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Original Findings Associated with Two Cases of Bovine Papular Stomatitis▽

F. Dal Pozzo,[1]§ L. Martinelle,[2]§ L. Gallina,[3]§ J. Mast,[4] P. Sarradin,[5] E. Thiry,[1] A. Scagliarini,[3] M. Büttner,[6] and C. Saegerman[2]*

Veterinary Virology and Animal Viral Diseases, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Boulevard de Colonster 20, B-4000 Liège, Belgium[1]; Research Unit of Epidemiology and Risk Analysis Applied to the Veterinary Sciences (UREAR), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Boulevard de Colonster 20, B-4000 Liège, Belgium[2]; Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna, via Tolara di Sopra 50, 40064 Ozzano Emilia, Bologna, Italy[3]; Veterinary and Agrochemical Research Centre, Interactions and Surveillance, EM-unit, Groeselemberg 99, B-1180 Uccle, Brussels, Belgium[4]; INRA UE 1277, Experimental Infectiology Platform, INRA—Research Center of Tours, Nouzilly, France[5]; and Bavarian Health and Food Safety Authority, 85764 Oberschleissheim, Germany[6]

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Bovine papular stomatitis virus was isolated from two calves in an animal house with biosafety level 3 confinement. The hypotheses on the origin of the infection, the interesting features of the partial amino acid sequences of the major envelope viral protein, and the importance of diagnostic tools available for animal diseases that are not listed by the World Organization for Animal Health (OIE) are discussed.

CASE REPORT

Two 7-month-old female Holstein calves were housed in an insect-secure zone of biosafety level 3 (A3). They originated from two different farms (located about 80 km away from each other), with no history of animal transfer or contacts. They were housed with 10 other calves of the same age, and they all tested seronegative and nonviremic for bluetongue virus and bovine viral diarrhea virus and seronegative for bovine herpesvirus 1. One month after the animal introduction in the A3 facility, lesions localized in the inner sides of the lips and gums were observed in one calf (9108). The lesions appeared flat, characterized by brownish erosions in the form of a ring or a horseshoe (Fig. 1A). This aspect allowed the veterinarian to formulate a suspicion of bovine papular stomatitis (BPS). Ten days after the first observation, similar lesions were observed in a second calf (3643) (Fig. 1B) and a biopsy specimen was taken for laboratory analysis.

The initial suspicion of BPS was supported by transmission electron microscopy (TEM) performed on scrapings of the lesions of calf 9108. Large amounts of particles with the characteristic size (approximately 320 nm by 190 nm) and ovoid morphology of parapoxviruses were observed. These demonstrated a typical criss-cross pattern of the filaments at their surface (Fig. 1C and D). A PCR was carried out on the DNA purified from the animal biopsy specimens using the pan-parapoxvirus primer pair PPP-1 and PPP-4 (7), and a unique and specific band of 594 bp, corresponding to a partial sequence of the B2L gene, was obtained, leading us to confirm a parapoxvirus infection. The amplification products were sequenced and compared, revealing 100% identity between the two isolates. The ClustalW alignment performed to compare the genomic sequences to those of other parapoxviruses showed that the highest nucleotide identity (97%) was found with bovine papular stomatitis virus (BPSV), followed by parapoxvirus of red deer in New Zealand (PVNZ) (85.9%), pseudocowpoxvirus (PCPV) (84.3 to 85.1%), and orf virus (OV) (83.8 to 83.9%) strains. In order to verify the BPSV viability, viral isolation was carried out on primary lamb keratinocytes (PLK) starting from homogenized specimens of the biopsy specimens, and after two blind passages, viral cytopathic effect was observed.

BPS is a widely distributed infection caused by BPSV, a member of the *Parapoxvirus* genus within the *Poxviridae* family. It is responsible for a mild and generalized disease in bovines, whereas a localized skin infection can develop in humans (2). The so-called "milker's node" characterized by papulae and pustulae is irrespective of the etiology (BPSV or PCPV) and is caused by close contact with an infected bovine, while the similar local dermatitis caused by OV is called contagious ecthyma and is due to contact with infected sheep or goats (10). Both are neglected zoonoses, often underestimated due to the frequent benign resolution of the lesions as well as the lack of specific etiology formulated by physicians (1). In this report, no human infection has been recorded thanks to the prophylactic measures adopted by the animal technicians and the veterinarian (systematic wear of gloves and working clothes). Furthermore, in order to reduce the risk of human transmission, calves were treated daily with a nonspecific iodine-based topical antiseptic.

