

## Phylogenetic tree analysis study of bovine papillomaviruses type 1 based on L1 gene in Al-Qadisiyah governorate, Iraq

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### Abstract

Bovine fibropapilloma and papilloma occur in different parts of the skin of animals. Bovine Papillomavirus (BPV) is an oncogenic virus making benign tumor lesion of together mucosal and cutaneous tissue in cattle. In order to confirm the clinical diagnosis; the study planned to make the molecular detection of BPV (DNA) using Polymerase Chain Reaction (PCR) from skin lesions and the phylogenetic analysis. Thirty-eight samples of skin lesions were collected from cattle clinically suspected to be infected with bovine papilloma virus from herds in Al-Qadisiyah Governorate in 2016, the primary clinical diagnosis depended on the morphological appearance and features of the lesion. Deoxyribonucleic Acid (DNA) was extracted from skin lesions; the DNA was examined by PCR technique using specific primer to BPV-1 /L gene-1. Twenty-two samples out of 38 (57,9%), which were collected from different regions in Al-Qadisiyah Governorate, were positive. The sequences of four positive samples of DNA product amplification of (BPV) type-1, L1 gene confirmed the PCR results. These samples had the DNA presented in four accession numbers KY662042-1, KY662043-1, KY662040-1 and KY662041-1. This study proofed that cutaneous bovine papillomatosis related with BPV-1 infection in the cattle herds has affinity to solid skin rather than other epithelial and mucosal tissue.

**Keywords:** Papilloma, Cattle, PCR, DNA Sequence  
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### دراسة تحليلية للشجرة النسيجية لفيروسات البابيلوما النوع الأول استناداً إلى L1 Gene في محافظة القادسية، العراق

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### الخلاصة

يحدث الورم الليفي الحليمي في أجزاء مختلفة من الجلد من الحيوانات، إن فيروس الثألول هو فيروس مسرطن يصنع آفة ورمية حميدة من الغشاء المخاطي والأنسجة الجلدية في الأبقار. تم إعداد هذه الدراسة للكشف الجزيئي للفايروس باستخدام تفاعل البلمرة المتسلسل من الآفات الجلدية المأخوذة من قطعان الأبقار في محافظة القادسية عام ٢٠١٦. وتم تحليل التسلسل في هذه الدراسة حيث جمعت ثمان وثلاثين عينة من الآفات الجلدية في الأبقار التي يشتبه سريريًا بأنها مصابة بفيروس الورم الحليمي البقري، وقد اعتمد التشخيص السريري الأساسي على المظهر الشكلي وملامح هذه الآفة. تم استخراج الحمض النووي الرايبوزي من الآفات الجلدية، وتم فحص الحمض النووي عن طريق تقنية تفاعل السلسلة المتبلورة باستخدام بادئات محدد لجين BPV-1/L. كانت هناك اثنتان وعشرون عينة موجبة للفحص من أصل ٣٨ عينة (بنسبة إصابة ٥٧,٨٩%) جمعت من مناطق مختلفة في محافظة القادسية. وقد أظهر تسلسل أربع عينات إيجابية من تضخيم حمض DNA للجين BPV type-1، L1 لتأكيد نتائج PCR. كانت هذه العينات تحمل الحمض النووي في أربعة أرقام مسجلة KY662042-1 و KY662043-1 و KY662040-1 و KY662041-1. وقد أثبتت هذه الدراسة أن الورم الحليمي الجلدي البقري المرتبط بالعدوى BPV-1 في قطعان الماشية له صلة بالجلد فقط بدلاً من الأنسجة الظهارية والأنسجة المخاطية الأخرى.

