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Molecular and epidemiological aspects related to bovine papular stomatitis in large ruminants in Assiut governorate, Egypt

Fatma E. Mahran[1], Khaled A.S. El-Khabaz[1,2], Osama A. Ali[1], Zainab M.A. Youssef[1*]

[1]*Infectious Diseases, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Postal code: 71526, Egypt.*
[2]*School of Veterinary Medicine, Badr University in Assiut (BUA), Assiut, Postal code: 71516, Egypt.*

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*Correspondence:

Corresponding author: Zainab M.A. Youssef
E-mail address: zeinabmohammed613@aun.edu.eg

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ABSTRACT

Bovine papular stomatitis (BPS) is a widespread epitheliotropic viral disease affects ruminants of all ages and considered a serious risk to public health. In Assiut governorate, there is little information on BPS despite their financial losses. Thus, the goal of this study was to confirm diagnosis on a molecular base, describe the clinical findings of the disease, and study the relationship between some factors that may play a role in the spread of the disease. The present investigation was conducted on 39 cattle and 11 buffaloes that belonged to different villages in Assiut governorate, Egypt. Whole blood and oral lesions swabs samples were taken for laboratory testing. Semi-nested polymerase chain reaction (semi-nested PCR) had been used for detection of BPS virus (BPSV). The viral DNA was detected in 32 cattle and 8 buffaloes. The result of clinical examination indicated that the clinical signs of BPS were oral (ulcers in gum, palate, papillae & tongue), commissure, muzzle, and nostrils lesions with one or more of other clinical signs such as fever, diarrhea, enlarged superficial lymph nodes, corneal opacity, respiratory distress and skin lesions in studied animals. Studying the effect of some factors on the spread of the disease revealed that the prevalence was significantly higher in middle areas of the governorate than in other areas. To lower the prevalence of BPSV, it is recommended to avoid eating hard hay and reeds that cause small abrasion in oral cavity and aid increasing frequency of BPSV infection.

Introduction

Oral lesions associate several viral diseases that affect cattle and buffaloes and can pose real diagnostic problems both clinically and at necropsy (Holliman, 2005). Which may be in the form of vesicles, erosion, ulceration, and necrosis of the oral mucosa (Radiostits *et al*., 2007). Viral diseases that causing oral lesions are BPS, foot and mouth disease, vesicular stomatitis, bluetongue, malignant catarrhal fever, bovine viral diarrhea and infectious bovine rhinotrachitis (Holliman, 2005). BPS is a common epitheliotropic viral disease affecting calves (Oem *et al*., 2013). BPS is caused by BPSV that belongs to genus *Parapoxvirus* (PPV), subfamily *Chordopoxvirinae* in *Poxviridae* family (Akbari *et al*., 2023). PPV differentiated into five species: BPSV of cattle, orf virus (OrfV) of sheep and goats, and PPV of red deer in New Zealand (PVNZ) as well as three tentative species of the genus, auzdyk disease virus, chamois contagious ecthyma virus and sealpox virus (Oem *et al*., 2013).

The viral genome consists of a linear double-stranded DNA of ~134 kb in length (Ebling *et al*., 2020). Using negative staining electron microscopy, the virus particles can appear to have a bamboo cage structure because a crisscross filament pattern covers their surface (Kato *et al*., 2021). BPSV induces papular lesions on muzzle, inside the nares, oral cavity, teats, and sometimes esophagus and forestomach in cattle (Akbari *et al*., 2023). The semi-nested PCR is the gold standard diagnostic method used to diagnose BPS depending on major envelope protein (B2L) gene (Hirano *et al*., 2021; Akbari *et al*., 2023). This method makes it possible to identify and distinguish between BPSV and Pseudocowpox virus, OrfV and PVNZ, which belong to the same genus and cause comparable lesions in ruminants other than cattle (Oem *et al*., 2013; Hirano *et al*., 2021). Still, there is no data available on studying BPS in Assiut Governorate, so the present study aimed to molecularly identify a B2L of the BPSV genome depended on semi-nested PCR, assess the clinical findings, and investigate the relationship between certain risk factors, including species, sex, age, seasonal variation and locality, and BPS infection rate.

