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Molecular genotyping, histopathological and immunohistochemical studies of bovine papillomatosis

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Abstract

Background: Bovine papillomatosis (BP) is considered the most common health problem in large cattle farms.

Aim: This study attempts to confirm clinically suspected BP in cattle by polymerase chain reaction (PCR) assay, histopathology, immunohistochemistry (IHC), and genotyping analysis of local isolates.

Methods: According to morphological appearance and lesion features, a cross sectional study of 54 clinically diagnosed BP cattle was assigned to this current investigation from May to August (2021) in Al-Kut district (Wasit Province, Iraq) private veterinary clinics using purposive sampling technique based on set criteria. The cattle were diagnosed clinically, and the tissues were collected and some fixed in 10% neutral buffered formalin and other stored frozen and examined by histopathological technique, IHC, and PCR assays.

Results: Using PCR assay, all cattle were positive for the BPV *L1* gene. According to detect the *L1* gene, analysis of the phylogenetic tree showed that local BPV cattle isolates were closely related to the NCBI-BLAST BPV type-1 and type-2 of the Polish equine isolate (KF284133.1) and BPV Brazilian *Bostaurus* isolate (MH187961.1), respectively. Histological detection showed there were acanthosis, hyperkeratosis, epidermal thickening, severe infiltration of mononuclear cells, massive hemorrhage, dermal fibroplasias, multifocal spongiosis, moderate neovascularization, moderate to severe elongation of the retention ridge towards the dermis, parakeratosis, rings of calcification, and necrosis with nuclear pyknosis of some spinosum cells. Immunohistochemical findings of tumor necrosis factor-alpha, epidermal growth factor receptor and Fascin showed a significant variation in values of immunoreaction in the dermis and epidermis. These results ranged from negative (0) to mild positive (+1) to moderate positive (+2) reactions.

Conclusion: The study provided essential molecular and genotyping data to improve our knowledge by emphasizing the crucial of IHC as an elegant diagnostic method to detect cellular alterations.

Keywords: Cattle warts, Iraq, Papillomavirus, PCR, Sequence.

Introduction

Bovine papillomatosis (BP) is a common skin-specific disease of cattle that is promoted by a paraphyletic group of circular double-stranded DNA viruses, bovine papillomaviruses (BPV). The latter belongs to the Papillomaviridae family which represents the oldest and widest family of viruses (Abouelkhair and Kennedy, 2022). There are several distinct BPVs in cattle that are classified based on their site and type of lesion including BPV-1 (Mathewos *et al*., 2021), BPV-2 (Mathewos *et al*., 2021), BPV-3 (Pfister *et al*., 1979), BPV-4 (Campo *et al*., 1980), BPV-5 (Campo *et al*., 1981), BPV6 (Jarrett *et al*., 1984) and BPV-7 (Ogawa *et al*., 2007).

The information of transmission method of BP between animals is limited due to unclear transmission mechanism (Pang *et al*., 2019). However, the animal populations in restricted spaces are more vulnerable and susceptible to infection due to the direct and indirect virus-spreading behavior (Ugochukwu *et al*., 2019). The possible mechanisms of transmission are vertical spreading, arthropod vectors, and direct skin contact (Roperto *et al*., 2019; Ata *et al*., 2021). The method of BPV spreads through the blood is attracting attention to extensively study the possible methods of transmission which can occur via non-epithelium tissues (Savini *et al*., 2019) and fluids (Meng *et al*., 2021). In adults, BPVs are unable to penetrate the host skin; thus, minimum abrasions in the skin are required to initiate the infection. Exposure of skin lesions to BPV leads to the initiation of infection followed by transformation and proliferation of infected epithelium's basal cells, which develop into benign papilloma or fibropapilloma (Mathewos *et al*., 2021). In the field, there are different types of papillomatosis. Cutaneous papilloma is the most prevalent type among cattle and displays distinct

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morphologies, such as the atypical filiform and typical pedunculate (cauliflower) forms that present the verrucous aspect (Constable *et al*., 2017; Daudt *et al*., 2018).

Although the clinical diagnosis of BP is usually performed when alterations are well characterized in the epidermis, histology and immunohistochemistry (IHC) for stained papilloma tissues can allow an identification of epidermal pathogenic alterations and reveal viral proteins, respectively (Russo *et al*., 2020; Hassanien *et al*., 2021). Molecular techniques, in particular polymerase chain reaction (PCR), are important and essential diagnostic tools that are usually applied to confirm infection through the detection of specific DNA of BPV (Emin *et al*., 2022). Due to the validity and accuracy of PCR test, applying this method can lead to exploring valuable insights into the characteristics of genomic data (Kiselev *et al*., 2020; Kubacki *et al*., 2021).

Worldwide, the disease is undoubtedly one of the most widely studied due to its significant effects on the veterinary sector. In Iraq, available data remain limited and need to be elucidated because the prevalence of disease has still developed greatly in the last 10 years (Hamad *et al*., 2017; Mansour *et al*., 2019; Al-Salihi *et al*., 2020). Therefore, the current study aimed to confirm clinically suspected BP cattle by using PCR assays, histology, and IHC, with genotyping of local isolates to be documented in the National Center for Biotechnology Information (NCBI).

Materials and Methods

Study animals, design, and topography

A gross sectional study was performed using 54 cattle arrived at the private veterinary clinics in Al-Kut district (Wasit province, Iraq) from May to August (2021). The topography of the current study is including all regions around Al-Kut district in addition to Al-Kut center. The animals were diagnosed clinically to be infected with papillomatosis based on morphological appearance and features of lesions. The set criteria that allowed only the cattle with obvious lesions and clinically diagnosed to employ in this study using purposive sampling technique.

Sample collection

After injection of lidocaine 5% (Cat No.# N01BB02, ADVANZ, UK) around each lesion, surgical removal of papillomas using a scalpel was performed under aseptic conditions. The collected samples of each animal were divided into two parts; one was kept into a plastic container containing 10% neutral buffered formalin for histology/IHC examination, while the second part was kept into a plastic tube and kept frozen for molecular examination.

Molecular genotyping

According to protocol (B) of G-spin™, DNAs were extracted from tissue samples using the total DNA Extraction Kit (Cat.No.#IBT-QMS-GT1704, Intron Biotechnology, South Korea). The DNA purity and concentration were measured using a Nanodrop system (Thermo-Scientific, UK). Targeting the conserved region of BPV *L1* gene (nt 7250 to 3225) with GenBank access number (KF284133.1), one set of primers was designed to amplify the region [F (5′-CAGTGTCTATCGGGGCCAAA-3′) and R (5′-AATTCAAGAGGAGGGCAAGGC-3′)] with 53.8°C annealing temperature, manufactured by Scientific Researcher Co. (Iraq). The PCR Premix Kit (Cat No.# 162770, Bioneer, South Korea) was used to prepare the mastermix with forwards and reverse gene sat a 20 µl final volume. PCR reaction was performed in thermocycler (Bio-Rad, USA) under the following conditions of: 1 cycle predenaturation (95°C/5 minutes), 35 cycles of denaturation (95°C/30 seconds), annealing (58°C/30 seconds) and extension (72°C/1 minute), and 1 cycle final extension (72°C/5 minutes). The electrophoresis of agarose-gel (1.5%) stained with Ethidium bromide was applied for the resulting PCR products, and then examined under a UV-transilluminator (Wised, South Korea). The samples were considered positive at 409 bp.

For genotyping, six positive PCR products were sent to the Macrogen Company (Seoul, South Korea) to be sequenced by the modified Sanger method. Fasta data of DNA sequences were subjected to MEGA software and the UPGMA program for phylogenetic tree analysis and multiple sequence alignment analysis. Finally, all the analyzed local isolates were named and documented in NCBI GenBank to obtain specific access numbers.

Histology

Following formalin fixation, tissues were exposed to ascending grades of ethanol for dehydration, followed by xylene for clearing, and exposure to paraffin for infiltration, embedding, and blocking. The block was sectioned using the Ultra-Thin Semiautomatic Rotary microtome (MRS3500, Histo-line, Italy) at a thickness of 4–5 µm and mounted on a slide. All prepared slides were stained with the hematoxylin and eosin (Cat. No.# ab245880, Abcam, India), and examined by a trinocular light microscope (MEIJI, Japan) at X10 and X40.

IHC

Envision FLEX IHC kits (Cat. No.# 126522-001, Dako, Denmark) were used to detect tumor necrosis factor-alpha (TNF-α), epidermal growth factor receptor (EGFR), and Fascin. Following the manufacturers' instructions, paraffin-embedded tissues were mounted on positively charged glass, deparaffinized by xylene, rinsed with distilled water and TBS, incubated with antigen retrieval solution at 60°C and then incubated in a water bath at 97°C for 25 minutes. The tissues were flooded with peroxidase block solution for 10 minutes and then with the anti- (TNF-α, EGFR and FASCIN) primary and secondary antibodies, followed by incubation with freshly prepared chromogen for 10 minutes. Then, the tissues were exposed to a counter stain (Mayer's hematoxylin) for 3 minutes.

After dehydration with three ascending ethanol concentrations, the slides were immersed in xylene, mounted with DPX, covered with cover slips and examined under a light microscope at ×10 and ×40. The results of IHC were classified based on their reactions as negative (−), mild positive (+1), moderate positive (+2), and strong positive (+3).

Statistical analysis

The obtained data was documented and managed using excel sheet (Microsoft excel, 2016). One-way analysis of variance was applied to analyze the IHC data by using GraphPad Prism (version 6.0.1) Software (GraphPad Software Inc., USA). The differences at $p < 0.05$ were considered significant among their values.

Ethical approval

The current study was approved by the Scientific Committees of both Colleges of Veterinary Medicine and Dentistry at the University of Wasit (Wasit Province, Iraq) as the work is under their guidelines.

Results

PCR identification and phylogenic tree recording

Molecular assessment by conventional PCR of all clinically suspected BP lesions (Fig. 1) revealed that all the studied cattle ($n = 54$) were positive for the BPV *L1* gene (Fig. 2). Analysis of the phylogenetic tree according to derive the *L1* gene alignments identified that the local BPV cattle isolates; Deltapapillomavirus 4 isolate Cattle-No. 1, Deltapapillomavirus 4 isolate Cattle-No. 3 and Deltapapillomavirus 4 isolate Cattle-No. 4 were closely related with NCBI-BLAST BPV type-1 (KF284133.1). Local BPV cattle isolates *Bostaurus* papillomavirus 2 isolate Cattle-No. 2, *Bostaurus* papillomavirus 2 isolate Cattle-No. 5, and *Bostaurus* papillomavirus 2 isolate Cattle-No. 6 were closely related with NCBI-BLAST BPV type-2 (MH187961.1) (Illustrations 1–6). NCBI-BLAST homology sequence analysis recorded a highly identity of the local Deltapapillomavirus 4 isolate Cattle-No. 1, No. 3 and No. 4 with the GenBank-NCBI BPV Polish equine isolate (KF284133.1) with 99.22%, 100% and 99.47% identity, respectively. However, the local *Bostaurus* papillomavirus 2 isolate Cattle-No. 2, No. 5 and No. 6 were more identical to the GenBank-NCBI BPV Brazilian *Bostaurus* isolate (MH187961.1) with 100%, 99.55% and 99.66% identity, respectively (Table 1, Fig. 3).

Fig. 1. Left lateral view of studied cattle clinically diagnosed with BP.

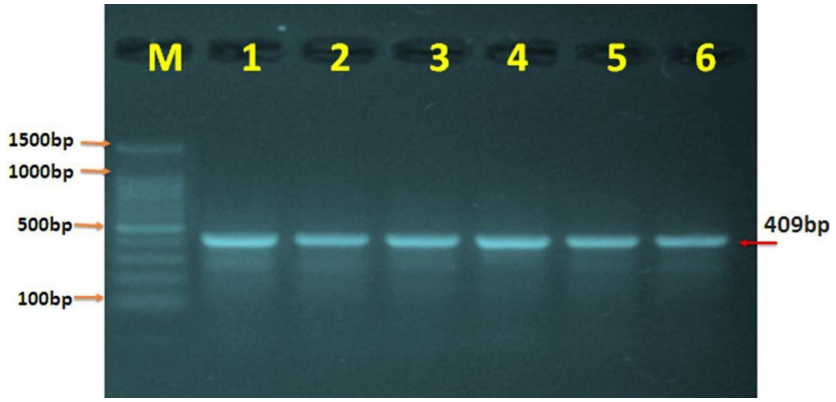
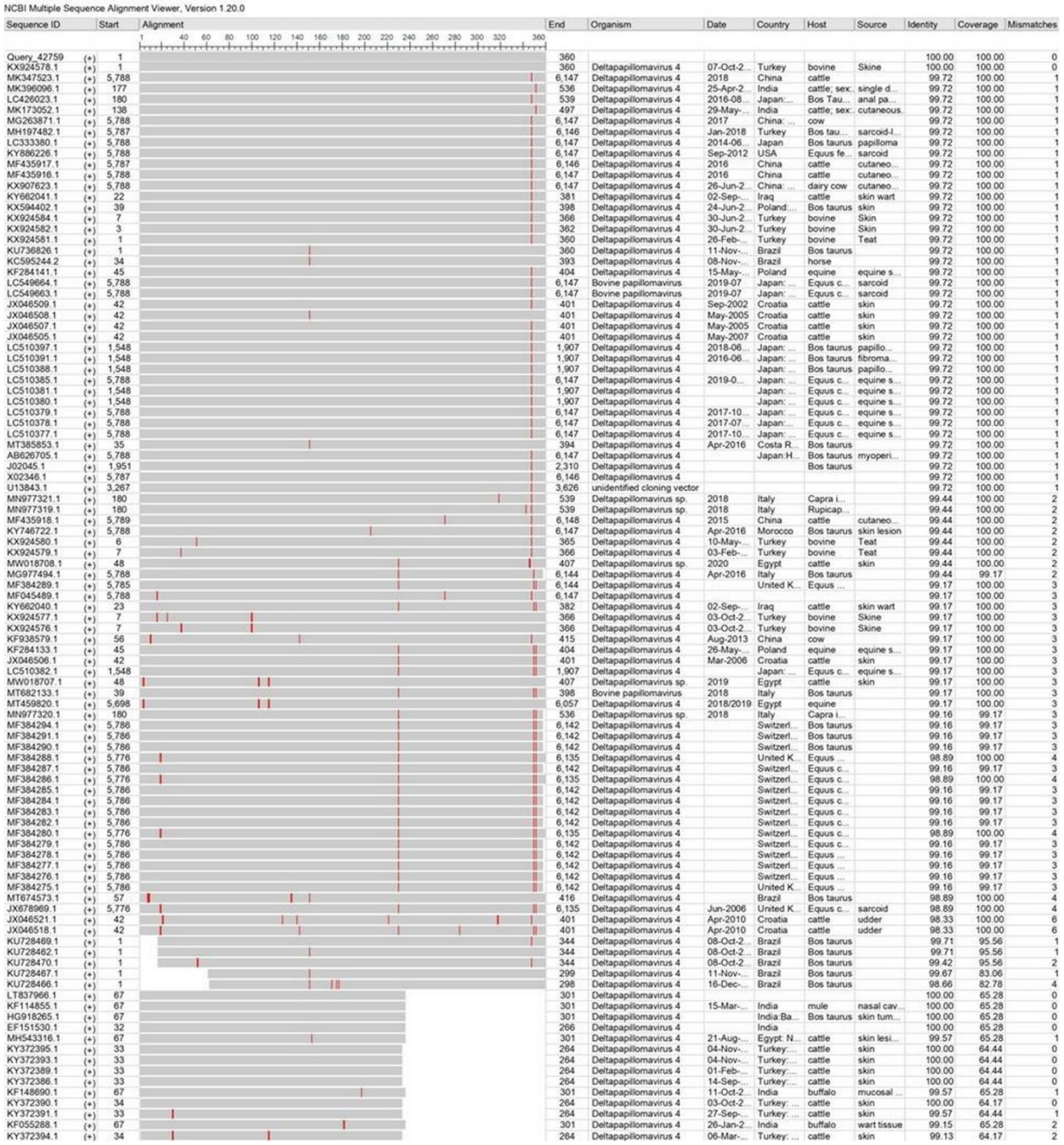


Fig. 2. PCR expression of the BPV L1 gene in agarose gel electrophoresis. M: ladder marker (1,500–100 bp); lanes 1–6: representative positive PCR samples at 409 bp.

