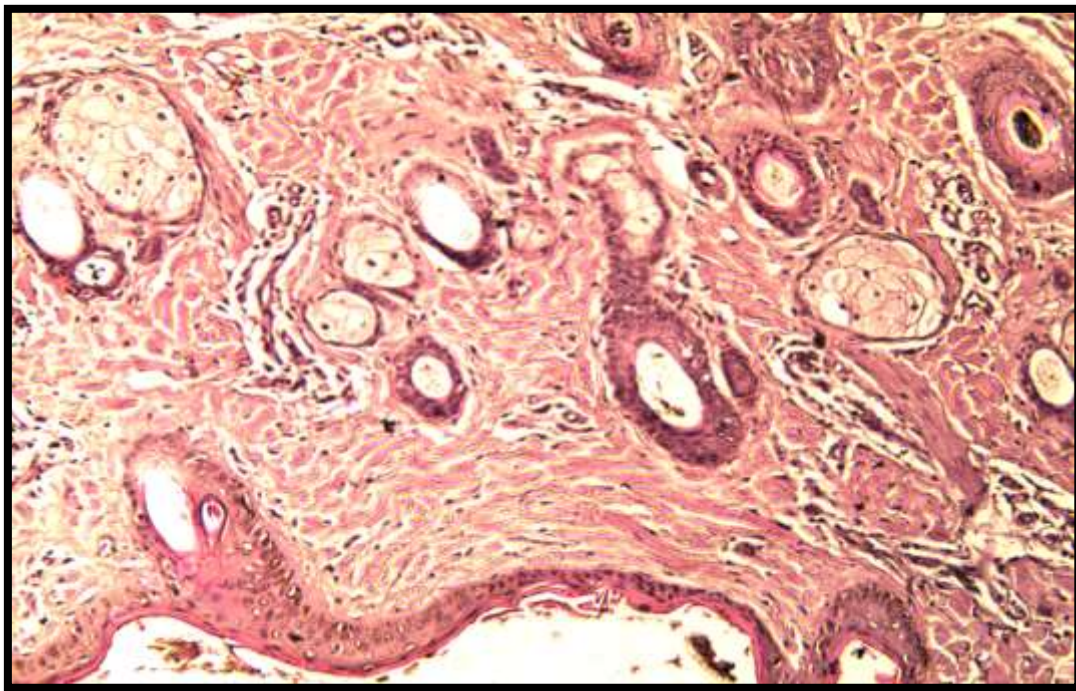


**Figure 6: Hyperkeratosis (thickening of keratinized layer ), thickening and hyperplasia of epidermis .**





**Figure7: Higher magnification , there is congestion of blood vessels with high infiltration of inflammatory cells mainly macrophages and proliferation of fibroblasts.**



**Figure, 8: Mild hyperplasia of epidermis with mild infiltration of inflammatory cells in the dermis**

## 5-discussion

The clinical diagnosis were depend on clinical inspection of infected animals . those animals Clinically examined , the temperature were normal (38.5 °C). The body condition of heavily infected cattle was poor. The appetite was normal .(Campo.,2003) .

The site of lesion were distributed on both or one side and appear like cauliflower with horny hard papilloma or roof shapes with smooth exterior surface in size (1-8 cm)in diameter and variable in number from one to multiples warts this result agreed with which deal with (Bravo *et al* .,2010). The common sites were forehead ,neck, shoulder ,back, ear ,around eyes ,abdomen ,legs and nose.This result agreed with (De Villiers *et al*.,2004) who indicated that the bovine papilloma differ in their tissue specify depending on genotyping of the virus.

Clinical evaluation shown varied lesions, with tumors some occasionally ulcerated, circumscribed and others have rough morphology, with dark and gray in color, some warts adhered to the skin with presence of hair, and flat, another pedunculated form with cauliflower aspect and intermediate neoformations that assumed characteristics in the sessile and pedunculated lesions .this result agreed with (Radostitis *et al*., 2007).

Histopathological changes of the affected skin (epidermis, dermis) layers showed, characteristic pathological changes as Severe infiltration of inflammatory cell , Presence of abundant collagen .marked, hyperkeratosis, , thickening and hyperplasia of epidermis .Higher magnification , there is congestion of blood vessels with high infiltration of inflammatory cells mainly macrophages and proliferation of fibroblasts, also there is ballooning swelling , vacuolation of stratum spinosum layer with downward hyperplasia of stratum basal and infiltration of inflammatory cell in the dermis. were in agreement with (Nenad *et al*,2005).Furthermore, there is marked ballooning degeneration of epidermal cell with eosinophilic intracytoplasmic inclusion

bodies were also show appeare. this finding same with(Giuseppe, *et al*, 2003)Also The Histopathological changes of epidermis layer, were similar to result in other studies that showed vaculotionand ballooning and swelling of the stratum layer and spinocytes layer with downward ( hyperplasis) of stratum layer. they were agreement with (Claudia, *et a.l* 2013).

On conclusion the bovine papillomatosis (cattle warts )are economic disease spread among cross breed cattle in different field in AL-Qadisiyah provinces , cattle warts , have clinical and histopathological signs that used in diagnosis of disease.

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## **Conclusion**

- 1- Bovine papillomatosis is seriously recorded in cross breed in Al-Qadisiyah province. And the local breed Holstein and cross breed ,Holstein Friesian of Iraqi cattle are the main class which were affected by BPV.
- 2- Histopathological examination revealed that the typical lesion of bovine papilloma virus infection in which include papilloma ,and showing epidermal and dermal inderditation.
- 3- The fibropapilloma and papilloma can be diagnosis depened on the clinical signs because there is no other disease that confused with typical clinical singes.

## **Recommendation**

- 1- Conducting molecular methods to confirmation the clinical and histopathological examination of BPV .
- 2- Establishing continuous cell line to culture from transformed cells required for further studies .
- 3- Conducting the sequencing and analysis of result to detecting the all genotyping that may be present in Al-Qadisiyah province.

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## الخلاصة

الورم الحلمي البقري من الامراض الواسعة الانتشار وله علاقة بالآفات الجلدية الحميدة او الخبيثة(الثآليل).مسبب المرض الفايروس البقري الحلمي .يصيب الثآليل بصورة رئيسية السلالات الخليطة المضربة بين الهوشتان والغريزيان في محافظة القادسية .في هذه الدراسة اربعة وعشرون بقرة تم فحصها حيث انها كانت متوقع اصابتها بالآفات الجلدية في مناطق مختلفة من محافظة القادسية من ايلول ولغاية تشرين الثاني 2017.اربعة عينات تم اخذها للفحص النسيجي وبأعمار تتراوح بي (16-28 شهر).اظهرت الفحص السريري ان الآفات منتشرة في مناطق مختلفة من الجسم (الراس ،الرقبة ،منطقة البطن ،الافخاذ وحول المستقيم).الآفات كانت تشبه القرنيبيط ولها شكل خشن ومتقرن وذات حجم يتراوح بين (1-8 سم) وبأعداد مختلفة .اظهرت نتائج الفحص النسيجي وجود بروتات حلمية متليفة في طبقات الجلد والبشرة . نستنتج ان الورم الحلمي البقري هو منتشر بين مجاميع سلالات الماشية وتسبب خسارة اقتصادية في صناعة الماشية في محافظة القادسية.