Materials and methods

Ethical approval

Every animal used in this study was handled ethically. The investigation was approved by the Faculty of Veterinary Medicine's Research Ethical Committee at Assiut University in Assiut, Egypt; the approval number was 06/2024/0253. This investigation is part of a master thesis focused on exploring the viral etiology of oral lesions in large ruminants in Assiut governorate (Mahran, 2025).

Animals

During the duration of study, from September 2023 to August 2024, a total of 39 cattle and 11 buffaloes of all ages and sexes from different villages in Assiut Governorate were came to Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University. The studied diseased cattle and buffaloes were assessed clinically in compliance with Jackson and Cockcroft (2002). The investigated animals had variable different signs with varying degrees of severity suggesting that they were infected with BPSV. The commonly noticed signs were lesions in oral cavity observed mainly on the gum with one or more of other clinical signs such as fever, diarrhea, enlarged superficial lymph nodes, corneal opacity, respiratory distress and skin lesions. From each diseased animal, whole blood and oral swabs samples were collected.

Sampling

From each diseased cattle and buffalo, in addition to oral swabs that obtained from oral lesions by using sterile cotton swab that put in phosphate buffer saline (PBS, pH7.4), 2 ml of whole blood were collected from jugular vein into sterile vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA). These samples were kept at -20°C for the DNA extraction subsequently.

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Molecular testing

Extraction of viral DNA

Fifty samples of whole blood and 50 swabs samples from oral lesions were utilized to extract viral DNA using ABT Genomic DNA Mini Extraction Kit (Cat. No. EX01, Applied Biotechnology, Egypt), following the directions provided by the manufacturer.

Primers

The current investigation assessed the specificities of the primers (Metabion International AG, Germany) selected for B2L gene of BPSV based on previous study (Inoshima *et al*., 2000). Primer sequences in the viral genome are illustrated in Table 1.

Table 1. Primer nucleotide sequences of B2L gene of BPSV and the sizes of the products obtained via semi-nested PCR.

Primer	Nucleotide sequences	Product size bp
PPP1 (F1)	5'-GTC GTC CAC GAT GAG CAG CT-3'	594
PPP4 (R)	5'-TAC GTG GGA AGC GCC TCG CT-3'	
PPP3 (F2)	5'-GCG AGT CCG AGA AGA ATA CG-3'	236
PPP4 (R)	5'-TAC GTG GGA AGC GCC TCG CT-3'	

Using semi-nested PCR to identify BPSV B2L gene

Potential of a particular semi-nested PCR used to amplify BPSV B2L gene. DNTPs and polymerase enzyme were obtained from the ABT red master mix (2X) (Applied Biotechnology, Egypt) for present work. PCR was conducted using a PCR thermocycler (Techine, UK) and subsequent reagents were utilized: A final volume of 16 µl that includes 8 µl ABT red master mix (2X), 0.5 µl of each primer (5 pmol), 3 µl DNA sample, and 4 µl RNase-free water in first round. While second round included 8 µl ABT red master mix (2X), 0.5 µl of each primer (5 pmol) ,1 µl PCR product of first round, and 6 µl RNase-free water. In briefly, the thermal cycling conditions were one 9 minutes initial denaturation at 95°C (30 cycles of denaturation at 94°C for one minute, 55°C for one minute for the annealing step, and 72°C for one minute for extension), and a final 10 minutes extension at 72°C.

PCR product evaluation and identification

Seven microliters of amplified PCR products were loaded for reaction observation. Before being examined using a gel UV transilluminator (Syngene, United Kingdom), the amplicons were subjected to a 60 minutes gel electrophoresis procedure at 90 V and 155 mA in a 1.5% agarose gel stained with ethidium bromide (10 mg/ml). The size of the amplicons was assessed using size of 100 bp DNA ladder.

Statistical analysis

The epidemiological results were obtained and analyzed using the Chi-square of independence (2007) utilizing the statistical package for the social sciences (SPSS) version 16 software.