## **Introduction**

There are several skin diseases of cattle, most common among such skin infections are dermatophilosis, lumpy skin disease and cutaneous papillomatosis known as warts (1). Bovine papillomaviruses (BPVs) are a heterogeneous group of viruses responsible for tumors of the skin, genital, paragenital area, eye, upper gastrointestinal tract and urinary bladder (2). BPVs, like all other papillomaviruses (PVs), are usually strictly species specific. However, cases of cross-species infection are known to occur. BPV type 1 (BPV-1) and BPV-2, belonging to the genus Delta papilloma virus (3), are responsible for infections in equines resulting in sarcoids (4,5), as well as in bison and water buffaloes leading to warts (6,7). Bovine papillomavirus (BPV) is an etiological agent associated with several forms of cutaneous and mucosal papilloma (8). Six different types of BPVs have been distinguished on the basis of DNA sequence relatedness, each BPV is associated with type-specific lesions (9). BPV-1 and BPV-2 are classified in the genus Delta papilloma virus and infect the epithelium and dermis, giving rise to fibropapilloma; while BPV-3, BPV-4 and BPV-6 are classified in the genus Xipapilloma virus and are strictly epitheliotropic, inducing true epithelial papillomas, and BPV-5 is classified in the genus Epsilon papillomavirus and infects the epithelium and dermis, inducing both fibropapillomas and true epithelial papilloma of the skin (10). The important types BPV1 and BPV2 are unusual in their capability to infect together the epithelial cells tissue and the fibroblasts of the basic dermis and epidermis, producing fibropapilloma of the different site of the body teats, skin and udders, in addition to urinary bladder tumor in the cow (11). Although the detection of bovine papilloma virus type-1, clinical and epidemiological studies have been providing a new vision for the therapeutic and prevention measures of papillomatosis infections (12). There are several molecular studies about the occurrence of BPV in Iraq; the objective of this study was to identify the BPV types in cattle population that associated with papillomatosis in several regions.

## **Materials and methods**

### **Sample collection**

Thirty eight skin samples (warts) were collected from animals that were suffering from disruption of skin lesion on different areas from the body included shoulder, neck, around eyes, chin, perianal region, and clinically suspected to be infected with cutaneous bovine papillomatosis. The samples were collected by cutting the whole mass by forceps and surgical blade; then they were put in container and sent to the laboratory in cool box as soon as the molecular analysis were done.

### **Viral DNA extraction**

Viral genomic DNA was extracted from frozen skin lesions (warts) samples using USA, Geneaid, DNA Mini Kit. The genomic viral DNA extraction was done based to company directions using tissue extraction procedure technique with proteinase K. The extracted DNA was tested using Nanodrop spectrophotometer that check DNA concentration and DNA purity through construing the absorbance at 160/210 nm.

### **PCR amplification**

PCR was done for recognition and genotyping of bovine papillomavirus; it was performed using definite primer for *L-major capsid protein gene*. The primers were designed according to (13).

Forward 5'-(CGGGGCCAAAACCTGTTTCCTA)-3'

Reverse 3'-(AATTCAAGAGAGGGCAGGC)-5'

The master mix tube of PCR was set using kit of PCR PreMix AccuPower®, Korea Bioneer. The premix tube of PCR which contains pellet freeze-dried of Taq polymerase 1 U, 10 mM pH 9.0 of Tris-HCl dNTPs 250 µM, MgCl<sub>2</sub> 1.5 mM KCl 30 mM, tracking dye and stabilizer.

The master mix of PCR reaction was set allowing to kit directions in 50 µl total volume by add 3µl of 10 pmole of reverse primer, 3 µl of 10 pmole of forward primer 10 µl of purified genomic DNA and, then complete the premix tube of PCR by deionized distilled water PCR water into 30 µl and fleetingly mixed by vortex centrifuge.

The reaction of PCR was done by setting up a thermocycler following conditions; the temperature of initial denaturation 95 °C for 5 min; the cycles 30 of denaturation in 95 °C for 30 s, annealing 58 °C for 30 s, and extension 72 °C for 30 s and final extension at 72 °C for 5 min. The PCR products 399 bp were tested by electrophoresis in an agarose gel 1.5% that stained with ethidium bromide, and pictured beneath UV illuminator.

### **DNA sequencer**

The partial DNA sequencing for phylogenetic tree analysis of bovine papilloma was done based on capsid protein L1 gene. The PCR product of positive samples were sent to Korea (Bioneer Company) for complete DNA sequencing depend on gpB forward primer by sequencing system (AB-DNA).

The partial Sequence of four positive samples for bovine papilloma virus was submitted at the GenBank database under the accession numbers KY662040.1, KY662041.1, KY662042.1 and KY662043.1. The analysis of Phylogenetic tree was performed according to (UPGMA tree) with Arithmetic Mean in (MEGA 6.0 version).

## Result

### Clinically

The BPV-1 has affinity for thick skin, therefore the lesion appears commonly with solids shaped, and the lesion looks most commonly on the skin rather than other mucosa or organs.

### PCR Amplification

The results of endpoint PCR for detection of bovine papilloma virus in ethidium bromide-stained agarose gel using specific primers for detection L1 gene at 399 bp had twenty-two positive tissue skin samples out of thirty-eight which were collected from different regions in Al-Qadisiyah Governorate as presented in figure 1.