In this report, the two infected calves experienced a local

* Corresponding author. Mailing address: Research Unit of Epidemiology and Risk Analysis Applied to the Veterinary Sciences (UREAR), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, Boulevard de Colonster 20, B42, B-4000 Liège, Belgium. Phone: 32 4 366 45 79. Fax: 32 4 366 42 61. E-mail: claude.saegerman@ulg.ac.be.
§ These three authors contributed equally to the research.
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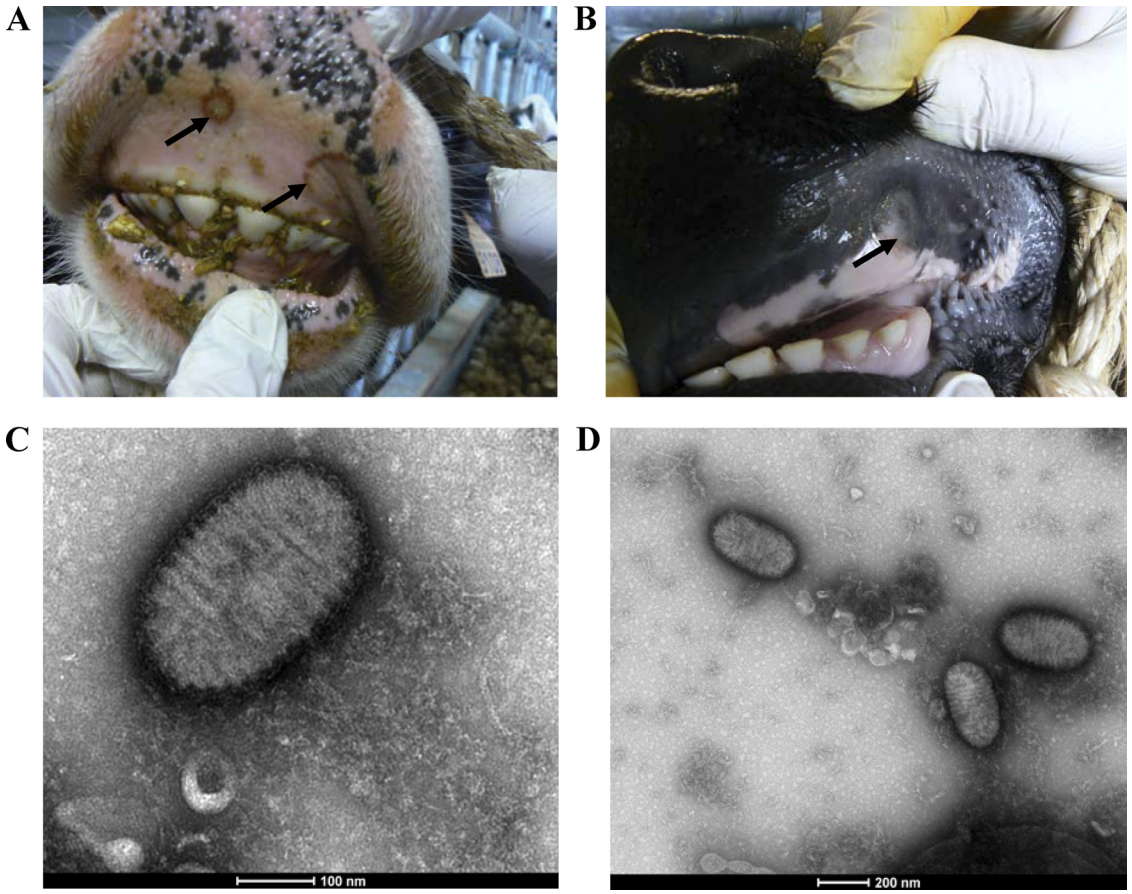