Results

Clinical findings of BPS in examined cases

BPS in cattle and buffaloes employed in this investigation displayed the usual clinical manifestations like ulcers in oral cavity noticed mainly on gum, palate, papillae & tongue beside ulcers on commissure, muzzle, and nostrils with one or more of other clinical signs such as fever, diarrhea, enlarged superficial lymph nodes, corneal opacity, respiratory distress and skin lesions (Table 2).

Molecular diagnosis of BPSV in diseased animals according to type of samples

DNA samples were tested during the first round of the semi-nested PCR based on B2L gene to produce the necessary bands at 594 bp, and products yielded from the first PCR reaction were tested throughout the second round to generate the specific diagnostic DNA band at 236 bp (Fig.1). BPSV was found in 80% (40/50) of the studied animals. Forty samples were proved positive (20 samples were detected in both first round at 594bp & second round at 236 bp and another 20 samples were detected in second round at 236bp). Additionally BPSV DNA was detected in different type of samples (whole blood and oral swabs samples) from diseased cattle and buffaloes (Table 3).

Comparison between the result of whole blood and oral swabs samples in detection of BPS in examined animals

There was no difference between blood and oral swabs samples for diagnosis of BPS (Table 4).

Table 2. Clinical signs of BPS in diseased animals.

Clinical findings	No. of investigated diseased animals	BPS	
		Positive	Negative
		No. (%)	No. (%)
Ulcers in gum and diarrhea	5	3 (60%)	2 (40%)
Ulcers in gum and fever	10	8 (80%)	2 (20%)
Ulcers in gum, diarrhea and fever	6	5 (83.33%)	1 (16.67%)
Ulcers in gum, commissure, muzzle & nostrils, respiratory signs and fever	4	2 (50%)	2 (50%)
Ulcers in gum, respiratory signs, fever and enlarged superficial lymph node	9	8 (88.89%)	1 (11.11%)
Ulcers in gum, respiratory signs, fever and corneal opacity	3	3 (100%)	0 (0%)
Ulcers in gum, diarrhea, respiratory signs, fever and enlarged superficial lymph node	3	3 (100%)	0 (0%)
Ulcers in gum, respiratory signs, fever, enlarged superficial lymph node and lameness	1	1 (100%)	0 (0%)
Ulcers in gum & papillae, respiratory signs and enlarged superficial lymph node	3	2 (66.67%)	1 (33.33%)
Ulcers in gum, palate, muzzle & nostrils, diarrhea, respiratory signs, fever, corneal opacity and skin exanthema	3	2 (66.67%)	1 (33.33%)
Ulcers in tongue & palate, respiratory signs and fever	3	3 (100%)	0 (0%)
Total	50	40 (80%)	10 (20%)

Table 3. Detection rate of BPSV DNA from diseased animals according to type of samples.

Animals	No.	Samples type	No.	Molecular results			
				+ve		-ve	
				No.	%	No.	%
Cattle	39	Oral swab	39	28	71.79	11	28.21
		Blood	39	26	66.67	13	33.33
Buffaloes	11	Oral swab	11	8	72.73	3	27.27
		Blood	11	7	63.64	4	36.36

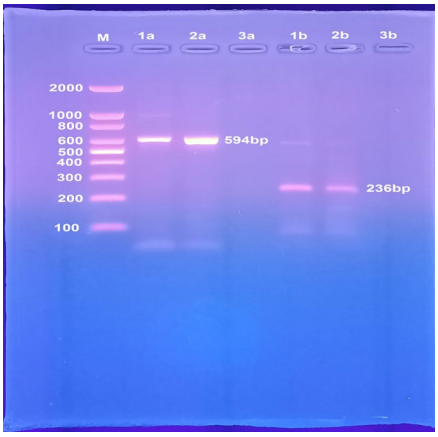

Fig. 1. Agarose gel electrophoresis of semi-nested PCR following B2L gene amplification of BPSV, Lane M: DNA ladder 100 bp, lanes 1a and 2a: positive samples of PCR first round and lanes 1b and 2b: positive samples of PCR second round samples and lanes 3a and 3b: negative sample in two PCR rounds.