The PCR assay came out to confirm clinical diagnosis of the disease, the analysis of PCR results showed that the bovine papillomatosis found in Al-Qadisiyah were induced by bovine papilloma virus type 1 (BPV-1).

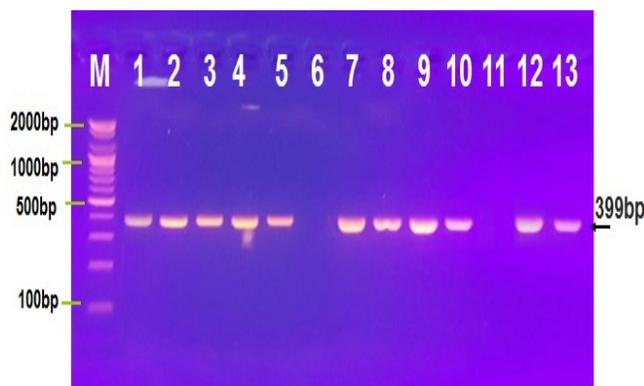


Figure 1: The results of endpoint of PCR products of BPV type-1, L1 gene in cattle warts. Lane 1, 2, 3, 4, 5, 7, 8, 9, 10, 12 and 13 were positive results. Lane 6 and 11 were negative results.

### Sequencing of local Iraqi isolate of bovine papillomavirus

The sequences of DNA amplification product of BPV type-1, L1 gene has come out to confirm PCR result mentioned above, the bovine papillomatosis caused by bovine papillomavirus type -1 in a crossbreed of the cattle population in a different area of Al-Qadisiyah Governorate. The sequence of four local Iraqi isolates were submitted by gene bank under number KY662042-1, KY662043-1, KY662040-1 and KY662041-1.

### Phylogenetic tree analysis

The phylogenetic tree was directed using the partial genome sequence of local Iraqi isolates between them. The tree showed that IQ-isolate KY662042-1, KY662043-1 and

KY662040-1 were arranged in the same node in the tree, but the KY662041-1 was located in a close branch of tree as presented in figure 2. The result of alignment showed a high identity 100% in sequence of Iraqi isolates KY662042-1, KY662043-1 and KY662040-1 where as small variety with KY662041-1 99% as showed in figure 3.

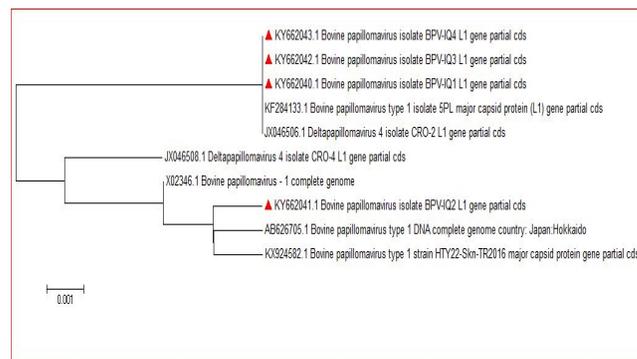


Figure 2: Phylogenetic tree of bovine papilloma virus type-1 of Iraqi isolates (partial genome) with other world reference using a neighbour joining method (Mega: 6 program).

According to sequences alignment and present identity, the Iraqi isolate KY 622041.1 indicate high similarity 100% to the sequences obtained from the Croatian isolate JX046508.1, Turkey isolate KX924582.1, and Japan isolate AB62605.1 as offered in figure 3. Analysis of the tree revealed that these isolates were clustered together in the identical node and in the same branches with the Iraqi isolate KY662041.1. The results of the tree analysis showed that the other three Iraqi isolate KY662042-1, KY662043-1 and KY662040-1 are clustered together with Poland isolate KF284133.1 and Croatia isolate JX046506.1.

## Discussion

Papillomatosis in cattle is associated with BPV type 1 and type 2 (14,15). The present study showed molecular detection of BPV type-1 in thirty-eight cutaneous samples of cattle papillomatosis in Al-Qadisiyah Governorates using specific primers, these results are similar to those presented by (14-17). The specific primers were used to identify genotype and characterize BPV type 1, and this finding was confirmed by partial sequencing and studying the phylogenetic tree analysis, so it could be the most suitable assay for characterizing BPV as discovered by (12,18).

There are several studies about BPV in many Iraqi Governorates (13,19,20) which have been led to determine the incidence of bovine papillomatosis infection in cattle, they had prevailed the types of the BPV, their geographic area distributions, methods of treatment. This study with a



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