FIG. 1. Typical brownish erosions in the form of a ring or a horseshoe on the muzzles of calves 9108 (A) and 3643 (B). Lesions are pointed out by the use of black arrows. (C and D) Representative micrographs of uranyl acetate-stained parapox-like particles in sample 9108.

infection with lesions in the inner side of the lips and gums, without significant variation of their appetites, body temperatures, and clinical patterns of the superficial lymph nodes. It is therefore very likely that the infection would have been unnoticed under field conditions. The detection of the lesions 1 month after the calves' introduction in the A3 facility raises questions on the origin of the infection. Viral DNA detection was performed on different environmental samples (commercial fodder produced under good manufacturing practice measures, litter, swabs from metallic stockyard), without finding evidence of a viral presence. Epidemiological investigations revealed that none of the 10 other calves came from the two farms from which calves 9108 and 3643 originated. Furthermore, each calf moved from its farm only at the moment of the introduction into the A3 facility. The 12 calves were kept in three groups, separated by plastic wall panels preventing all contacts. Calves 9108 and 3643 were in two different groups. The high biosafety measures applied during the management of the calves imposed a change of gloves, a passage though a disinfectant footbath, and the use of clean attire before working with each group. Despite the contagious nature of BPSV and the high vigilance of the veterinarian during the daily inspection of the calves, further clinical cases were not observed. Concerning the origin of the infection, the negative findings of the epidemiological investigation on the environmental samples and the limited number of clinical cases are not sufficient to exclude the hypothesis of an acute infection during the housing in the A3 zone. Parapoxviruses are highly resistant, and an undefined passive carrier (infected animal or contaminated objects) could have brought the virus inside the A3 zone. Another hypothesis is a chronic BPSV infection with lesions periodically produced under stress conditions. A short viremic phase with the isolation of a parapoxvirus from peripheral blood leukocytes in a cow treated with gamma interferon has been reported (15). This unique description raises the question of the potential occurrence of systemic virus spread, although in the field the most common findings are associated with the presence of BPSV in the skin and mucous membranes (2). The potential sites of viral persistence are not described, but the hypothesis that the virus establishes chronic subclinical infection with occasional clinical episodes may be supported by outbreaks of overt BPS in calves following experimental thymectomy, antilymphocyte globulin treatment, and sham thymectomy (16). Parapoxviruses are very stable agents especially when double enveloped particles are present; their longevity in mucous mem-

TABLE 1. Parapoxvirus species used for the phylogenetic analyses of the major envelope protein B2L gene

Parapoxvirus species and strain	Geographic origin	Host	GenBank accession no.
PCPV			
IT1303/05	Italy	Bovine	JN171852
B035/00	Germany	Bovine	JN171853
V 619	Germany	Bovine	JN191575
00/03	Cameroon	Bovine	JN171855
1/07	Germany	Bovine	JN171856
F00.128	Finland	Reindeer	AY453653
F00.91	Finland	Reindeer	AY453658
F00.120	Finland	Reindeer	GQ329669
VR634	New Zealand	Human	GQ329670
F99.177C	Finland	Bovine	AY453663
27/03	Cameroon	Bovine	JN191577
21/03	Cameroon	Bovine	JN171858
35	Cameroon	Bovine	JN171859
OV			
NZ-2	New Zealand	Sheep	U06671
AICHI	Japan	Japanese serow	AB521165
F92.849	Cameroon	Reindeer	AY453659
373/08	Italy	Ibex	HQ239072
PVNZ			
168/09	Italy	Red deer	HQ239068
BPSV			
B040/01	Germany	Bovine	JN171854
V 380/74	Germany	Bovine	JN171860
CE 41	Sudan	Camel	JN171861
9108[a]	France	Bovine	JN162119
30/03	Cameroon	Bovine	JN191576
Aomori	Japan	Bovine	AB044797
Chiba	Japan	Bovine	AB044798
AR02	United States	Bovine	AY386265
V660	Japan	Bovine	AY453664

[a] The sequence of sample 3643 was not submitted to GenBank because it was 100% identical to the BPSV 9108 sequence.

branes in the oral cavity and in the skin needs to be determined.