Table 4. Comparison between the results of BPS detection in whole blood and oral swabs samples in the examined animals.

		Whole blood		Total
		Positive	Negative	
Oral swab	Positive	29	7	36
	Negative	4	10	14
Total		33	17	50

Potential risk factors

This study was dealt with some risk factors such as species, age, sex, season and locality that influence the prevalence of BPSV infection (Table 5). No discernible variation was seen in the percentages of BPSV infection by species, age, sex, and season that were subjected to molecular testing but middle Assiut region had a greater rate of BPSV infection than other regions (Table 5).

Discussion

BPS is a widespread epitheliotropic viral disease affects cattle of all ages and considered a serious risk to public health and characterized by developing of papules or nodules that progress to vesicles and then crusts or scabs on the lips, gingiva and tongue (Khudhair *et al.*, 2024). In this study, by using semi-nested PCR based on B2L gene, 40 samples were proved positive (20 samples were detected in first and second rounds at 594bp and 236 bp, respectively and another 20 samples were detected in second round at 236bp). This result may be attributed to semi-nested PCR was more sensitive than conventional PCR for diagnosis of BPSV infection. The semi-nested PCR is the gold standard diagnostic method used to diagnose BPS depending on B2L gene (Hirano *et al.*, 2021; Akbari *et al.*, 2023).

According to findings of the current study, detection rate of BPSV infection in the studied animals in Assiut Governorate was 82.05% (32/39) in cattle and 72.73% (8/11) in buffaloes with overall prevalence 80% (40/50). This finding was almost similar to the previously recorded by Micheloud *et al.* (2020) and Khudhair *et al.* (2024). The higher prevalence of BPSV

Table 5. Association between BPSV infection in examined diseased animals and potential risk factors according to semi-nested PCR result.

Variant		No. of examined animals	PCR				P-value
			Positive		Negative		
			No.	%	No.	%	
Species	Cattle	39	32	82.05	7	17.95	0.5
	Buffalo	11	8	72.73	3	27.27	
	Total	50	40	80.00	10	20.00	
Age	3 months - 1.5 year	16	10	62.50	6	37.50	0.1
	>1.5 - 3 years	20	18	90.00	2	10.00	
	>3 - 5years	14	12	85.71	2	14.29	
	Total	50	40	80.00	10	20.00	
Sex	Male	20	14	70.00	6	30.00	0.15
	Female	30	26	86.67	4	13.33	
	Total	50	40	80.00	10	20.00	
Season	Summer	7	4	57.14	3	42.86	0.1
	Autumn	22	16	72.73	6	27.27	
	Winter	14	13	92.86	1	7.14	
	Spring	7	7	100.00	0	0.00	
	Total	50	40	80.00	10	20.00	
Locality	North Assiut region	12	5	41.67	7	58.33	0.00
	Middle Assiut region	29	27	93.10**	2	6.90	
	South Assiut region	9	8	88.89	1	11.11	
	Total	50	40	80.00	10	20.00	

No significant variation at $p<0.05$ **Highly significant increase at $p<0.001$ (0.000)

may be attributed to high resistance of BPSV to environmental factors and is a contagious virus outside the body for an extended period of time (Robinson and Lyttle, 1992; Hsu *et al*., 2024), so there's a chance that more large ruminants would contract BPSV (Oem *et al*., 2013). It is believed that BPS is spread by minor scratches when an animal comes into close contact with the injury and animals can also become indirectly infected by instruments contaminated with lesion or scab tissue (Hsu *et al*., 2024), as well as in breeding facilities and grazing grounds where scabs have fallen off and small scratches in the oral cavity which caused by hard hay (Sudan grass) was fed to the calves in the cattle pen where the outbreak occurred (Kato *et al*., 2021). This result was higher than those earlier studies by De Sant'Ana *et al*. (2012); Oem *et al*. (2013); Akbari *et al*. (2023) and Hsu *et al*. (2024). The variation in the findings may be related to various sample numbers and distinct study areas. Furthermore, there is currently no vaccine in our country to prevent BPSV infection, different sample types and using different technique.