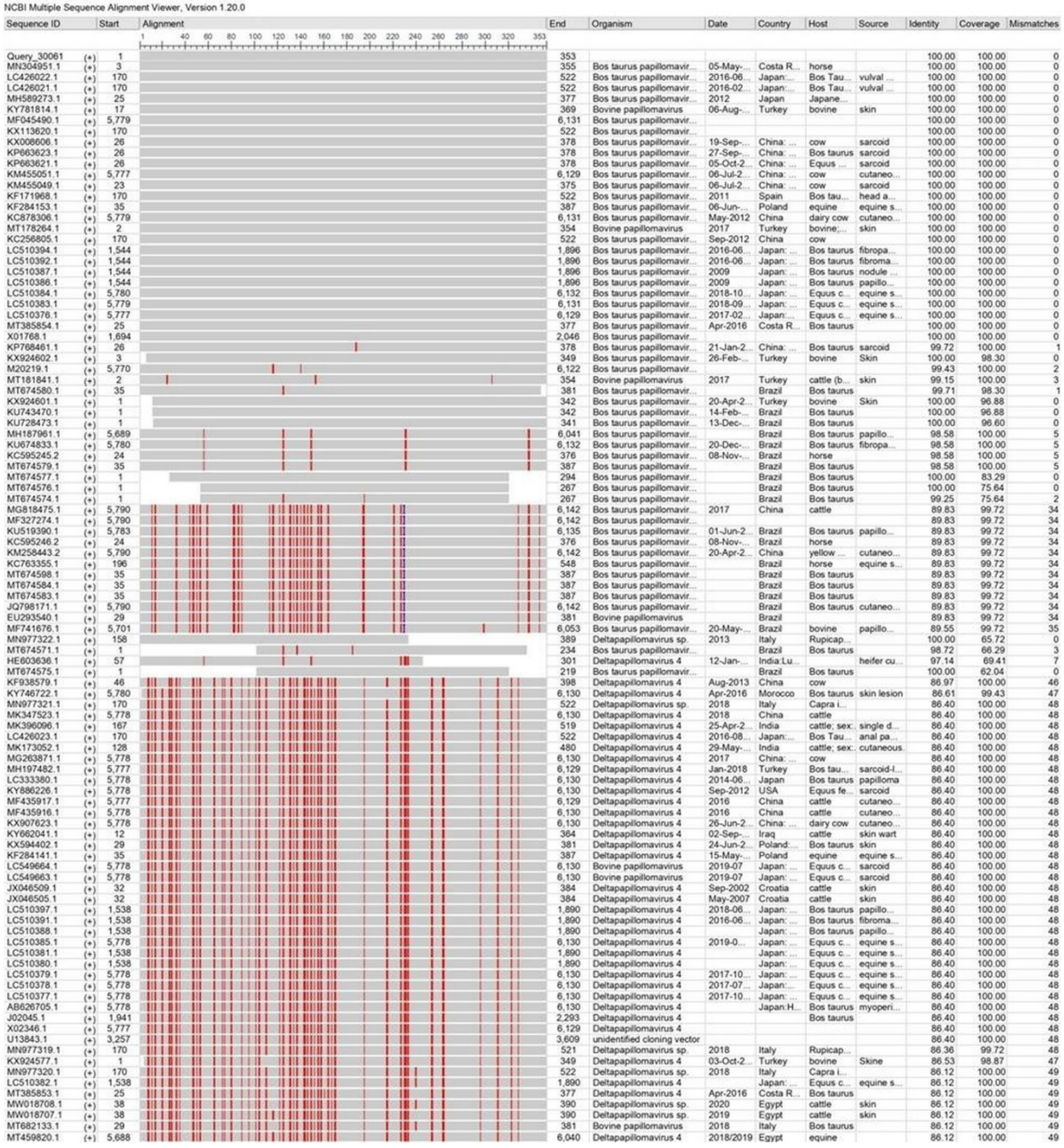

NCBI Multiple Sequence Alignment Viewer, Version 1.20.0

Sequence ID		Start	Alignment	End	Organism	Date	Country	Host	Source	Identity	Coverage	Mismatches
Query_42759	(+)	1		360						100.00	100.00	0
KX924578.1	(+)	1		360	Deltapapillomavirus 4	07-Oct-2...	Turkey	bovine	Skine	100.00	100.00	0
MK347523.1	(+)	5,788		6,147	Deltapapillomavirus 4	2018	China	cattle		99.72	100.00	1
MK396096.1	(+)	177		536	Deltapapillomavirus 4	25-Apr-2...	India	cattle; sex...	single d...	99.72	100.00	1
LC426023.1	(+)	180		539	Deltapapillomavirus 4	2016-08...	Japan:...	Bos Tau...	anal pa...	99.72	100.00	1
MK173052.1	(+)	138		497	Deltapapillomavirus 4	29-May-...	India	cattle; sex...	cutaneous	99.72	100.00	1
MG263871.1	(+)	5,788		6,147	Deltapapillomavirus 4	2017	China: ...	cow		99.72	100.00	1
MH197482.1	(+)	5,787		6,146	Deltapapillomavirus 4	Jan-2018	Turkey	Bos tau...	sarcoid-l...	99.72	100.00	1
LC333380.1	(+)	5,788		6,147	Deltapapillomavirus 4	2014-06...	Japan	Bos taurus	papilloma	99.72	100.00	1
KY886226.1	(+)	5,788		6,147	Deltapapillomavirus 4	Sep-2012	USA	Equus fe...	sarcoid	99.72	100.00	1
MF435917.1	(+)	5,787		6,146	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	99.72	100.00	1
MF435916.1	(+)	5,788		6,147	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	99.72	100.00	1
KX907623.1	(+)	5,788		6,147	Deltapapillomavirus 4	26-Jun-2...	China: ...	dairy cow	cutaneo...	99.72	100.00	1
KY662041.1	(+)	22		381	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	99.72	100.00	1
KX594402.1	(+)	39		398	Deltapapillomavirus 4	24-Jun-2...	Poland:...	Bos taurus	skin	99.72	100.00	1
KX924584.1	(+)	7		366	Deltapapillomavirus 4	30-Jun-2...	Turkey	bovine	Skin	99.72	100.00	1
KX924582.1	(+)	3		362	Deltapapillomavirus 4	30-Jun-2...	Turkey	bovine	Skin	99.72	100.00	1
KX924581.1	(+)	1		360	Deltapapillomavirus 4	26-Feb-...	Turkey	bovine	Teat	99.72	100.00	1
KU736826.1	(+)	1		360	Deltapapillomavirus 4	11-Nov-...	Brazil	Bos taurus		99.72	100.00	1
KC595244.2	(+)	34		393	Deltapapillomavirus 4	08-Nov-...	Brazil	horse		99.72	100.00	1
KF284141.1	(+)	45		404	Deltapapillomavirus 4	15-May-...	Poland	equine	equine s...	99.72	100.00	1
LC549664.1	(+)	5,788		6,147	Bovine papillomavirus	2019-07	Japan: ...	Equus c...	sarcoid	99.72	100.00	1
LC549663.1	(+)	5,788		6,147	Bovine papillomavirus	2019-07	Japan: ...	Equus c...	sarcoid	99.72	100.00	1
JX046509.1	(+)	42		401	Deltapapillomavirus 4	Sep-2002	Croatia	cattle	skin	99.72	100.00	1
JX046508.1	(+)	42		401	Deltapapillomavirus 4	May-2005	Croatia	cattle	skin	99.72	100.00	1
JX046507.1	(+)	42		401	Deltapapillomavirus 4	May-2005	Croatia	cattle	skin	99.72	100.00	1
JX046505.1	(+)	42		401	Deltapapillomavirus 4	May-2007	Croatia	cattle	skin	99.72	100.00	1
LC510397.1	(+)	1,548		1,907	Deltapapillomavirus 4	2018-06...	Japan: ...	Bos taurus	papillo...	99.72	100.00	1
LC510391.1	(+)	1,548		1,907	Deltapapillomavirus 4	2016-06...	Japan: ...	Bos taurus	fibroma...	99.72	100.00	1
LC510388.1	(+)	1,548		1,907	Deltapapillomavirus 4		Japan: ...	Bos taurus	papillo...	99.72	100.00	1
LC510385.1	(+)	5,788		6,147	Deltapapillomavirus 4	2019-0...	Japan: ...	Equus c...	equine s...	99.72	100.00	1
LC510381.1	(+)	1,548		1,907	Deltapapillomavirus 4		Japan: ...	Equus c...	equine s...	99.72	100.00	1
LC510380.1	(+)	1,548		1,907	Deltapapillomavirus 4		Japan: ...	Equus c...	equine s...	99.72	100.00	1
LC510379.1	(+)	5,788		6,147	Deltapapillomavirus 4	2017-10...	Japan: ...	Equus c...	equine s...	99.72	100.00	1
LC510378.1	(+)	5,788		6,147	Deltapapillomavirus 4	2017-07...	Japan:...	Equus c...	equine s...	99.72	100.00	1
LC510377.1	(+)	5,788		6,147	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	99.72	100.00	1
MT385853.1	(+)	35		394	Deltapapillomavirus 4	Apr-2016	Costa R...	Bos taurus		99.72	100.00	1
AB626705.1	(+)	5,788		6,147	Deltapapillomavirus 4		Japan:H...	Bos taurus	myoperi...	99.72	100.00	1
J02045.1	(+)	1,951		2,310	Deltapapillomavirus 4			Bos taurus		99.72	100.00	1
X02346.1	(+)	5,787		6,146	Deltapapillomavirus 4					99.72	100.00	1
U13843.1	(+)	3,267		3,626	unidentified cloning vector					99.72	100.00	1
MN977321.1	(+)	180		539	Deltapapillomavirus sp.	2018	Italy	Capra i...		99.44	100.00	2
MN977319.1	(+)	180		539	Deltapapillomavirus sp.	2018	Italy	Rupicap...		99.44	100.00	2
MF435918.1	(+)	5,789		6,148	Deltapapillomavirus 4	2015	China	cattle	cutaneo...	99.44	100.00	2
KY746722.1	(+)	5,788		6,147	Deltapapillomavirus 4	Apr-2016	Morocco	Bos taurus	skin lesion	99.44	100.00	2
KX924580.1	(+)	6		365	Deltapapillomavirus 4	10-May-...	Turkey	bovine	Teat	99.44	100.00	2
KX924579.1	(+)	7		366	Deltapapillomavirus 4	03-Feb-...	Turkey	bovine	Teat	99.44	100.00	2
MW018708.1	(+)	48		407	Deltapapillomavirus sp.	2020	Egypt	cattle	skin	99.44	100.00	2
MG977494.1	(+)	5,788		6,144	Deltapapillomavirus 4	Apr-2016	Italy	Bos taurus		99.44	99.17	2
MF384289.1	(+)	5,785		6,144	Deltapapillomavirus 4		United K...	Equus ...		99.17	100.00	3
MF045489.1	(+)	5,788		6,147	Deltapapillomavirus 4					99.17	100.00	3
KY662040.1	(+)	23		382	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	99.17	100.00	3
KX924577.1	(+)	7		366	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	99.17	100.00	3
KX924576.1	(+)	7		366	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	99.17	100.00	3
KF938579.1	(+)	56		415	Deltapapillomavirus 4	Aug-2013	China	cow		99.17	100.00	3
KF284133.1	(+)	45		404	Deltapapillomavirus 4	26-May-...	Poland	equine	equine s...	99.17	100.00	3
JX046506.1	(+)	42		401	Deltapapillomavirus 4	Mar-2006	Croatia	cattle	skin	99.17	100.00	3
LC510382.1	(+)	1,548		1,907	Deltapapillomavirus 4		Japan: ...	Equus c...	equine s...	99.17	100.00	3
MW018707.1	(+)	48		407	Deltapapillomavirus sp.	2019	Egypt	cattle	skin	99.17	100.00	3
MT682133.1	(+)	39		398	Bovine papillomavirus	2018	Italy	Bos taurus		99.17	100.00	3
MT459820.1	(+)	5,698		6,057	Deltapapillomavirus 4	2018/2019	Egypt	equine		99.17	100.00	3
MN977320.1	(+)	180		536	Deltapapillomavirus sp.	2018	Italy	Capra i...		99.16	99.17	3
MF384294.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		99.16	99.17	3
MF384291.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		99.16	99.17	3
MF384290.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		99.16	99.17	3
MF384288.1	(+)	5,776		6,135	Deltapapillomavirus 4		United K...	Equus ...		98.89	100.00	4
MF384287.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		99.16	99.17	3
MF384286.1	(+)	5,776		6,135	Deltapapillomavirus 4		Switzerl...	Equus c...		98.89	100.00	4
MF384285.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		99.16	99.17	3
MF384284.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		99.16	99.17	3
MF384283.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		99.16	99.17	3
MF384282.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		99.16	99.17	3
MF384280.1	(+)	5,776		6,135	Deltapapillomavirus 4		Switzerl...	Equus c...		98.89	100.00	4
MF384279.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		99.16	99.17	3
MF384278.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		99.16	99.17	3
MF384277.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		99.16	99.17	3
MF384276.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		99.16	99.17	3
MF384275.1	(+)	5,786		6,142	Deltapapillomavirus 4		United K...	Equus ...		99.16	99.17	3
MT674573.1	(+)	57		416	Deltapapillomavirus 4		Brazil	Bos taurus		98.89	100.00	4
JX678969.1	(+)	5,776		6,135	Deltapapillomavirus 4	Jun-2006	United K...	Equus c...	sarcoid	98.89	100.00	4
JX046521.1	(+)	42		401	Deltapapillomavirus 4	Apr-2010	Croatia	cattle	udder	98.33	100.00	6
JX046518.1	(+)	42		401	Deltapapillomavirus 4	Apr-2010	Croatia	cattle	udder	98.33	100.00	6
KU728469.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		99.71	95.56	1
KU728462.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		99.71	95.56	1
KU728470.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		99.42	95.56	2
KU728467.1	(+)	1		299	Deltapapillomavirus 4	11-Nov-...	Brazil	Bos taurus		99.67	83.06	1
KU728466.1	(+)	1		298	Deltapapillomavirus 4	16-Dec-...	Brazil	Bos taurus		98.66	82.78	4
LT837966.1	(+)	67		301	Deltapapillomavirus 4					100.00	65.28	0
KF114855.1	(+)	67		301	Deltapapillomavirus 4	15-Mar-...	India	mule	nasal cav...	100.00	65.28	0
HG918265.1	(+)	67		301	Deltapapillomavirus 4		India:Ba...	Bos taurus	skin tum...	100.00	65.28	0
EF151530.1	(+)	32		266	Deltapapillomavirus 4		India			100.00	65.28	0
MH543316.1	(+)	67		301	Deltapapillomavirus 4	21-Aug-...	Egypt: N...	cattle	skin lesi...	99.57	65.28	1
KY372395.1	(+)	33		264	Deltapapillomavirus 4	04-Nov-...	Turkey:...	cattle	skin	100.00	64.44	0
KY372393.1	(+)	33		264	Deltapapillomavirus 4	04-Nov-...	Turkey:...	cattle	skin	100.00	64.44	0
KY372389.1	(+)	33		264	Deltapapillomavirus 4	01-Feb-...	Turkey:...	cattle	skin	100.00	64.44	0
KY372386.1	(+)	33		264	Deltapapillomavirus 4	14-Sep-...	Turkey:...	cattle	skin	100.00	64.44	0
KF148690.1	(+)	67		301	Deltapapillomavirus 4	11-Oct-2...	India	buffalo	mucosal ...	99.57	65.28	1
KY372390.1	(+)	34		264	Deltapapillomavirus 4	03-Oct-2...	Turkey: ...	cattle	skin	100.00	64.17	0
KY372391.1	(+)	33		264	Deltapapillomavirus 4	27-Sep-...	Turkey: ...	cattle	skin	99.57	64.44	1
KF055288.1	(+)	67		301	Deltapapillomavirus 4	26-Jan-2...	India	buffalo	wart tissue	99.15	65.28	2
KY372394.1	(+)	34		264	Deltapapillomavirus 4	06-Mar-...	Turkey: ...	cattle	skin	99.13	64.17	2

Illustration 1. NCBI multiple sequence alignment for local isolate 1 (MW658347.1).