The partial amino acid sequences of the major envelope protein encoded by the B2L gene of isolates 9108 and 3643 were aligned by ClustalW and compared to other BPSV strains, showing 96.7% identity. This partial protein sequence has been frequently used for phylogenetic analyses of parapoxviruses (5, 6, 14, 18), showing that PCPV and OV strains are normally characterized by a certain level of variability, while PVNZ (14) and BPSV strains demonstrate 100% identity, in spite of the different geographic origins and years of isolation (Table 1 and Fig. 2). The viruses isolated in this study showed several residue substitutions at positions 168 (Y/R), 196 (D/N), 261 (L/V), 288 (V/A), and 301 (I/S) in the major envelope protein; however, the evolutionary analyses confirmed that isolates 9108 and 3643 belonged to the BPSV species (Fig. 2).

The exact identification of the etiological agent was made possible through an interactive collaboration of teams, sharing their expertise in different domains (clinicians, virologists, epidemiologists). As for other zoonotic poxviruses, BPS is not listed among the animal diseases to be reported to the World Organization for Animal Health (OIE) and the infection in humans is considered a neglected zoonosis since its incidence is often unknown or greatly underestimated. In the last few years, several case reports (8, 11, 12, 18) have underlined the presence and wide distribution of the parapoxvirus infections in cattle. BPS, as a part of the differential diagnosis of several OIE listed diseases, does not rely on readily available diagnostic assays, leading to misdiagnosis with other pathogens causing vesicular disease in ruminants, and practical problems emerged during the 2001 outbreak of the pan-Asiatic type O foot-and-mouth disease virus in the United Kingdom (4).

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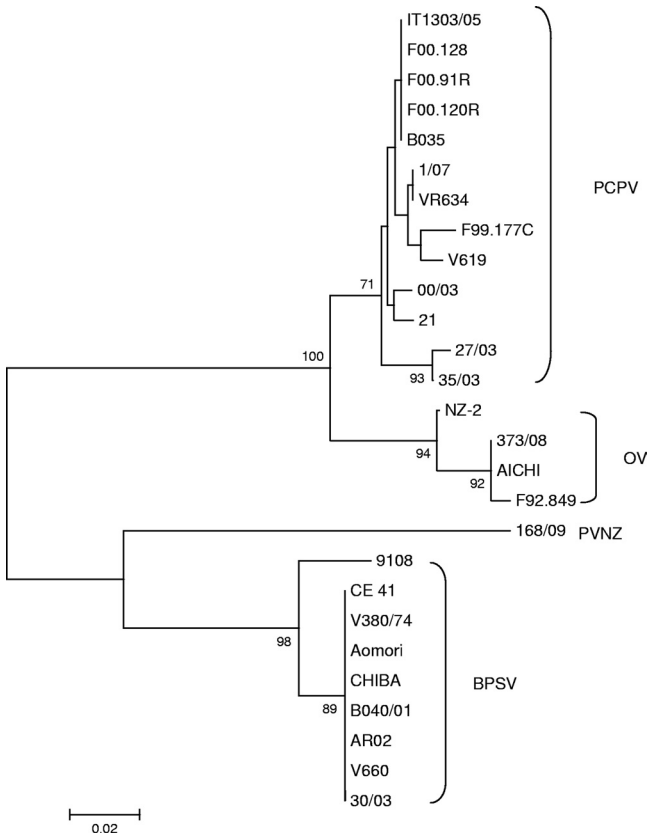

FIG. 2. Phylogenetic tree generated using the neighbor-joining method (13) after ClustalW alignment of the amino acid sequences of the major envelope protein encoded by the B2L gene segment. The different BPSV, OV, PCPV, and PVNZ isolates used in this analysis are listed in Table 1 with the corresponding GenBank accession numbers. The evolutionary distances were computed using the JTT matrix-based method (9). The rate variation among sites was modeled with a gamma distribution. Numbers on the nodes show the percentage of bootstrap calculated for 500 replicates (3) and only values higher than 70% are shown. Evolutionary analyses were conducted with MEGA 5 (17).

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