Regarding to our result, most of diseased animals had BPSV infection was detected in both whole blood and oral swab samples. This finding indicating that no difference between whole blood and oral swabs samples for diagnosis of BPS and presence of this virus in oral swab samples suggests that communication patterns may be one way for its spread. The existence of BPSV in whole blood samples may be explained by entrance of the virus into bloodstream causing a primary viraemia followed by a secondary viraemia (Yesari *et al*., 2020).This result was nearly similar to the previous recorded investigation by Hsu *et al*. (2024) who recorded that the existence of BPSV in the esophageal-pharyngeal fluid, nasal swabs, and oral swabs of affected calves.

The observed clinical signs of BPSV infection were ulcers in gum, palate, papillae, commissure, muzzle & nostrils, cough, enlarged superficial lymph node, nasal discharge, fever, diarrhea, corneal opacity, lameness and skin exanthema. These clinical findings were the same as those described in previous studies recorded by Micheloud *et al*. (2020); Yesari *et al*. (2020); Akbari *et al*. (2023) and Hsu *et al*. (2024). After skin invasion, the BPSV enters the bloodstream causing a primary viremia followed by a secondary viremia leading to the spread of the virus back to the skin and other target organs and this virus induces degenerative changes by their proliferation in endothelial cells or in the epithelium leading to the formation of typical lesions (Yesari *et al*., 2020). Broken or damaged skin is typically the first sign of BPSV infection, which is followed by virus replication in keratinocytes along with nearby focally extensive ulcers in the epidermis (Brown *et al*., 2007; Micheloud *et al*., 2020).

The current work was dealt with the role of some risk factors with the prevalence rate of BPS in large ruminants. These studied risk factors include species, age and sex of examined animals; additionally the seasonal variation and locality were also involved. In respect to the susceptibility of species, the rate of BPSV infection in cattle and buffaloes under study was similarly susceptible with no statistical difference. Cattle are BPS's primary host, even though the current study found no species-specific differences. Our results indicated that although there were differences in number of positive BPS animals in the different age groups, the statistical analysis showed that these differences were not significant. Our results suggest that the age groups of animals under study are equally susceptible to contracting BPS. Our result concurred with Cargnelutti *et al*. (2012) and Khudhair *et al*. (2024) who reported that all ages of cattle can contract BPSV. In the present study, we observed that the old aged animals was higher mathematically in susceptibility to BPSV infection than young aged animals, this may be attributed to breeders deal with old aged animals by giving them rough foods (hard hay and reeds) that causing small abrasions in oral cavity which act as an entry point for BPSV. However, these breeders take more care of the young ones by giving them easily digested green fodders. In terms of sex susceptibility, there was no statistically significant difference in the rate of BPSV infection between the male and female animals under study, this may indicate that BPS is not a sex-related disease and that male and female animals are equally likely to get it. Our result concurred with Khudhair *et al*. (2024) who recorded that no significant variation between sex of cattle and infection of BPSV. When the frequency of BPSV infection and seasonal variations were examined in our study, there was no statistically significant variation between the four seasons. This may be attributed to BPSV can withstand the wide variation in an ambient temperature all over the year (Robinson and Lyttle, 1992). Our result corresponded with Khudhair *et al*. (2024) who recorded that no significant variation between seasons (winter and summer season) and infection of BPSV. According to locality, the detection rate of BPSV infection in investigated animals was statistically higher in middle Assiut region than other regions. This outcome may be due to the economic situation of the people in the central regions of the governorate , some farmers had starting using dry corn stalks to feed their animals, which may result in oral abrasions that are an entry point for BPSV infection.

Conclusion

The current investigation found cattle and buffaloes in Assiut Governorate, Egypt were infected with BPS. These findings have an impact on the economy and emphasize the need for effective prevention and control measures to be put in place throughout Egypt in order to lower the prevalence of BPSV. Furthermore, laboratory testing is required to validate any clinical suspicions of BPS in order to rule out diseases that share clinical similarities with BPS, such as foot-and-mouth disease, vesicular stomatitis, blue tongue, malignant catarrhal fever, bovine viral diarrhea, and infectious bovine rhinotracheitis.

Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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