Histological determination

Histological investigation of papilloma under light microscopy showed mature finger-like projection papillae with grown rete pegs expressed in the stratum corneum of the skin epidermis (Fig. 4A). Thickening of the epidermis due to diffuse hyperplasia in stratum spinosum layer (acanthosis) with hyperkeratosis was observed (Fig. 4B). Furthermore, severe infiltration of mononuclear cells (MNCs) mainly macrophages, lymphocytes and fibroblasts, was observed in epidermis and dermis with massive hemorrhage in the epidermal layer in addition to dermal fibroplasias (Fig. 4C). Moreover, multifocal spongiosis and hyperkeratosis (Fig. 4D), with moderate neovascularization and fibroplasias due to fibrous connective proliferation (Fig. 4E), were observed. In addition, there was marked


NCBI Multiple Sequence Alignment Viewer, Version 1.20.0

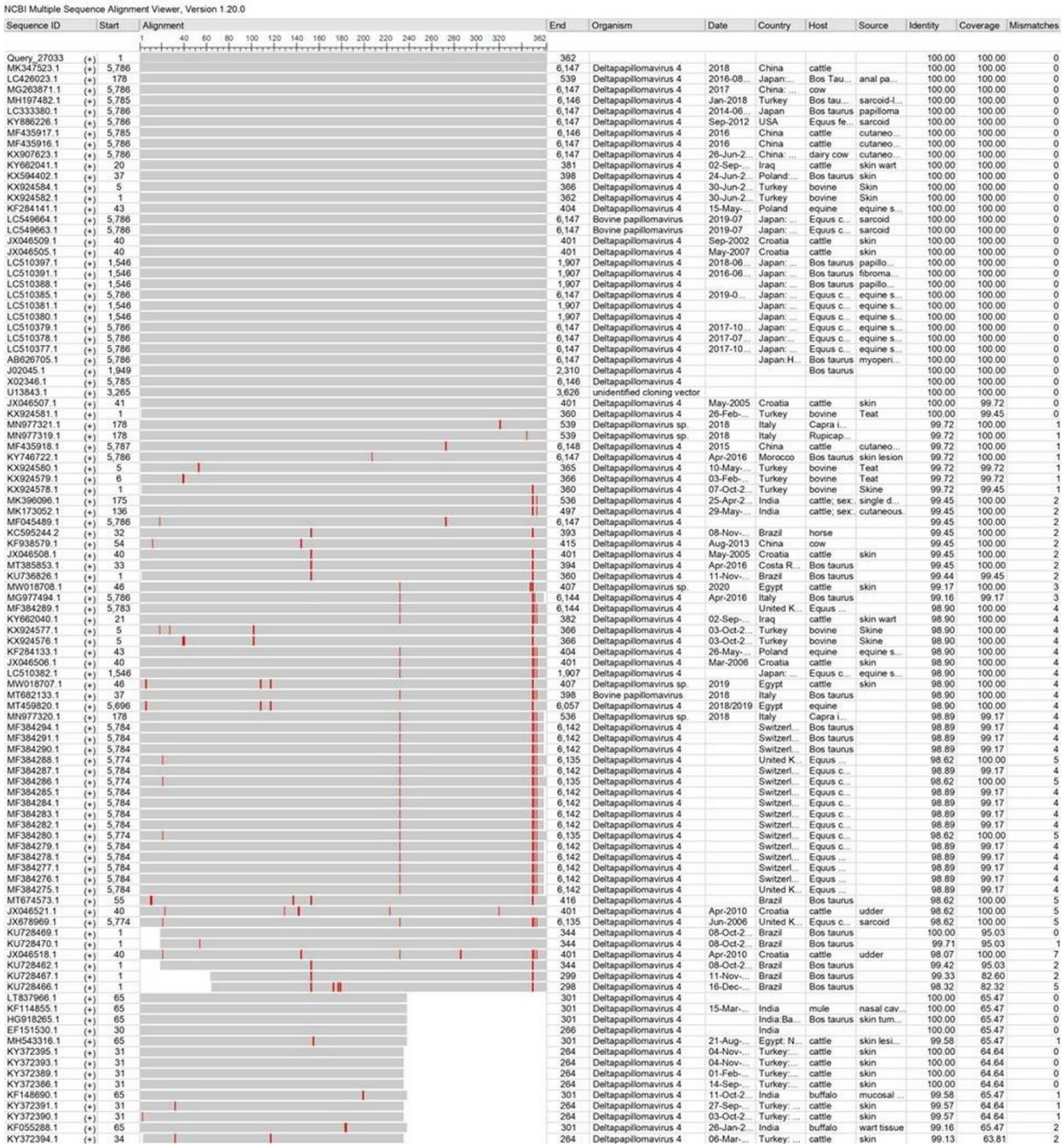
Sequence ID		Start	Alignment	End	Organism	Date	Country	Host	Source	Identity	Coverage	Mismatches
Query_30061	(+)	1		353						100.00	100.00	0
MN304951.1	(+)	3		355	Bos taurus papillomavir...	05-May-...	Costa R...	horse		100.00	100.00	0
LC426022.1	(+)	170		522	Bos taurus papillomavir...	2016-06...	Japan:...	Bos Tau...	vulval ...	100.00	100.00	0
LC426021.1	(+)	170		522	Bos taurus papillomavir...	2016-02...	Japan:...	Bos Tau...	vulval ...	100.00	100.00	0
MH589273.1	(+)	25		377	Bos taurus papillomavir...	2012	Japan	Japane...		100.00	100.00	0
KY781814.1	(+)	17		369	Bovine papillomavirus	06-Aug-...	Turkey	bovine	skin	100.00	100.00	0
MF045490.1	(+)	5,779		6,131	Bos taurus papillomavir...					100.00	100.00	0
KX113620.1	(+)	170		522	Bos taurus papillomavir...					100.00	100.00	0
KX008606.1	(+)	26		378	Bos taurus papillomavir...	19-Sep-...	China: ...	cow	sarcoid	100.00	100.00	0
KP663623.1	(+)	26		378	Bos taurus papillomavir...	27-Sep-...	China: ...	Bos taurus	sarcoid	100.00	100.00	0
KP663621.1	(+)	26		378	Bos taurus papillomavir...	05-Oct-2...	China: ...	Equus ...	sarcoid	100.00	100.00	0
KM455051.1	(+)	5,777		6,129	Bos taurus papillomavir...	06-Jul-2...	China: ...	cow	cutaneo...	100.00	100.00	0
KM455049.1	(+)	23		375	Bos taurus papillomavir...	06-Jul-2...	China: ...	cow	sarcoid	100.00	100.00	0
KF171968.1	(+)	170		522	Bos taurus papillomavir...	2011	Spain	Bos tau...	head a...	100.00	100.00	0
KF284153.1	(+)	35		387	Bos taurus papillomavir...	06-Jun-...	Poland	equine	equine s...	100.00	100.00	0
KC878306.1	(+)	5,779		6,131	Bos taurus papillomavir...	May-2012	China	dairy cow	cutaneo...	100.00	100.00	0
MT178264.1	(+)	2		354	Bovine papillomavirus	2017	Turkey	bovine;...	skin	100.00	100.00	0
KC256805.1	(+)	170		522	Bos taurus papillomavir...	Sep-2012	China	cow		100.00	100.00	0
LC510394.1	(+)	1,544		1,896	Bos taurus papillomavir...	2016-06...	Japan:...	Bos taurus	fibropa...	100.00	100.00	0
LC510392.1	(+)	1,544		1,896	Bos taurus papillomavir...	2016-06...	Japan:...	Bos taurus	fibroma...	100.00	100.00	0
LC510387.1	(+)	1,544		1,896	Bos taurus papillomavir...	2009	Japan:...	Bos taurus	nodule ...	100.00	100.00	0
LC510386.1	(+)	1,544		1,896	Bos taurus papillomavir...	2009	Japan:...	Bos taurus	papillo...	100.00	100.00	0
LC510384.1	(+)	5,780		6,132	Bos taurus papillomavir...	2018-10...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510383.1	(+)	5,779		6,131	Bos taurus papillomavir...	2018-09...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510376.1	(+)	5,777		6,129	Bos taurus papillomavir...	2017-02...	Japan:...	Equus c...	equine s...	100.00	100.00	0
MT385854.1	(+)	25		377	Bos taurus papillomavir...	Apr-2016	Costa R...	Bos taurus		100.00	100.00	0
X01768.1	(+)	1,694		2,046	Bos taurus papillomavir...					100.00	100.00	0
KP768461.1	(+)	26		378	Bos taurus papillomavir...	21-Jan-2...	China: ...	Bos taurus	sarcoid	99.72	100.00	1
KX924602.1	(+)	3		349	Bos taurus papillomavir...	26-Feb-...	Turkey	bovine	Skin	100.00	98.30	0
M20219.1	(+)	5,770		6,122	Bos taurus papillomavir...					99.43	100.00	2
MT181841.1	(+)	2		354	Bovine papillomavirus	2017	Turkey	cattle (b...	skin	99.15	100.00	3
MT674580.1	(+)	35		381	Bos taurus papillomavir...		Brazil	Bos taurus		99.71	98.30	1
KX924601.1	(+)	1		342	Bos taurus papillomavir...	20-Apr-2...	Turkey	bovine	Skin	100.00	96.88	0
KU743470.1	(+)	1		342	Bos taurus papillomavir...	14-Feb-...	Brazil	Bos taurus		100.00	96.88	0
KU728473.1	(+)	1		341	Bos taurus papillomavir...	13-Dec-...	Brazil	Bos taurus		100.00	96.60	0
MH187961.1	(+)	5,689		6,041	Bos taurus papillomavir...		Brazil	Bos taurus	papillo...	98.58	100.00	5
KU674833.1	(+)	5,780		6,132	Bos taurus papillomavir...	20-Dec-...	Brazil	Bos taurus	fibropa...	98.58	100.00	5
KC595245.2	(+)	24		376	Bos taurus papillomavir...	08-Nov-...	Brazil	horse		98.58	100.00	5
MT674579.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		98.58	100.00	5
MT674577.1	(+)	1		294	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	83.29	0
MT674576.1	(+)	1		267	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	75.64	0
MT674574.1	(+)	1		267	Bos taurus papillomavir...		Brazil	Bos taurus		99.25	75.64	2
MG818475.1	(+)	5,790		6,142	Bos taurus papillomavir...	2017	China	cattle		89.83	99.72	34
MF327274.1	(+)	5,790		6,142	Bos taurus papillomavir...					89.83	99.72	34
KU519390.1	(+)	5,783		6,135	Bos taurus papillomavir...	01-Jun-2...	Brazil	Bos taurus	papillo...	89.83	99.72	34
KC595246.2	(+)	24		376	Bos taurus papillomavir...	08-Nov-...	Brazil	horse		89.83	99.72	34
KM258443.2	(+)	5,790		6,142	Bos taurus papillomavir...	20-Apr-2...	China	yellow ...	cutaneo...	89.83	99.72	34
KC763355.1	(+)	196		548	Bos taurus papillomavir...		Brazil	horse	equine s...	89.83	99.72	34
MT674598.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
MT674584.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
MT674583.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
JQ798171.1	(+)	5,790		6,142	Bos taurus papillomavir...		Brazil	Bos taurus	cutaneo...	89.83	99.72	34
EU293540.1	(+)	29		381	Bovine papillomavirus		Brazil			89.83	99.72	34
MF741676.1	(+)	5,701		6,053	Bos taurus papillomavir...	20-May-...	Brazil	bovine	papillo...	89.55	99.72	35
MN977322.1	(+)	158		389	Deltapapillomavirus sp.	2013	Italy	Rupicap...		100.00	65.72	0
MT674571.1	(+)	1		234	Bos taurus papillomavir...		Brazil	Bos taurus		98.72	66.29	3
HE603636.1	(+)	57		301	Deltapapillomavirus 4	12-Jan-...	India:Lu...		heifer cu...	97.14	69.41	7
MT674575.1	(+)	1		219	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	62.04	0
KF938579.1	(+)	46		398	Deltapapillomavirus 4	Aug-2013	China	cow		86.97	100.00	46
KY746722.1	(+)	5,780		6,130	Deltapapillomavirus 4	Apr-2016	Morocco	Bos taurus	skin lesion	86.61	99.43	47
MN977321.1	(+)	170		522	Deltapapillomavirus sp.	2018	Italy	Capra i...		86.40	100.00	48
MK347523.1	(+)	5,778		6,130	Deltapapillomavirus 4	2018	China	cattle		86.40	100.00	48
MK396096.1	(+)	167		519	Deltapapillomavirus 4	25-Apr-2...	India	cattle; sex...	single d...	86.40	100.00	48
LC426023.1	(+)	170		522	Deltapapillomavirus 4	2016-08...	Japan:...	Bos Tau...	anal pa...	86.40	100.00	48
MK173052.1	(+)	128		480	Deltapapillomavirus 4	29-May-...	India	cattle; sex...	cutaneous	86.40	100.00	48
MG263871.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017	China:...	cow		86.40	100.00	48
MH197482.1	(+)	5,777		6,129	Deltapapillomavirus 4	Jan-2018	Turkey	Bos tau...	sarcoid-l...	86.40	100.00	48
LC333380.1	(+)	5,778		6,130	Deltapapillomavirus 4	2014-06...	Japan	Bos taurus	papilloma	86.40	100.00	48
KY886226.1	(+)	5,778		6,130	Deltapapillomavirus 4	Sep-2012	USA	Equus fe...	sarcoid	86.40	100.00	48
MF435917.1	(+)	5,777		6,129	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	86.40	100.00	48
MF435916.1	(+)	5,778		6,130	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	86.40	100.00	48
KX907623.1	(+)	5,778		6,130	Deltapapillomavirus 4	26-Jun-2...	China: ...	dairy cow	cutaneo...	86.40	100.00	48
KY662041.1	(+)	12		364	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	86.40	100.00	48
KX594402.1	(+)	29		381	Deltapapillomavirus 4	24-Jun-2...	Poland:	Bos taurus	skin	86.40	100.00	48
KF284141.1	(+)	35		387	Deltapapillomavirus 4	15-May-...	Poland	equine	equine s...	86.40	100.00	48
LC549664.1	(+)	5,778		6,130	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	86.40	100.00	48
LC549663.1	(+)	5,778		6,130	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	86.40	100.00	48
JX046509.1	(+)	32		384	Deltapapillomavirus 4	Sep-2002	Croatia	cattle	skin	86.40	100.00	48
JX046505.1	(+)	32		384	Deltapapillomavirus 4	May-2007	Croatia	cattle	skin	86.40	100.00	48
LC510397.1	(+)	1,538		1,890	Deltapapillomavirus 4	2018-06...	Japan:...	Bos taurus	papillo...	86.40	100.00	48
LC510391.1	(+)	1,538		1,890	Deltapapillomavirus 4	2016-06...	Japan:...	Bos taurus	fibroma...	86.40	100.00	48
LC510388.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Bos taurus	papillo...	86.40	100.00	48
LC510385.1	(+)	5,778		6,130	Deltapapillomavirus 4	2019-0...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510381.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510380.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510379.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510378.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-07...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510377.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	86.40	100.00	48
AB626705.1	(+)	5,778		6,130	Deltapapillomavirus 4		Japan:H...	Bos taurus	myoperi...	86.40	100.00	48
J02045.1	(+)	1,941		2,293	Deltapapillomavirus 4			Bos taurus		86.40	100.00	48
X02346.1	(+)	5,777		6,129	Deltapapillomavirus 4					86.40	100.00	48
U13843.1	(+)	3,257		3,609	unidentified cloning vector					86.40	100.00	48
MN977319.1	(+)	170		521	Deltapapillomavirus sp.	2018	Italy	Rupicap...		86.36	99.72	48
KX924577.1	(+)	1		349	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	86.53	98.87	47
MN977320.1	(+)	170		522	Deltapapillomavirus sp.	2018	Italy	Capra i...		86.12	100.00	49
LC510382.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.12	100.00	49
MT385853.1	(+)	25		377	Deltapapillomavirus 4	Apr-2016	Costa R...	Bos taurus		86.12	100.00	49
MW018708.1	(+)	38		390	Deltapapillomavirus sp.	2020	Egypt	cattle	skin	86.12	100.00	49
MW018707.1	(+)	38		390	Deltapapillomavirus sp.	2019	Egypt	cattle	skin	86.12	100.00	49
MT682133.1	(+)	29		381	Bovine papillomavirus	2018	Italy	Bos taurus		86.12	100.00	49
MT459820.1	(+)	5,688		6,040	Deltapapillomavirus 4	2018/2019	Egypt	equine		86.12	100.00	49

Illustration 2. NCBI multiple sequence alignment for local isolate 2 (MW658348.1).

acanthosis with moderate elongation of the retention ridge towards the dermis that showed dense fibrous tissue with infiltration of inflammatory cells (Fig. 4F). Hyperkeratosis, parakeratosis, severe elongation of the retention ridge towards the dermis and signs of calcification observed on the epidermal surface (Fig. 4G). Finally, necrosis in the epidermal layer, mainly in basal cells with nuclear pyknosis of some spinosum cells, was observed (Fig. 4H).

Immunohistochemical detection

In the present study, the expression of TNF-α, EGFR and FASCIN markers was targeted using IHC. The results of the immunoreactions interestingly displayed significant variation among the examined skin layers (dermis and epidermis), which ranged from negative (0) to mild positive (+1) to moderate positive (+2) reactions (Figs. 5–7).

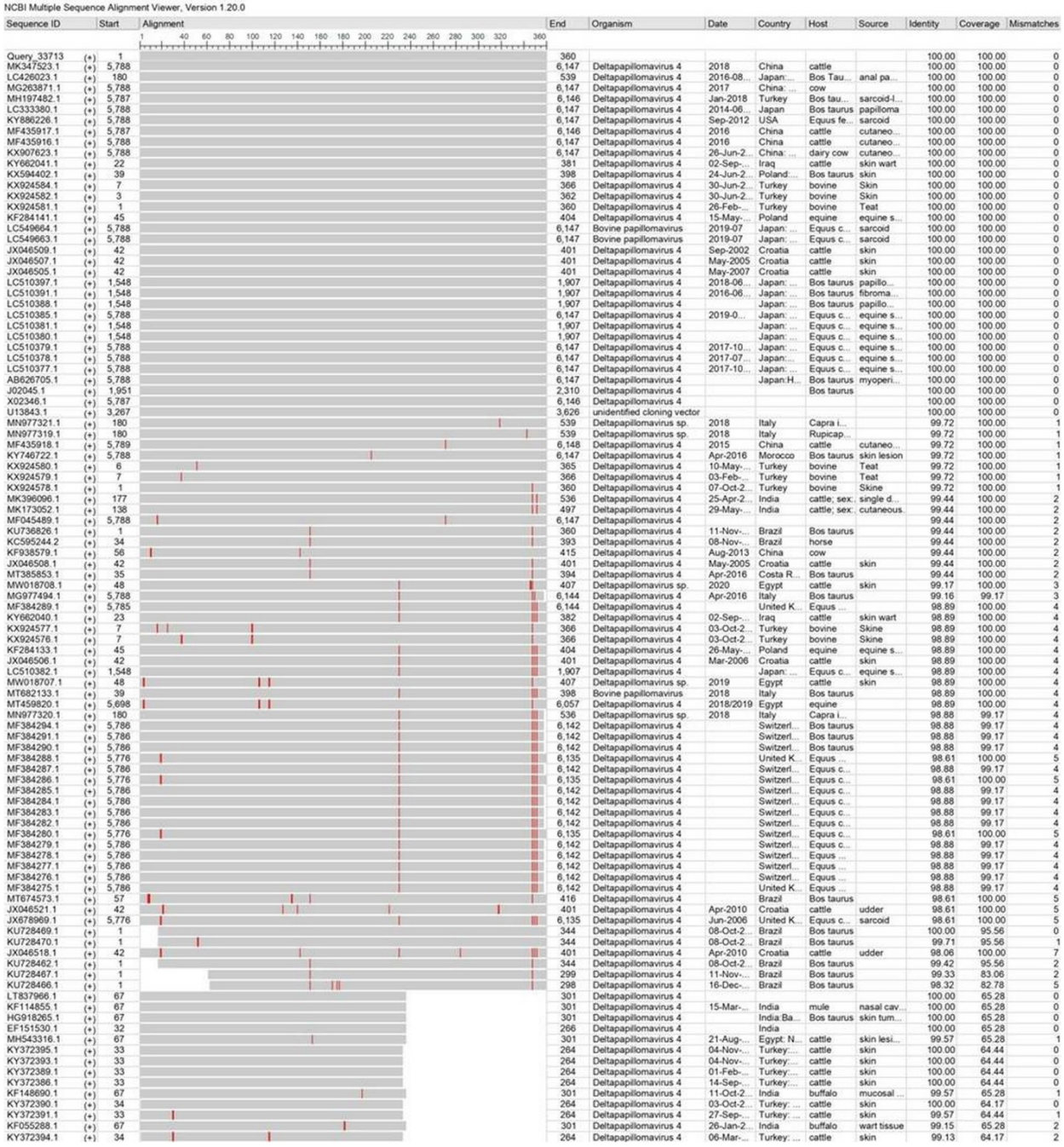

NCBI Multiple Sequence Alignment Viewer, Version 1.20.0

Sequence ID		Start	Alignment	End	Organism	Date	Country	Host	Source	Identity	Coverage	Mismatches
Query_27033	(+)	1		362						100.00	100.00	0
MK347523.1	(+)	5,786		6,147	Deltapapillomavirus 4	2018	China	cattle		100.00	100.00	0
LC426023.1	(+)	178		539	Deltapapillomavirus 4	2016-08...	Japan:...	Bos Tau...	anal pa...	100.00	100.00	0
MG263871.1	(+)	5,786		6,147	Deltapapillomavirus 4	2017	China:...	cow		100.00	100.00	0
MH197482.1	(+)	5,785		6,146	Deltapapillomavirus 4	Jan-2018	Turkey	Bos tau...	sarcoid-l...	100.00	100.00	0
LC333380.1	(+)	5,786		6,147	Deltapapillomavirus 4	2014-06...	Japan	Bos taurus	papilloma	100.00	100.00	0
KY886226.1	(+)	5,786		6,147	Deltapapillomavirus 4	Sep-2012	USA	Equus fe...	sarcoid	100.00	100.00	0
MF435917.1	(+)	5,785		6,146	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	100.00	100.00	0
MF435916.1	(+)	5,786		6,147	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	100.00	100.00	0
KX907623.1	(+)	5,786		6,147	Deltapapillomavirus 4	26-Jun-2...	China:...	dairy cow	cutaneo...	100.00	100.00	0
KY662041.1	(+)	20		381	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	100.00	100.00	0
KX594402.1	(+)	37		398	Deltapapillomavirus 4	24-Jun-2...	Poland:...	Bos taurus	skin	100.00	100.00	0
KX924584.1	(+)	5		366	Deltapapillomavirus 4	30-Jun-2...	Turkey	bovine	Skin	100.00	100.00	0
KX924582.1	(+)	1		362	Deltapapillomavirus 4	30-Jun-2...	Turkey	bovine	Skin	100.00	100.00	0
KF284141.1	(+)	43		404	Deltapapillomavirus 4	15-May-...	Poland	equine	equine s...	100.00	100.00	0
LC549664.1	(+)	5,786		6,147	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	100.00	100.00	0
LC549663.1	(+)	5,786		6,147	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	100.00	100.00	0
JX046509.1	(+)	40		401	Deltapapillomavirus 4	Sep-2002	Croatia	cattle	skin	100.00	100.00	0
JX046505.1	(+)	40		401	Deltapapillomavirus 4	May-2007	Croatia	cattle	skin	100.00	100.00	0
LC510397.1	(+)	1,546		1,907	Deltapapillomavirus 4	2018-06...	Japan:...	Bos taurus	papillo...	100.00	100.00	0
LC510391.1	(+)	1,546		1,907	Deltapapillomavirus 4	2016-06...	Japan:...	Bos taurus	fibroma...	100.00	100.00	0
LC510388.1	(+)	1,546		1,907	Deltapapillomavirus 4		Japan:...	Bos taurus	papillo...	100.00	100.00	0
LC510385.1	(+)	5,786		6,147	Deltapapillomavirus 4	2019-0...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510381.1	(+)	1,546		1,907	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510380.1	(+)	1,546		1,907	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510379.1	(+)	5,786		6,147	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510378.1	(+)	5,786		6,147	Deltapapillomavirus 4	2017-07...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510377.1	(+)	5,786		6,147	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	100.00	100.00	0
AB626705.1	(+)	5,786		6,147	Deltapapillomavirus 4		Japan:H...	Bos taurus	myoperi...	100.00	100.00	0
J02045.1	(+)	1,949		2,310	Deltapapillomavirus 4			Bos taurus		100.00	100.00	0
X02346.1	(+)	5,785		6,146	Deltapapillomavirus 4					100.00	100.00	0
U13843.1	(+)	3,265		3,626	unidentified cloning vector					100.00	100.00	0
JX046507.1	(+)	41		401	Deltapapillomavirus 4	May-2005	Croatia	cattle	skin	100.00	99.72	0
KX924581.1	(+)	1		360	Deltapapillomavirus 4	26-Feb-...	Turkey	bovine	Teat	100.00	99.45	0
MN977321.1	(+)	178		539	Deltapapillomavirus sp.	2018	Italy	Capra i...		99.72	100.00	1
MN977319.1	(+)	178		539	Deltapapillomavirus sp.	2018	Italy	Rupicap...		99.72	100.00	1
MF435918.1	(+)	5,787		6,148	Deltapapillomavirus 4	2015	China	cattle	cutaneo...	99.72	100.00	1
KY746722.1	(+)	5,786		6,147	Deltapapillomavirus 4	Apr-2016	Morocco	Bos taurus	skin lesion	99.72	100.00	1
KX924580.1	(+)	5		365	Deltapapillomavirus 4	10-May-...	Turkey	bovine	Teat	99.72	99.72	1
KX924579.1	(+)	6		366	Deltapapillomavirus 4	03-Feb-...	Turkey	bovine	Teat	99.72	99.72	1
KX924578.1	(+)	1		360	Deltapapillomavirus 4	07-Oct-2...	Turkey	bovine	Skine	99.72	99.45	1
MK396096.1	(+)	175		536	Deltapapillomavirus 4	25-Apr-2...	India	cattle; sex...	single d...	99.45	100.00	2
MK173052.1	(+)	136		497	Deltapapillomavirus 4	29-May-...	India	cattle; sex...	cutaneous	99.45	100.00	2
MF045489.1	(+)	5,786		6,147	Deltapapillomavirus 4					99.45	100.00	2
KC595244.2	(+)	32		393	Deltapapillomavirus 4	08-Nov-...	Brazil	horse		99.45	100.00	2
KF938579.1	(+)	54		415	Deltapapillomavirus 4	Aug-2013	China	cow		99.45	100.00	2
JX046508.1	(+)	40		401	Deltapapillomavirus 4	May-2005	Croatia	cattle	skin	99.45	100.00	2
MT385853.1	(+)	33		394	Deltapapillomavirus 4	Apr-2016	Costa R...	Bos taurus		99.45	100.00	2
KU736826.1	(+)	1		360	Deltapapillomavirus 4	11-Nov-...	Brazil	Bos taurus		99.44	99.45	2
MW018708.1	(+)	46		407	Deltapapillomavirus sp.	2020	Egypt	cattle	skin	99.17	100.00	3
MG977494.1	(+)	5,786		6,144	Deltapapillomavirus 4	Apr-2016	Italy	Bos taurus		99.16	99.17	3
MF384289.1	(+)	5,783		6,144	Deltapapillomavirus 4		United K...	Equus ...		98.90	100.00	4
KY662040.1	(+)	21		382	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	98.90	100.00	4
KX924577.1	(+)	5		366	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	98.90	100.00	4
KX924576.1	(+)	5		366	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	98.90	100.00	4
KF284133.1	(+)	43		404	Deltapapillomavirus 4	26-May-...	Poland	equine	equine s...	98.90	100.00	4
JX046506.1	(+)	40		401	Deltapapillomavirus 4	Mar-2006	Croatia	cattle	skin	98.90	100.00	4
LC510382.1	(+)	1,546		1,907	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	98.90	100.00	4
MW018707.1	(+)	46		407	Deltapapillomavirus sp.	2019	Egypt	cattle	skin	98.90	100.00	4
MT682133.1	(+)	37		398	Bovine papillomavirus	2018	Italy	Bos taurus		98.90	100.00	4
MT459820.1	(+)	5,696		6,057	Deltapapillomavirus 4	2018/2019	Egypt	equine		98.90	100.00	4
MN977320.1	(+)	178		536	Deltapapillomavirus sp.	2018	Italy	Capra i...		98.89	99.17	4
MF384294.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		98.89	99.17	4
MF384291.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		98.89	99.17	4
MF384290.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		98.89	99.17	4
MF384288.1	(+)	5,774		6,135	Deltapapillomavirus 4		United K...	Equus ...		98.62	100.00	5
MF384287.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.89	99.17	4
MF384286.1	(+)	5,774		6,135	Deltapapillomavirus 4		Switzerl...	Equus c...		98.62	100.00	5
MF384285.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.89	99.17	4
MF384284.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.89	99.17	4
MF384283.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.89	99.17	4
MF384282.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.89	99.17	4
MF384280.1	(+)	5,774		6,135	Deltapapillomavirus 4		Switzerl...	Equus c...		98.62	100.00	5
MF384279.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.89	99.17	4
MF384278.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		98.89	99.17	4
MF384277.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		98.89	99.17	4
MF384276.1	(+)	5,784		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		98.89	99.17	4
MF384275.1	(+)	5,784		6,142	Deltapapillomavirus 4		United K...	Equus ...		98.89	99.17	4
MT674573.1	(+)	55		416	Deltapapillomavirus 4		Brazil	Bos taurus		98.62	100.00	5
JX046521.1	(+)	40		401	Deltapapillomavirus 4	Apr-2010	Croatia	cattle	udder	98.62	100.00	5
JX678969.1	(+)	5,774		6,135	Deltapapillomavirus 4	Jun-2006	United K...	Equus c...	sarcoid	98.62	100.00	5
KU728469.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		100.00	95.03	0
KU728470.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		99.71	95.03	1
JX046518.1	(+)	40		401	Deltapapillomavirus 4	Apr-2010	Croatia	cattle	udder	98.07	100.00	7
KU728462.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		99.42	95.03	2
KU728467.1	(+)	1		299	Deltapapillomavirus 4	11-Nov-...	Brazil	Bos taurus		99.33	82.60	2
KU728466.1	(+)	1		298	Deltapapillomavirus 4	16-Dec-...	Brazil	Bos taurus		98.32	82.32	5
LT837966.1	(+)	65		301	Deltapapillomavirus 4					100.00	65.47	0
KF114855.1	(+)	65		301	Deltapapillomavirus 4	15-Mar-...	India	mule	nasal cav...	100.00	65.47	0
HG918265.1	(+)	65		301	Deltapapillomavirus 4		India:Ba...	Bos taurus	skin tum...	100.00	65.47	0
EF151530.1	(+)	30		266	Deltapapillomavirus 4		India			100.00	65.47	0
MH543316.1	(+)	65		301	Deltapapillomavirus 4	21-Aug-...	Egypt: N...	cattle	skin lesi...	99.58	65.47	1
KY372395.1	(+)	31		264	Deltapapillomavirus 4	04-Nov-...	Turkey:...	cattle	skin	100.00	64.64	0
KY372393.1	(+)	31		264	Deltapapillomavirus 4	04-Nov-...	Turkey:...	cattle	skin	100.00	64.64	0
KY372389.1	(+)	31		264	Deltapapillomavirus 4	01-Feb-...	Turkey:...	cattle	skin	100.00	64.64	0
KY372386.1	(+)	31		264	Deltapapillomavirus 4	14-Sep-...	Turkey:...	cattle	skin	100.00	64.64	0
KF148690.1	(+)	65		301	Deltapapillomavirus 4	11-Oct-2...	India	buffalo	mucosal ...	99.58	65.47	1
KY372391.1	(+)	31		264	Deltapapillomavirus 4	27-Sep-...	Turkey: ...	cattle	skin	99.57	64.64	1
KY372390.1	(+)	31		264	Deltapapillomavirus 4	03-Oct-2...	Turkey: ...	cattle	skin	99.57	64.64	1
KF055288.1	(+)	65		301	Deltapapillomavirus 4	26-Jan-2...	India	buffalo	wart tissue	99.16	65.47	2
KY372394.1	(+)	34		264	Deltapapillomavirus 4	06-Mar-...	Turkey: ...	cattle	skin	99.13	63.81	2

Illustration 3. NCBI multiple sequence alignment for local isolate 3 (MW658349.1).

Discussion

BP is a highly prevalent skin disease of cattle in many countries worldwide including Iraq. However, the global distribution of other types of BPVs still needs to be established. The molecular detection in the current study revealed that all clinically suspected cattle were diagnosed with BV; while genotyping analysis confirmed that the local isolates were closely related to Type 1 and Type 2 BPV. In India, a specific molecular study was performed on normal and BP skin lesions in cattle. The results identified that the normal and BP skin lesions were infected with diagnosed type 1 and 2 BPV, which suggested a wide distribution of the virus that caused a high incidence of the disease (Pangty *et al*., 2010). Several studies conducted on cattle in the same

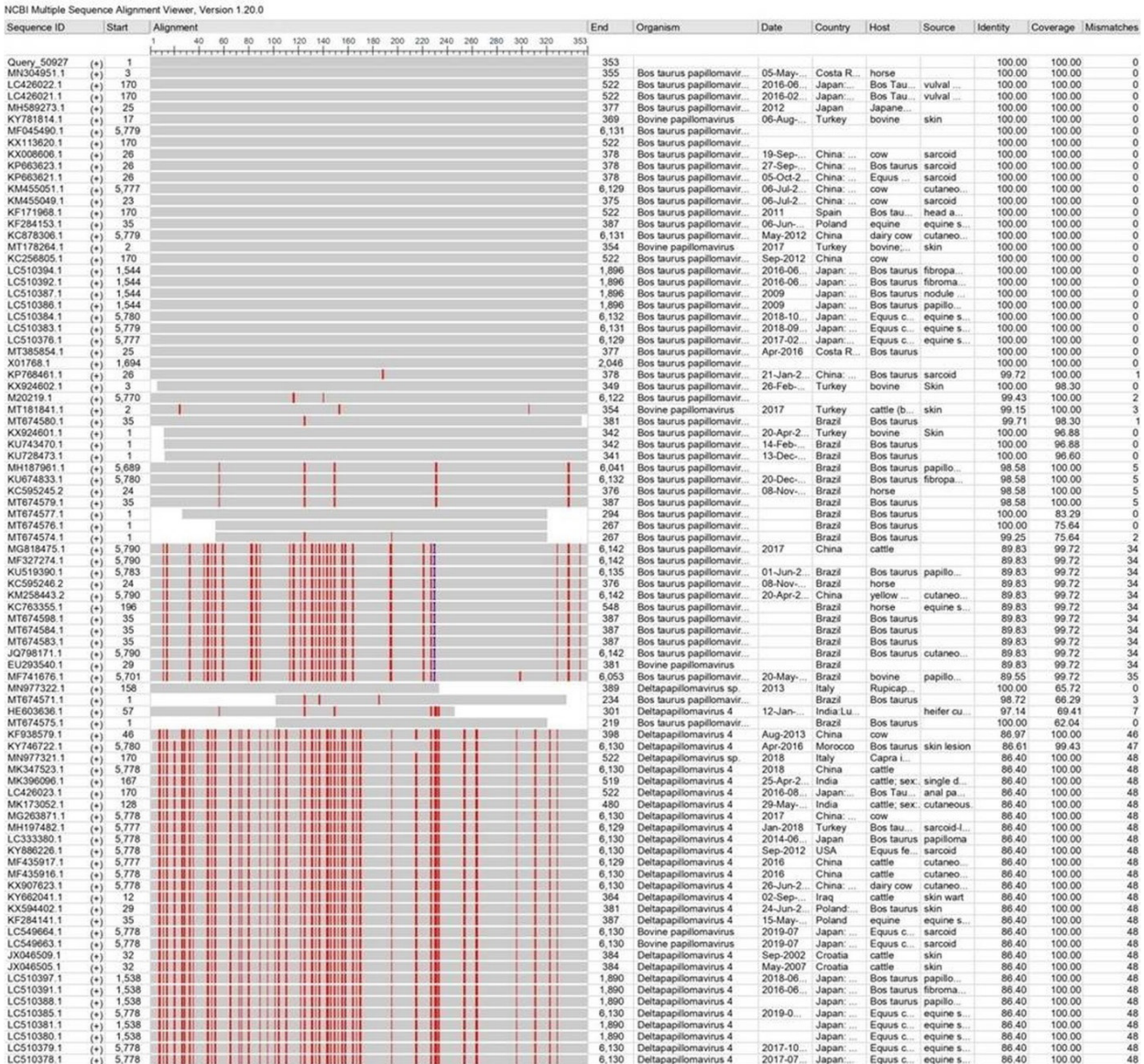


Sequence ID		Start	Alignment	End	Organism	Date	Country	Host	Source	Identity	Coverage	Mismatches
Query_33713	(+)	1		360						100.00	100.00	0
MK347523.1	(+)	5,788		6,147	Deltapapillomavirus 4	2018	China	cattle		100.00	100.00	0
LC426023.1	(+)	180		539	Deltapapillomavirus 4	2016-08...	Japan:...	Bos Tau...	anal pa...	100.00	100.00	0
MG263871.1	(+)	5,788		6,147	Deltapapillomavirus 4	2017	China:...	cow		100.00	100.00	0
MH197482.1	(+)	5,787		6,146	Deltapapillomavirus 4	Jan-2018	Turkey	Bos tau...	sarcoid-l...	100.00	100.00	0
LC333380.1	(+)	5,788		6,147	Deltapapillomavirus 4	2014-06...	Japan	Bos taurus	papilloma	100.00	100.00	0
KY886226.1	(+)	5,788		6,147	Deltapapillomavirus 4	Sep-2012	USA	Equus fe...	sarcoid	100.00	100.00	0
MF435917.1	(+)	5,787		6,146	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	100.00	100.00	0
MF435916.1	(+)	5,788		6,147	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	100.00	100.00	0
KX907623.1	(+)	5,788		6,147	Deltapapillomavirus 4	26-Jun-2...	China:...	dairy cow	cutaneo...	100.00	100.00	0
KY662041.1	(+)	22		381	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	100.00	100.00	0
KX594402.1	(+)	39		398	Deltapapillomavirus 4	24-Jun-2...	Poland:...	Bos taurus	skin	100.00	100.00	0
KX924584.1	(+)	7		366	Deltapapillomavirus 4	30-Jun-2...	Turkey	bovine	Skin	100.00	100.00	0
KX924582.1	(+)	3		362	Deltapapillomavirus 4	30-Jun-2...	Turkey	bovine	Skin	100.00	100.00	0
KX924581.1	(+)	1		360	Deltapapillomavirus 4	26-Feb-...	Turkey	bovine	Teat	100.00	100.00	0
KF284141.1	(+)	45		404	Deltapapillomavirus 4	15-May-...	Poland	equine	equine s...	100.00	100.00	0
LC549664.1	(+)	5,788		6,147	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	100.00	100.00	0
LC549663.1	(+)	5,788		6,147	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	100.00	100.00	0
JX046509.1	(+)	42		401	Deltapapillomavirus 4	Sep-2002	Croatia	cattle	skin	100.00	100.00	0
JX046507.1	(+)	42		401	Deltapapillomavirus 4	May-2005	Croatia	cattle	skin	100.00	100.00	0
JX046505.1	(+)	42		401	Deltapapillomavirus 4	May-2007	Croatia	cattle	skin	100.00	100.00	0
LC510397.1	(+)	1,548		1,907	Deltapapillomavirus 4	2018-06...	Japan:...	Bos taurus	papillo...	100.00	100.00	0
LC510391.1	(+)	1,548		1,907	Deltapapillomavirus 4	2016-06...	Japan:...	Bos taurus	fibroma...	100.00	100.00	0
LC510388.1	(+)	1,548		1,907	Deltapapillomavirus 4		Japan:...	Bos taurus	papillo...	100.00	100.00	0
LC510385.1	(+)	5,788		6,147	Deltapapillomavirus 4	2019-0...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510381.1	(+)	1,548		1,907	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510380.1	(+)	1,548		1,907	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510379.1	(+)	5,788		6,147	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510378.1	(+)	5,788		6,147	Deltapapillomavirus 4	2017-07...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510377.1	(+)	5,788		6,147	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	100.00	100.00	0
AB626705.1	(+)	5,788		6,147	Deltapapillomavirus 4		Japan:H...	Bos taurus	myoperi...	100.00	100.00	0
J02045.1	(+)	1,951		2,310	Deltapapillomavirus 4			Bos taurus		100.00	100.00	0
X02346.1	(+)	5,787		6,146	Deltapapillomavirus 4					100.00	100.00	0
U13843.1	(+)	3,267		3,626	unidentified cloning vector					100.00	100.00	0
MN977321.1	(+)	180		539	Deltapapillomavirus sp.	2018	Italy	Capra i...		99.72	100.00	1
MN977319.1	(+)	180		539	Deltapapillomavirus sp.	2018	Italy	Rupicap...		99.72	100.00	1
MF435918.1	(+)	5,789		6,148	Deltapapillomavirus 4	2015	China	cattle	cutaneo...	99.72	100.00	1
KY746722.1	(+)	5,788		6,147	Deltapapillomavirus 4	Apr-2016	Morocco	Bos taurus	skin lesion	99.72	100.00	1
KX924580.1	(+)	6		365	Deltapapillomavirus 4	10-May-...	Turkey	bovine	Teat	99.72	100.00	1
KX924579.1	(+)	7		366	Deltapapillomavirus 4	03-Feb-...	Turkey	bovine	Teat	99.72	100.00	1
KX924578.1	(+)	1		360	Deltapapillomavirus 4	07-Oct-2...	Turkey	bovine	Skine	99.72	100.00	1
MK396096.1	(+)	177		536	Deltapapillomavirus 4	25-Apr-2...	India	cattle; sex...	single d...	99.44	100.00	2
MK173052.1	(+)	138		497	Deltapapillomavirus 4	29-May-...	India	cattle; sex...	cutaneous	99.44	100.00	2
MF045489.1	(+)	5,788		6,147	Deltapapillomavirus 4					99.44	100.00	2
KU736826.1	(+)	1		360	Deltapapillomavirus 4	11-Nov-...	Brazil	Bos taurus		99.44	100.00	2
KC595244.2	(+)	34		393	Deltapapillomavirus 4	08-Nov-...	Brazil	horse		99.44	100.00	2
KF938579.1	(+)	56		415	Deltapapillomavirus 4	Aug-2013	China	cow		99.44	100.00	2
JX046508.1	(+)	42		401	Deltapapillomavirus 4	May-2005	Croatia	cattle	skin	99.44	100.00	2
MT385853.1	(+)	35		394	Deltapapillomavirus 4	Apr-2016	Costa R...	Bos taurus		99.44	100.00	2
MW018708.1	(+)	48		407	Deltapapillomavirus sp.	2020	Egypt	cattle	skin	99.17	100.00	3
MG977494.1	(+)	5,788		6,144	Deltapapillomavirus 4	Apr-2016	Italy	Bos taurus		99.16	99.17	3
MF384289.1	(+)	5,785		6,144	Deltapapillomavirus 4		United K...	Equus ...		98.89	100.00	4
KY662040.1	(+)	23		382	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	98.89	100.00	4
KX924577.1	(+)	7		366	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	98.89	100.00	4
KX924576.1	(+)	7		366	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	98.89	100.00	4
KF284133.1	(+)	45		404	Deltapapillomavirus 4	26-May-...	Poland	equine	equine s...	98.89	100.00	4
JX046506.1	(+)	42		401	Deltapapillomavirus 4	Mar-2006	Croatia	cattle	skin	98.89	100.00	4
LC510382.1	(+)	1,548		1,907	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	98.89	100.00	4
MW018707.1	(+)	48		407	Deltapapillomavirus sp.	2019	Egypt	cattle	skin	98.89	100.00	4
MT682133.1	(+)	39		398	Bovine papillomavirus	2018	Italy	Bos taurus		98.89	100.00	4
MT459820.1	(+)	5,698		6,057	Deltapapillomavirus 4	2018/2019	Egypt	equine		98.89	100.00	4
MN977320.1	(+)	180		536	Deltapapillomavirus sp.	2018	Italy	Capra i...		98.88	99.17	4
MF384294.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		98.88	99.17	4
MF384291.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		98.88	99.17	4
MF384290.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Bos taurus		98.88	99.17	4
MF384288.1	(+)	5,776		6,135	Deltapapillomavirus 4		United K...	Equus ...		98.61	100.00	5
MF384287.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.88	99.17	4
MF384286.1	(+)	5,776		6,135	Deltapapillomavirus 4		Switzerl...	Equus c...		98.61	100.00	5
MF384285.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.88	99.17	4
MF384284.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.88	99.17	4
MF384283.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.88	99.17	4
MF384282.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.88	99.17	4
MF384280.1	(+)	5,776		6,135	Deltapapillomavirus 4		Switzerl...	Equus c...		98.61	100.00	5
MF384279.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus c...		98.88	99.17	4
MF384278.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		98.88	99.17	4
MF384277.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		98.88	99.17	4
MF384276.1	(+)	5,786		6,142	Deltapapillomavirus 4		Switzerl...	Equus ...		98.88	99.17	4
MF384275.1	(+)	5,786		6,142	Deltapapillomavirus 4		United K...	Equus ...		98.88	99.17	4
MT674573.1	(+)	57		416	Deltapapillomavirus 4		Brazil	Bos taurus		98.61	100.00	5
JX046521.1	(+)	42		401	Deltapapillomavirus 4	Apr-2010	Croatia	cattle	udder	98.61	100.00	5
JX678969.1	(+)	5,776		6,135	Deltapapillomavirus 4	Jun-2006	United K...	Equus c...	sarcoid	98.61	100.00	5
KU728469.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		100.00	95.56	0
KU728470.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		99.71	95.56	1
JX046518.1	(+)	42		401	Deltapapillomavirus 4	Apr-2010	Croatia	cattle	udder	98.06	100.00	7
KU728462.1	(+)	1		344	Deltapapillomavirus 4	08-Oct-2...	Brazil	Bos taurus		99.42	95.56	2
KU728467.1	(+)	1		299	Deltapapillomavirus 4	11-Nov-...	Brazil	Bos taurus		99.33	83.06	2
KU728466.1	(+)	1		298	Deltapapillomavirus 4	16-Dec-...	Brazil	Bos taurus		98.32	82.78	5
LT837966.1	(+)	67		301	Deltapapillomavirus 4					100.00	65.28	0
KF114855.1	(+)	67		301	Deltapapillomavirus 4	15-Mar-...	India	mule	nasal cav...	100.00	65.28	0
HG918265.1	(+)	67		301	Deltapapillomavirus 4		India:Ba...	Bos taurus	skin tum...	100.00	65.28	0
EF151530.1	(+)	32		266	Deltapapillomavirus 4		India			100.00	65.28	0
MH543316.1	(+)	67		301	Deltapapillomavirus 4	21-Aug-...	Egypt: N...	cattle	skin lesi...	99.57	65.28	1
KY372395.1	(+)	33		264	Deltapapillomavirus 4	04-Nov-...	Turkey:...	cattle	skin	100.00	64.44	0
KY372393.1	(+)	33		264	Deltapapillomavirus 4	04-Nov-...	Turkey:...	cattle	skin	100.00	64.44	0
KY372389.1	(+)	33		264	Deltapapillomavirus 4	01-Feb-...	Turkey:...	cattle	skin	100.00	64.44	0
KY372386.1	(+)	33		264	Deltapapillomavirus 4	14-Sep-...	Turkey:...	cattle	skin	100.00	64.44	0
KF148690.1	(+)	67		301	Deltapapillomavirus 4	11-Oct-2...	India	buffalo	mucosal ...	99.57	65.28	1
KY372390.1	(+)	34		264	Deltapapillomavirus 4	03-Oct-2...	Turkey: ...	cattle	skin	100.00	64.17	0
KY372391.1	(+)	33		264	Deltapapillomavirus 4	27-Sep-...	Turkey: ...	cattle	skin	99.57	64.44	1
KF055288.1	(+)	67		301	Deltapapillomavirus 4	26-Jan-2...	India	buffalo	wart tissue	99.15	65.28	2
KY372394.1	(+)	34		264	Deltapapillomavirus 4	06-Mar-...	Turkey: ...	cattle	skin	99.13	64.17	2

Illustration 4. NCBI multiple sequence alignment for local isolate 4 (MW658350.1).

field showed various spreading of papillomaviruses (PVs) in cattle with clear symptoms and asymptomatic cattle. These results suggested that the cattle without symptoms have a crucial role in spreading the disease, as the virus is endemic (Ataseven *et al*., 2016; Wassilew, 2018; Kwok *et al*., 2020).

PCR assays, which are important and successful diagnostic methods, have high specificity and sensitivity for detecting agents and pathogens (MacLachlan *et al*., 2017; Dörttaş and Dağalp, 2020). The conserved region of the L1 gene of PV was used in this study to detect BPVs by PCR (Melo *et al*., 2014; Ataseven *et al*., 2016). PVs are classically used in the classification and construction of phylogenetic trees according to biological and medical characterization and sequence alignment of L1 nucleotides (Bocaneti *et al*., 2016).

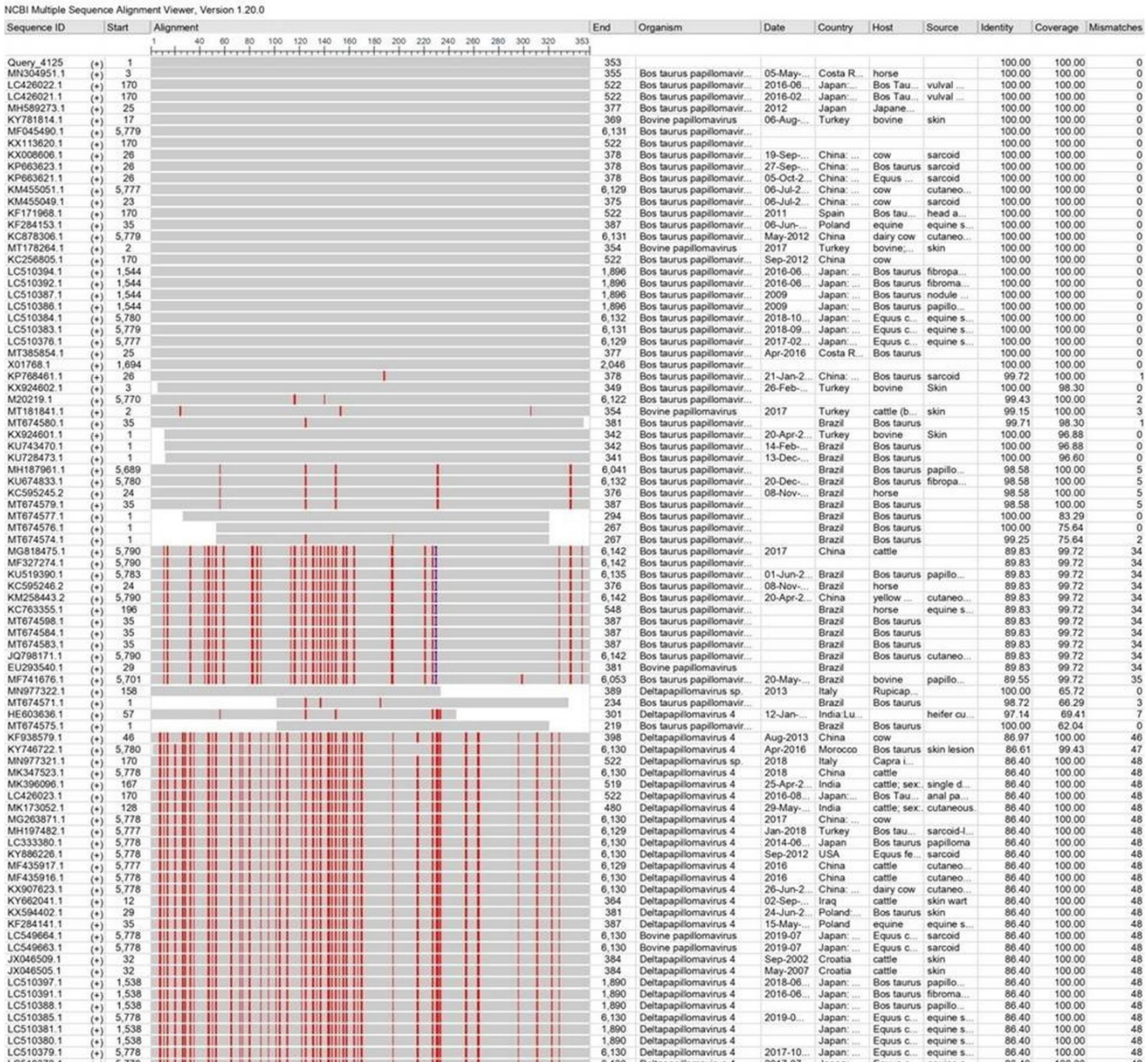

NCBI Multiple Sequence Alignment Viewer, Version 1.20.0

Sequence ID		Start	Alignment	End	Organism	Date	Country	Host	Source	Identity	Coverage	Mismatches
Query_50927	(+)	1		353						100.00	100.00	0
MN304951.1	(+)	3		355	Bos taurus papillomavir...	05-May-...	Costa R...	horse		100.00	100.00	0
LC426022.1	(+)	170		522	Bos taurus papillomavir...	2016-06...	Japan:...	Bos Tau...	vulval ...	100.00	100.00	0
LC426021.1	(+)	170		522	Bos taurus papillomavir...	2016-02...	Japan:...	Bos Tau...	vulval ...	100.00	100.00	0
MH589273.1	(+)	25		377	Bos taurus papillomavir...	2012	Japan	Japane...		100.00	100.00	0
KY781814.1	(+)	17		369	Bovine papillomavirus	06-Aug-...	Turkey	bovine	skin	100.00	100.00	0
MF045490.1	(+)	5,779		6,131	Bos taurus papillomavir...					100.00	100.00	0
KX113620.1	(+)	170		522	Bos taurus papillomavir...					100.00	100.00	0
KX008606.1	(+)	26		378	Bos taurus papillomavir...	19-Sep-...	China: ...	cow	sarcoid	100.00	100.00	0
KP663623.1	(+)	26		378	Bos taurus papillomavir...	27-Sep-...	China: ...	Bos taurus	sarcoid	100.00	100.00	0
KP663621.1	(+)	26		378	Bos taurus papillomavir...	05-Oct-2...	China: ...	Equus ...	sarcoid	100.00	100.00	0
KM455051.1	(+)	5,777		6,129	Bos taurus papillomavir...	06-Jul-2...	China: ...	cow	cutaneo...	100.00	100.00	0
KM455049.1	(+)	23		375	Bos taurus papillomavir...	06-Jul-2...	China: ...	cow	sarcoid	100.00	100.00	0
KF171968.1	(+)	170		522	Bos taurus papillomavir...	2011	Spain	Bos tau...	head a...	100.00	100.00	0
KF284153.1	(+)	35		387	Bos taurus papillomavir...	06-Jun-...	Poland	equine	equine s...	100.00	100.00	0
KC878306.1	(+)	5,779		6,131	Bos taurus papillomavir...	May-2012	China	dairy cow	cutaneo...	100.00	100.00	0
MT178264.1	(+)	2		354	Bovine papillomavirus	2017	Turkey	bovine;...	skin	100.00	100.00	0
KC256805.1	(+)	170		522	Bos taurus papillomavir...	Sep-2012	China	cow		100.00	100.00	0
LC510394.1	(+)	1,544		1,896	Bos taurus papillomavir...	2016-06...	Japan:...	Bos taurus	fibropa...	100.00	100.00	0
LC510392.1	(+)	1,544		1,896	Bos taurus papillomavir...	2016-06...	Japan:...	Bos taurus	fibroma...	100.00	100.00	0
LC510387.1	(+)	1,544		1,896	Bos taurus papillomavir...	2009	Japan:...	Bos taurus	nodule ...	100.00	100.00	0
LC510386.1	(+)	1,544		1,896	Bos taurus papillomavir...	2009	Japan:...	Bos taurus	papillo...	100.00	100.00	0
LC510384.1	(+)	5,780		6,132	Bos taurus papillomavir...	2018-10...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510383.1	(+)	5,779		6,131	Bos taurus papillomavir...	2018-09...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510376.1	(+)	5,777		6,129	Bos taurus papillomavir...	2017-02...	Japan:...	Equus c...	equine s...	100.00	100.00	0
MT385854.1	(+)	25		377	Bos taurus papillomavir...	Apr-2016	Costa R...	Bos taurus		100.00	100.00	0
X01768.1	(+)	1,694		2,046	Bos taurus papillomavir...					100.00	100.00	0
KP768461.1	(+)	26		378	Bos taurus papillomavir...	21-Jan-2...	China: ...	Bos taurus	sarcoid	99.72	100.00	1
KX924602.1	(+)	3		349	Bos taurus papillomavir...	26-Feb-...	Turkey	bovine	Skin	100.00	98.30	0
M20219.1	(+)	5,770		6,122	Bos taurus papillomavir...					99.43	100.00	2
MT181841.1	(+)	2		354	Bovine papillomavirus	2017	Turkey	cattle (b...	skin	99.15	100.00	3
MT674580.1	(+)	35		381	Bos taurus papillomavir...		Brazil	Bos taurus		99.71	98.30	1
KX924601.1	(+)	1		342	Bos taurus papillomavir...	20-Apr-2...	Turkey	bovine	Skin	100.00	96.88	0
KU743470.1	(+)	1		342	Bos taurus papillomavir...	14-Feb-...	Brazil	Bos taurus		100.00	96.88	0
KU728473.1	(+)	1		341	Bos taurus papillomavir...	13-Dec-...	Brazil	Bos taurus		100.00	96.60	0
MH187961.1	(+)	5,689		6,041	Bos taurus papillomavir...		Brazil	Bos taurus	papillo...	98.58	100.00	5
KU674833.1	(+)	5,780		6,132	Bos taurus papillomavir...	20-Dec-...	Brazil	Bos taurus	fibropa...	98.58	100.00	5
KC595245.2	(+)	24		376	Bos taurus papillomavir...	08-Nov-...	Brazil	horse		98.58	100.00	5
MT674579.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		98.58	100.00	5
MT674577.1	(+)	1		294	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	83.29	0
MT674576.1	(+)	1		267	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	75.64	0
MT674574.1	(+)	1		267	Bos taurus papillomavir...		Brazil	Bos taurus		99.25	75.64	2
MG818475.1	(+)	5,790		6,142	Bos taurus papillomavir...	2017	China	cattle		89.83	99.72	34
MF327274.1	(+)	5,790		6,142	Bos taurus papillomavir...					89.83	99.72	34
KU519390.1	(+)	5,783		6,135	Bos taurus papillomavir...	01-Jun-2...	Brazil	Bos taurus	papillo...	89.83	99.72	34
KC595246.2	(+)	24		376	Bos taurus papillomavir...	08-Nov-...	Brazil	horse		89.83	99.72	34
KM258443.2	(+)	5,790		6,142	Bos taurus papillomavir...	20-Apr-2...	China	yellow ...	cutaneo...	89.83	99.72	34
KC763355.1	(+)	196		548	Bos taurus papillomavir...		Brazil	horse	equine s...	89.83	99.72	34
MT674598.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
MT674584.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
MT674583.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
JQ798171.1	(+)	5,790		6,142	Bos taurus papillomavir...		Brazil	Bos taurus	cutaneo...	89.83	99.72	34
EU293540.1	(+)	29		381	Bovine papillomavirus		Brazil			89.83	99.72	34
MF741676.1	(+)	5,701		6,053	Bos taurus papillomavir...	20-May-...	Brazil	bovine	papillo...	89.55	99.72	35
MN977322.1	(+)	158		389	Deltapapillomavirus sp.	2013	Italy	Rupicap...		100.00	65.72	0
MT674571.1	(+)	1		234	Bos taurus papillomavir...		Brazil	Bos taurus		98.72	66.29	3
HE603636.1	(+)	57		301	Deltapapillomavirus 4	12-Jan-...	India:Lu...		heifer cu...	97.14	69.41	7
MT674575.1	(+)	1		219	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	62.04	0
KF938579.1	(+)	46		398	Deltapapillomavirus 4	Aug-2013	China	cow		86.97	100.00	46
KY746722.1	(+)	5,780		6,130	Deltapapillomavirus 4	Apr-2016	Morocco	Bos taurus	skin lesion	86.61	99.43	47
MN977321.1	(+)	170		522	Deltapapillomavirus sp.	2018	Italy	Capra i...		86.40	100.00	48
MK347523.1	(+)	5,778		6,130	Deltapapillomavirus 4	2018	China	cattle		86.40	100.00	48
MK396096.1	(+)	167		519	Deltapapillomavirus 4	25-Apr-2...	India	cattle; sex:...	single d...	86.40	100.00	48
LC426023.1	(+)	170		522	Deltapapillomavirus 4	2016-08...	Japan:...	Bos Tau...	anal pa...	86.40	100.00	48
MK173052.1	(+)	128		480	Deltapapillomavirus 4	29-May-...	India	cattle; sex:...	cutaneous...	86.40	100.00	48
MG263871.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017	China: ...	cow		86.40	100.00	48
MH197482.1	(+)	5,777		6,129	Deltapapillomavirus 4	Jan-2018	Turkey	Bos tau...	sarcoid-l...	86.40	100.00	48
LC333380.1	(+)	5,778		6,130	Deltapapillomavirus 4	2014-06...	Japan	Bos taurus	papilloma	86.40	100.00	48
KY886226.1	(+)	5,778		6,130	Deltapapillomavirus 4	Sep-2012	USA	Equus fe...	sarcoid	86.40	100.00	48
MF435917.1	(+)	5,777		6,129	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	86.40	100.00	48
MF435916.1	(+)	5,778		6,130	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	86.40	100.00	48
KX907623.1	(+)	5,778		6,130	Deltapapillomavirus 4	26-Jun-2...	China: ...	dairy cow	cutaneo...	86.40	100.00	48
KY662041.1	(+)	12		384	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	86.40	100.00	48
KX594402.1	(+)	29		381	Deltapapillomavirus 4	24-Jun-2...	Poland:...	Bos taurus	skin	86.40	100.00	48
KF284141.1	(+)	35		387	Deltapapillomavirus 4	15-May-...	Poland	equine	equine s...	86.40	100.00	48
LC549664.1	(+)	5,778		6,130	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	86.40	100.00	48
LC549663.1	(+)	5,778		6,130	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	86.40	100.00	48
JX046509.1	(+)	32		384	Deltapapillomavirus 4	Sep-2002	Croatia	cattle	skin	86.40	100.00	48
JX046505.1	(+)	32		384	Deltapapillomavirus 4	May-2007	Croatia	cattle	skin	86.40	100.00	48
LC510397.1	(+)	1,538		1,890	Deltapapillomavirus 4	2018-06...	Japan:...	Bos taurus	papillo...	86.40	100.00	48
LC510391.1	(+)	1,538		1,890	Deltapapillomavirus 4	2016-06...	Japan:...	Bos taurus	fibroma...	86.40	100.00	48
LC510388.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Bos taurus	papillo...	86.40	100.00	48
LC510385.1	(+)	5,778		6,130	Deltapapillomavirus 4	2019-0...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510381.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510380.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510379.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510378.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-07...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510377.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	86.40	100.00	48
AB626705.1	(+)	5,778		6,130	Deltapapillomavirus 4		Japan:H...	Bos taurus	myoperi...	86.40	100.00	48
J02045.1	(+)	1,941		2,293	Deltapapillomavirus 4			Bos taurus		86.40	100.00	48
X02346.1	(+)	5,777		6,129	Deltapapillomavirus 4					86.40	100.00	48
U13843.1	(+)	3,257		3,609	unidentified cloning vector					86.40	100.00	48
MN977319.1	(+)	170		521	Deltapapillomavirus sp.	2018	Italy	Rupicap...		86.36	99.72	48
KX924577.1	(+)	1		349	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	86.53	98.87	47
MN977320.1	(+)	170		522	Deltapapillomavirus sp.	2018	Italy	Capra i...		86.12	100.00	49
LC510382.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.12	100.00	49
MT385853.1	(+)	25		377	Deltapapillomavirus 4	Apr-2016	Costa R...	Bos taurus		86.12	100.00	49
MW018708.1	(+)	38		390	Deltapapillomavirus sp.	2020	Egypt	cattle	skin	86.12	100.00	49
MW018707.1	(+)	38		390	Deltapapillomavirus sp.	2019	Egypt	cattle	skin	86.12	100.00	49
MT682133.1	(+)	29		381	Bovine papillomavirus	2018	Italy	Bos taurus		86.12	100.00	49
MT459820.1	(+)	5,688		6,040	Deltapapillomavirus 4	2018/2019	Egypt	equine		86.12	100.00	49

Illustration 5. NCBI multiple sequence alignment for local isolate 5 (MW658351.1).

Our genotyping findings showed that the local isolates were closely related to Type 1 and Type 2 BPV, which is in line with the results of several worldwide studies (Pangty *et al*., 2010; Bam *et al*., 2013; Grindatto *et al*., 2015). Other studies reported that BPV- 1 and BPV2 have the ability to infect buffaloes, as well as other species, such as equines inducing fibropapillomas and fibroplastic tumors (Silvestre *et al*., 2009). In this study, although Type 2 BPV was detected genotypically in some local isolates, no one of these infected animals showed bladder cancer clinical signs, such as enzootic hematuria. This could be due to a lack of Pteridium aquilinum bracken fernsin in the fields of the study animals.

Histological analysis of stained papillomas indicated the presence of tissue changes suggesting cytopathic


NCBI Multiple Sequence Alignment Viewer, Version 1.20.0

Sequence ID		Start	Alignment	End	Organism	Date	Country	Host	Source	Identity	Coverage	Mismatches
Query_4125	(+)	1		353						100.00	100.00	0
MN304951.1	(+)	3		355	Bos taurus papillomavir...	05-May-...	Costa R...	horse		100.00	100.00	0
LC426022.1	(+)	170		522	Bos taurus papillomavir...	2016-06...	Japan:...	Bos Tau...	vulval ...	100.00	100.00	0
LC426021.1	(+)	170		522	Bos taurus papillomavir...	2016-02...	Japan:...	Bos Tau...	vulval ...	100.00	100.00	0
MH589273.1	(+)	25		377	Bos taurus papillomavir...	2012	Japan	Japane...		100.00	100.00	0
KY781814.1	(+)	17		369	Bovine papillomavirus	06-Aug-...	Turkey	bovine	skin	100.00	100.00	0
MF045490.1	(+)	5,779		6,131	Bos taurus papillomavir...					100.00	100.00	0
KX113620.1	(+)	170		522	Bos taurus papillomavir...					100.00	100.00	0
KX008606.1	(+)	26		378	Bos taurus papillomavir...	19-Sep-...	China: ...	cow	sarcoid	100.00	100.00	0
KP663623.1	(+)	26		378	Bos taurus papillomavir...	27-Sep-...	China: ...	Bos taurus	sarcoid	100.00	100.00	0
KP663621.1	(+)	26		378	Bos taurus papillomavir...	05-Oct-2...	China: ...	Equus ...	sarcoid	100.00	100.00	0
KM455051.1	(+)	5,777		6,129	Bos taurus papillomavir...	06-Jul-2...	China: ...	cow	cutaneo...	100.00	100.00	0
KM455049.1	(+)	23		375	Bos taurus papillomavir...	06-Jul-2...	China: ...	cow	sarcoid	100.00	100.00	0
KF171968.1	(+)	170		522	Bos taurus papillomavir...	2011	Spain	Bos tau...	head a...	100.00	100.00	0
KF284153.1	(+)	35		387	Bos taurus papillomavir...	06-Jun-...	Poland	equine	equine s...	100.00	100.00	0
KC878306.1	(+)	5,779		6,131	Bos taurus papillomavir...	May-2012	China	dairy cow	cutaneo...	100.00	100.00	0
MT178264.1	(+)	2		354	Bovine papillomavirus	2017	Turkey	bovine;...	skin	100.00	100.00	0
KC256805.1	(+)	170		522	Bos taurus papillomavir...	Sep-2012	China	cow		100.00	100.00	0
LC510394.1	(+)	1,544		1,896	Bos taurus papillomavir...	2016-06...	Japan:...	Bos taurus	fibropa...	100.00	100.00	0
LC510392.1	(+)	1,544		1,896	Bos taurus papillomavir...	2016-06...	Japan:...	Bos taurus	fibroma...	100.00	100.00	0
LC510387.1	(+)	1,544		1,896	Bos taurus papillomavir...	2009	Japan:...	Bos taurus	nodule ...	100.00	100.00	0
LC510386.1	(+)	1,544		1,896	Bos taurus papillomavir...	2009	Japan:...	Bos taurus	papillo...	100.00	100.00	0
LC510384.1	(+)	5,780		6,132	Bos taurus papillomavir...	2018-10...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510383.1	(+)	5,779		6,131	Bos taurus papillomavir...	2018-09...	Japan:...	Equus c...	equine s...	100.00	100.00	0
LC510376.1	(+)	5,777		6,129	Bos taurus papillomavir...	2017-02...	Japan:...	Equus c...	equine s...	100.00	100.00	0
MT385854.1	(+)	25		377	Bos taurus papillomavir...	Apr-2016	Costa R...	Bos taurus		100.00	100.00	0
X01768.1	(+)	1,694		2,046	Bos taurus papillomavir...					100.00	100.00	0
KP768461.1	(+)	26		378	Bos taurus papillomavir...	21-Jan-2...	China: ...	Bos taurus	sarcoid	99.72	100.00	1
KX924602.1	(+)	3		349	Bos taurus papillomavir...	26-Feb-...	Turkey	bovine	Skin	100.00	98.30	0
M20219.1	(+)	5,770		6,122	Bos taurus papillomavir...					99.43	100.00	2
MT181841.1	(+)	2		354	Bovine papillomavirus	2017	Turkey	cattle (b...	skin	99.15	100.00	3
MT674580.1	(+)	35		381	Bos taurus papillomavir...		Brazil	Bos taurus		99.71	98.30	1
KX924601.1	(+)	1		342	Bos taurus papillomavir...	20-Apr-2...	Turkey	bovine	Skin	100.00	96.88	0
KU743470.1	(+)	1		342	Bos taurus papillomavir...	14-Feb-...	Brazil	Bos taurus		100.00	96.88	0
KU728473.1	(+)	1		341	Bos taurus papillomavir...	13-Dec-...	Brazil	Bos taurus		100.00	96.60	0
MH187961.1	(+)	5,689		6,041	Bos taurus papillomavir...		Brazil	Bos taurus	papillo...	98.58	100.00	5
KU674833.1	(+)	5,780		6,132	Bos taurus papillomavir...	20-Dec-...	Brazil	Bos taurus	fibropa...	98.58	100.00	5
KC595245.2	(+)	24		376	Bos taurus papillomavir...	08-Nov-...	Brazil	horse		98.58	100.00	5
MT674579.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		98.58	100.00	5
MT674577.1	(+)	1		294	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	83.29	0
MT674576.1	(+)	1		267	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	75.64	0
MT674574.1	(+)	1		267	Bos taurus papillomavir...		Brazil	Bos taurus		99.25	75.64	2
MG818475.1	(+)	5,790		6,142	Bos taurus papillomavir...	2017	China	cattle		89.83	99.72	34
MF327274.1	(+)	5,790		6,142	Bos taurus papillomavir...					89.83	99.72	34
KU519390.1	(+)	5,783		6,135	Bos taurus papillomavir...	01-Jun-2...	Brazil	Bos taurus	papillo...	89.83	99.72	34
KC595246.2	(+)	24		376	Bos taurus papillomavir...	08-Nov-...	Brazil	horse		89.83	99.72	34
KM258443.2	(+)	5,790		6,142	Bos taurus papillomavir...	20-Apr-2...	China	yellow ...	cutaneo...	89.83	99.72	34
KC763355.1	(+)	196		548	Bos taurus papillomavir...		Brazil	horse	equine s...	89.83	99.72	34
MT674598.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
MT674584.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
MT674583.1	(+)	35		387	Bos taurus papillomavir...		Brazil	Bos taurus		89.83	99.72	34
JQ798171.1	(+)	5,790		6,142	Bos taurus papillomavir...		Brazil	Bos taurus	cutaneo...	89.83	99.72	34
EU293540.1	(+)	29		381	Bovine papillomavirus		Brazil			89.83	99.72	34
MF741676.1	(+)	5,701		6,053	Bos taurus papillomavir...	20-May-...	Brazil	bovine	papillo...	89.55	99.72	35
MN977322.1	(+)	158		389	Deltapapillomavirus sp.	2013	Italy	Rupicap...		100.00	65.72	0
MT674571.1	(+)	1		234	Bos taurus papillomavir...		Brazil	Bos taurus		98.72	66.29	3
HE603636.1	(+)	57		301	Deltapapillomavirus 4	12-Jan-...	India:Lu...		heifer cu...	97.14	69.41	7
MT674575.1	(+)	1		219	Bos taurus papillomavir...		Brazil	Bos taurus		100.00	62.04	0
KF938579.1	(+)	46		398	Deltapapillomavirus 4	Aug-2013	China	cow		86.97	100.00	46
KY746722.1	(+)	5,780		6,130	Deltapapillomavirus 4	Apr-2016	Morocco	Bos taurus	skin lesion	86.61	99.43	47
MN977321.1	(+)	170		522	Deltapapillomavirus sp.	2018	Italy	Capra i...		86.40	100.00	48
MK347523.1	(+)	5,778		6,130	Deltapapillomavirus 4	2018	China	cattle		86.40	100.00	48
MK396096.1	(+)	167		519	Deltapapillomavirus 4	25-Apr-2...	India	cattle; sex:...	single d...	86.40	100.00	48
LC426023.1	(+)	170		522	Deltapapillomavirus 4	2016-08...	Japan:...	Bos Tau...	anal pa...	86.40	100.00	48
MK173052.1	(+)	128		480	Deltapapillomavirus 4	29-May-...	India	cattle; sex:...	cutaneous...	86.40	100.00	48
MG263871.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017	China: ...	cow		86.40	100.00	48
MH197482.1	(+)	5,777		6,129	Deltapapillomavirus 4	Jan-2018	Turkey	Bos tau...	sarcoid-l...	86.40	100.00	48
LC333380.1	(+)	5,778		6,130	Deltapapillomavirus 4	2014-06...	Japan	Bos taurus	papilloma	86.40	100.00	48
KY886226.1	(+)	5,778		6,130	Deltapapillomavirus 4	Sep-2012	USA	Equus fe...	sarcoid	86.40	100.00	48
MF435917.1	(+)	5,777		6,129	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	86.40	100.00	48
MF435916.1	(+)	5,778		6,130	Deltapapillomavirus 4	2016	China	cattle	cutaneo...	86.40	100.00	48
KX907623.1	(+)	5,778		6,130	Deltapapillomavirus 4	26-Jun-2...	China: ...	dairy cow	cutaneo...	86.40	100.00	48
KY662041.1	(+)	12		384	Deltapapillomavirus 4	02-Sep-...	Iraq	cattle	skin wart	86.40	100.00	48
KX594402.1	(+)	29		381	Deltapapillomavirus 4	24-Jun-2...	Poland:...	Bos taurus	skin	86.40	100.00	48
KF284141.1	(+)	35		387	Deltapapillomavirus 4	15-May-...	Poland	equine	equine s...	86.40	100.00	48
LC549664.1	(+)	5,778		6,130	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	86.40	100.00	48
LC549663.1	(+)	5,778		6,130	Bovine papillomavirus	2019-07	Japan:...	Equus c...	sarcoid	86.40	100.00	48
JX046509.1	(+)	32		384	Deltapapillomavirus 4	Sep-2002	Croatia	cattle	skin	86.40	100.00	48
JX046505.1	(+)	32		384	Deltapapillomavirus 4	May-2007	Croatia	cattle	skin	86.40	100.00	48
LC510397.1	(+)	1,538		1,890	Deltapapillomavirus 4	2018-06...	Japan:...	Bos taurus	papillo...	86.40	100.00	48
LC510391.1	(+)	1,538		1,890	Deltapapillomavirus 4	2016-06...	Japan:...	Bos taurus	fibroma...	86.40	100.00	48
LC510388.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Bos taurus	papillo...	86.40	100.00	48
LC510385.1	(+)	5,778		6,130	Deltapapillomavirus 4	2019-0...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510381.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510380.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510379.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510378.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-07...	Japan:...	Equus c...	equine s...	86.40	100.00	48
LC510377.1	(+)	5,778		6,130	Deltapapillomavirus 4	2017-10...	Japan:...	Equus c...	equine s...	86.40	100.00	48
AB626705.1	(+)	5,778		6,130	Deltapapillomavirus 4		Japan:H...	Bos taurus	myoperi...	86.40	100.00	48
J02045.1	(+)	1,941		2,293	Deltapapillomavirus 4			Bos taurus		86.40	100.00	48
X02346.1	(+)	5,777		6,129	Deltapapillomavirus 4					86.40	100.00	48
U13843.1	(+)	3,257		3,609	unidentified cloning vector					86.40	100.00	48
MN977319.1	(+)	170		521	Deltapapillomavirus sp.	2018	Italy	Rupicap...		86.36	99.72	48
KX924577.1	(+)	1		349	Deltapapillomavirus 4	03-Oct-2...	Turkey	bovine	Skine	86.53	98.87	47
MN977320.1	(+)	170		522	Deltapapillomavirus sp.	2018	Italy	Capra i...		86.12	100.00	49
LC510382.1	(+)	1,538		1,890	Deltapapillomavirus 4		Japan:...	Equus c...	equine s...	86.12	100.00	49
MT385853.1	(+)	25		377	Deltapapillomavirus 4	Apr-2016	Costa R...	Bos taurus		86.12	100.00	49
MW018708.1	(+)	38		390	Deltapapillomavirus sp.	2020	Egypt	cattle	skin	86.12	100.00	49
MW018707.1	(+)	38		390	Deltapapillomavirus sp.	2019	Egypt	cattle	skin	86.12	100.00	49
MT682133.1	(+)	29		381	Bovine papillomavirus	2018	Italy	Bos taurus		86.12	100.00	49
MT459820.1	(+)	5,688		6,040	Deltapapillomavirus 4	2018/2019	Egypt	equine		86.12	100.00	49

Illustration 6. NCBI multiple sequence alignment for local isolate 6 (MW658352.1).

action of BP. Acanthosis was the most microscopic skin alteration observed in stained biopsies, which might be due to the mitogenic role of BPVs. Araldi *et al*. (2015) determined that the epithelium and connective tissues are directly affected by BPV, and chromosomal alterations and DNA breaks were seen incomet assay, suggesting cellular hyperactivity of the virus. However, Özsoy *et al*. (2011) identified epidermal hyperplasia and dermal irregular papillary projection to various degrees in animals infected with BP. Grindatto *et al*. (2015) demonstrated that there were no connections between the involved viral types and histopathological findings.

In the current study, mild to moderate positive immunoreactions were observed in the tissues assigned to specific immunohistochemical staining.

Table 1. Identity (%) sequencing between local BPV isolates and NCBI-BLAST BPV isolates based on NCBI-BLAST homology.

Local BPV isolate			BPV NCBI BLAST isolate		
Name	**Access no.**	**Type**	**Access no.**	**Source-host, country**	**Identity (%)**
Deltapapillomavirus 4 isolate Cattle-No. 1	MW658347.1	Type 1	KF284133.1	Sarcoid, Equine, Poland	99.22
Bostaurus papillomavirus 2 isolate Cattle-No.2	MW658348.1	Type 2	MH187961.1	Papilloma, *Bos taurus*, Brazil	100
Deltapapillomavirus 4 isolate Cattle-No. 3	MW658349.1	Type 1	KF284133.1	Sarcoid, Equine, Poland	100
Deltapapillomavirus 4 isolate Cattle-No. 4	MW658350.1	Type 1	KF284133.1	Sarcoid, Equine, Poland	99.47
Bostaurus papillomavirus 2 isolate Cattle-No. 5	MW658351.1	Type 2	MH187961.1	Papilloma, *Bos taurus*, Brazil	99.55
Bostaurus papillomavirus 2 isolate Cattle-No. 6	MW658352.1	Type 2	MH187961.1	Papilloma, *Bos taurus*, Brazil	99.66

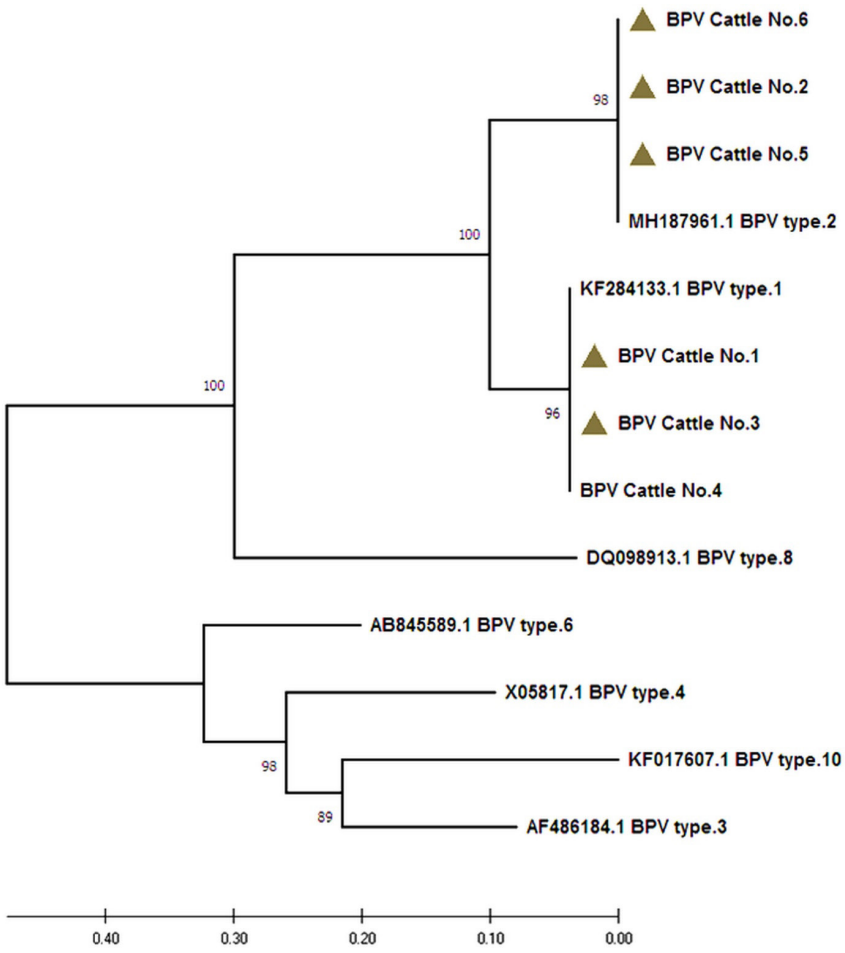


Fig. 3. Analysis of phylogenetic tree according to the L1 gene in local BPV isolates. A UPGMA tree in MEGA (version 6.0) was applied. The local BPV isolates exhibited a close relationship to NCBI-BLAST isolates in terms of total genetic changes (0.4%–0.10%).

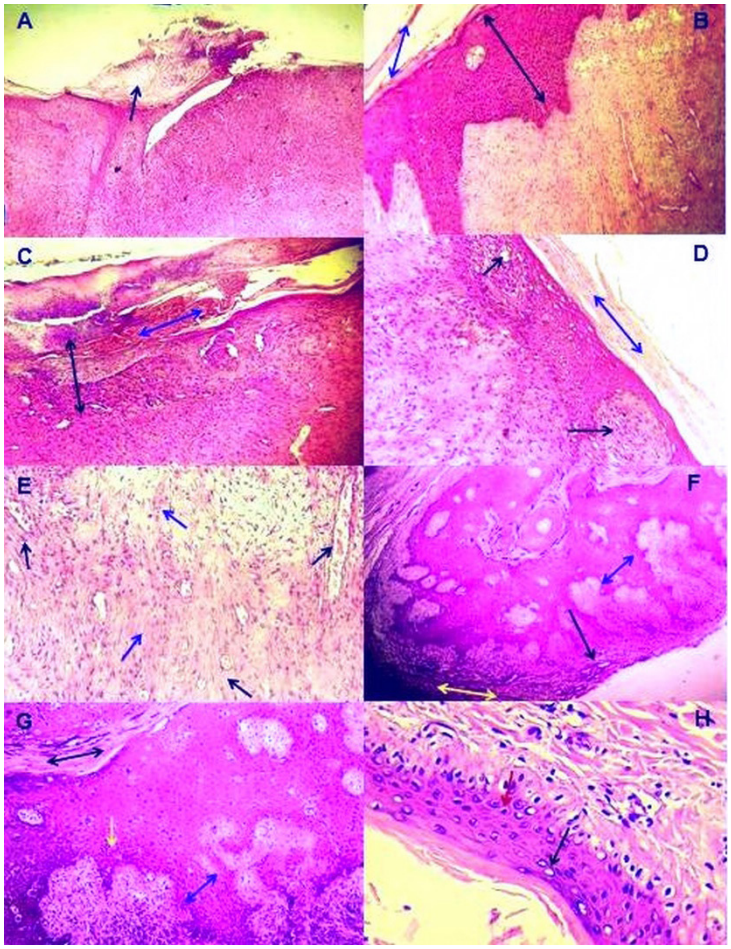


Fig. 4. Histopathological changes in skin lesions infected with BP. (A): Mature finger like projection papillae with rete pegs which grown in the stratum corneum in the epidermis of skin (×400); (B): Thickening of epidermis due to diffuse hyperplasia in the stratum spinosum layer (Black dual arrow) with hyperkeratosis (Blue dual arrow) (×100); (C): Severe infiltration of MNCs in epidermis and dermis of skin (Black dual arrow) with massive hemorrhage in epidermal layer (Blue dual arrow) in addition to dermal fibroplasia (×100); (D): Multifocal spongiosis (Black arrow) with hyperkeratosis (Blue dual arrow), (×200); (E): Dermis with moderate neovascularization (Black arrow) and fibroplasia due to fibrous connective proliferation (Blue arrow), (×200); (F): Marked acanthosis (Yellow dual arrow) with moderate elongation of retention ridge towards dermis (Blue dual arrow) and rings of calcification observed in epidermal surface (Black arrow), (×100); (G): Hyperkeratosis, parakeratosis, severe elongation of retention ridge towards dermis, acanthosis (Blue dual arrow) and infiltration of inflammatory cells (Yellow arrow), (×200); (H): Necrotic finding in epidermal layer mainly in basal cells (Black arrow) with nuclear pyknosis of some spinosum cells (Blue arrow), (×400). All sections were stained with H&E, and the images represent all study animals.

El-Mandrawy and Alam (2018) identified that infected cells were more susceptible to TNF stimulation by releasing various cytokines and inducing inflammation in various tissues at the viral stage of the disease. Başbuğ *et al*. (2016) suggested that high levels of TNF-α may be involved in the assessment of the clinical course of the disease. Goodman *et al*. (2011) determined that the combination of TNF and recombinant vaccine based on virus protein rapidly reduced infection; however, the overall picture obtained during anti-TNF treatment of infected animals is unclear and may reveal some differences. It has been suggested that the convenient support for TNF antiviral effects comes from the fact that the family of viruses contains a variety of factors that regulate TNF-related activities. Many poxviruses encode soluble versions of TNF receptors and, more importantly, disrupt the viral TNFR gene in a virus, leading to a reduction in virus virulence (Kerr *et al*., 2015; Alvarez-de Miranda *et al*., 2021).

Growth factors are crucial for the growth, development and homeostasis of almost all organisms and are required for tissue specialization, apoptosis, cell survival and viability, and fate determination (Mukai *et al*., 2018; Koons and Mikos, 2019). Growth factor receptors transmit extracellular signals by activating intracellular messengers or by transporting the receptors

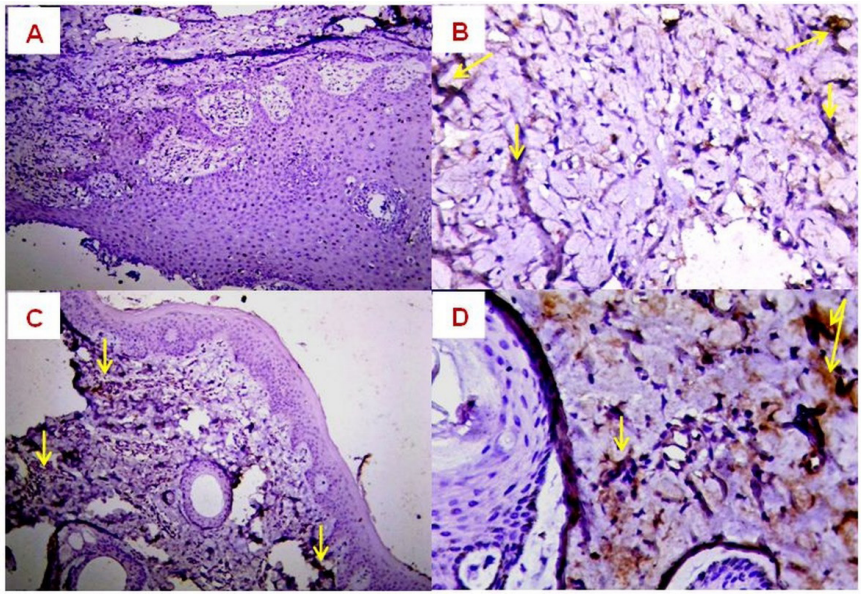


Fig. 5. Expression ofTNF-α in BP skin lesions using IHC with Mayer's hematoxylin. (A): 0 (×100); (B): +1 (×400); (C): +2 (×200); (D): +2 (×400) reactions.

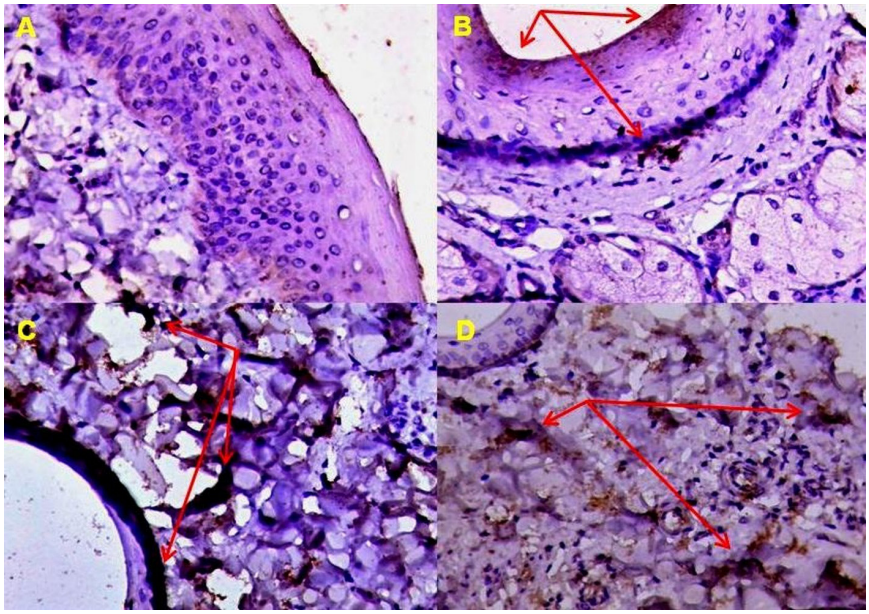


Fig. 6. Expression of EGFR in BP skin lesions using IHC with Mayer's hematoxylin. (A): 0 (×100); (B): +1 (×200); (C): +1 (×400); (D): +2 (×200) reactions.

directly to the nucleus (Liu *et al*., 2020). EGFR is a specific factor receptor of transmembrane proteins that share structural and functional similarities (Nagano *et al*., 2018). Although, EGFR is a key indicator involved in predicting the diseases and their prognostic value, however, the EGFR-IHC role is still unclear. Several studies have reported that reduced signals of EGFR and other tyrosine kinase receptors are associated with disease, while increased activity is associated with the development of various tumors (Lian *et al*., 2019; Xia *et al*., 2020; Hong *et al*., 2022). Suppression of binding sites of EGFR signaling at the level of extracellular receptor or tyrosine kinase intracellular activity may inhibit EGFR expression networks and thus improve patient outcomes (Kitamura *et al*., 2020; Wingert *et al*., 2021). Several authors have suggested that the increased

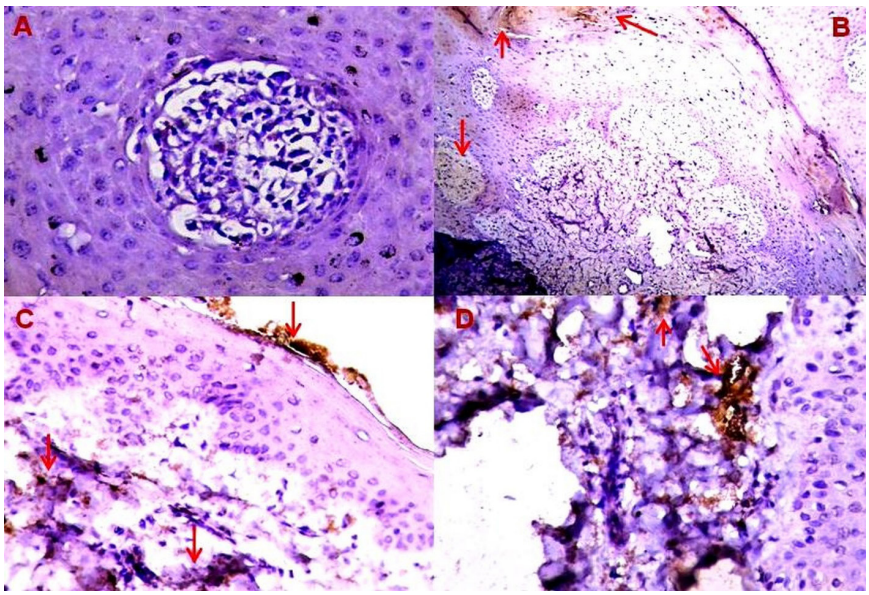


Fig. 7. Expression of Fascin in BP skin lesions using IHC with Mayer's hematoxylin. (A): 0 (×400); (B): +1 (×100); (C): +1 (×400); (D): +2 (×400) reactions.

expression of EGFR has negative impacts (Kwon *et al*., 2018; Hashmi *et al*., 2019; London and Gallo, 2020), while others maintain the opposite (Pidugu *et al*., 2019; Feng *et al*., 2020; Lamtha *et al*., 2022). However, meta-analysis data summarizing the results of various studies show that exposure to EGFR is not an independent predictor of survival in various diseases (Yang *et al*., 2019; Nastały *et al*., 2020; Atef *et al*., 2021).

Fascin represents a family of active binding proteins that are localized in stress fibers and throughout the proliferation of various cells, such as myoblasts and glioma cells (Ogunlade *et al*., 2020). This protein is unique to muscle and cell types, such as glial cells, neurons, antigen-presenting dendritic cells, and endothelial cells (Zhang *et al*., 2022). This observation suggested that Fascin plays a role in cell movement or interaction with other cell types (Chung *et al*., 2022).

Conclusion

Interestingly, the BPVs exhibited a cytopathic role that interacted with cellular structure and function and promoted cytological and histomorphological changes; however, the number of available studies remains low. Thus, the current study highlights the interesting essential data that provide insight into mechanisms to acknowledge the possible oncogenesis and pathogenesis of BPV. Furthermore, the study emphasized that IHC is a specific crucial diagnostic tool that provides precise detection of the cellular and intracellular distribution of viral and antigenic proteins using specific antibodies. Moreover, targeting other PV types in cattle and other animal species, disease prevalence, animal breeding biosafety evaluation and fundamental vaccine roles and production are of great interest.

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Authors' contribution

HAJG: Clinical examination of study animals, collection of papilloma samples, molecular examination and documentation of local isolates in the NCBI. SJJA: Histological and immunohistochemical processing of papilloma tissue samples, and created stained slides. TJH: Histopathological and immunohistochemical examination and reading of the slides by light microscopy. Data analysis was performed by both HAJG and SJJA. The authors have read and approved the final version of this manuscript as they participated in the writing of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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