Original findings associated with two cases of bovine papular stomatitis

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Abstract

Bovine papular stomatitis virus was isolated from two calves in an animal house with biosecurity 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 as well as the importance of diagnostic tools available for World Organization for Animal Health (OIE) not listed animal diseases, 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 ten other calves of the same age and they were all tested seronegative and non-viraemic for bluetongue virus, bovine viral diarrhoea virus and seronegative for bovine herpesvirus 1. One month after the animal introduction in the A3 facility, lesions localized in the inner side 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 (Figure 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) (Figure 1B) and a biopsy 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. High 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 filament at their surface (Figure 1C, 1D). A PCR was carried out on the DNA purified from the animal biopsies 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 BPSV, followed by parapoxvirus of red deer in New Zealand (PVNZ) (85.9%), pseudocowpoxvirus (PCPV) (84.3-85.1%) and orf virus strains (OV) (83.8-83.9%). In order to verify the BPSV viability, viral isolation was carried out on primary lamb keratinocytes (PLK) starting from homogenized specimens of the biopsies 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 bovine, while a localized skin infection can develop in humans (2). The so-called "milker's node" characterized by papulae and pustulae is irrespective of the aetiology (BPSV or PCPV) and is caused by close contact with 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 aetiology 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 non-specific iodine-based topical antiseptic.

In this report, the two infected calves underwent a local infection with lesions in the inner side of the lips and gums, without significant variation of their appetite, 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 one 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 evidences of viral presence. Epidemiological investigations revealed that none of the ten other calves came from the two farms from which originated 9108 and 3643. Furthermore, each calf moved from its farm only at the moment of the introduction in the A3 facility. The twelve 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 biosecurity measures applied during the management of the calves imposed change of gloves, passage though a disinfectant footbath and use of clean attire between each group. Despite the contagious feature 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 BSL-3 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 upon stress situations. A short viraemic phase with the isolation of a parapoxvirus from peripheral blood leucocytes in a cow treated with interferon gamma has been once 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 to 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 membranes 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

114 analyses of parapoxviruses (5; 6; 14; 18), showing that PCPV and OV strains are normally
115 characterized by a certain level of variability, while PVNZ (14) and BPSV strains demonstrate
116 100% identity, in spite of the different geographic origin and the year of isolation (Table 1 and
117 Figure 2). The viruses isolated in this study showed several residue substitutions at position 168
118 (Y/R), 196 (D/N), 261 (L/V), 288 (V/A) and 301 (I/S) in the major envelope protein, however,
119 the evolutionary analyses confirmed that isolates 9108 and 3643 belonged to the BPSV species
120 (Figure 2).

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

132

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Figures and table

Figure 1: Typical brownish erosions in the form of a ring or a horseshoe on the muzzle of calves 9108 and 3643, in A and B respectively. 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.

Figure 2: Phylogenetic tree generated using the Neighbor-Joining method (13) after ClustalW alignment of the aminoacid 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 number. 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) andonly values higher than 70% are shown. Evolutionary analyses were conducted with MEGA 5 (17).

TABLE 1: Parapoxvirus species used for the phylogenetic analyses of the major envelope protein B2L gene. The sequence of the sample 3643 was not submitted to GenBank because was 100% identical to the BPSV 9108 sequence.

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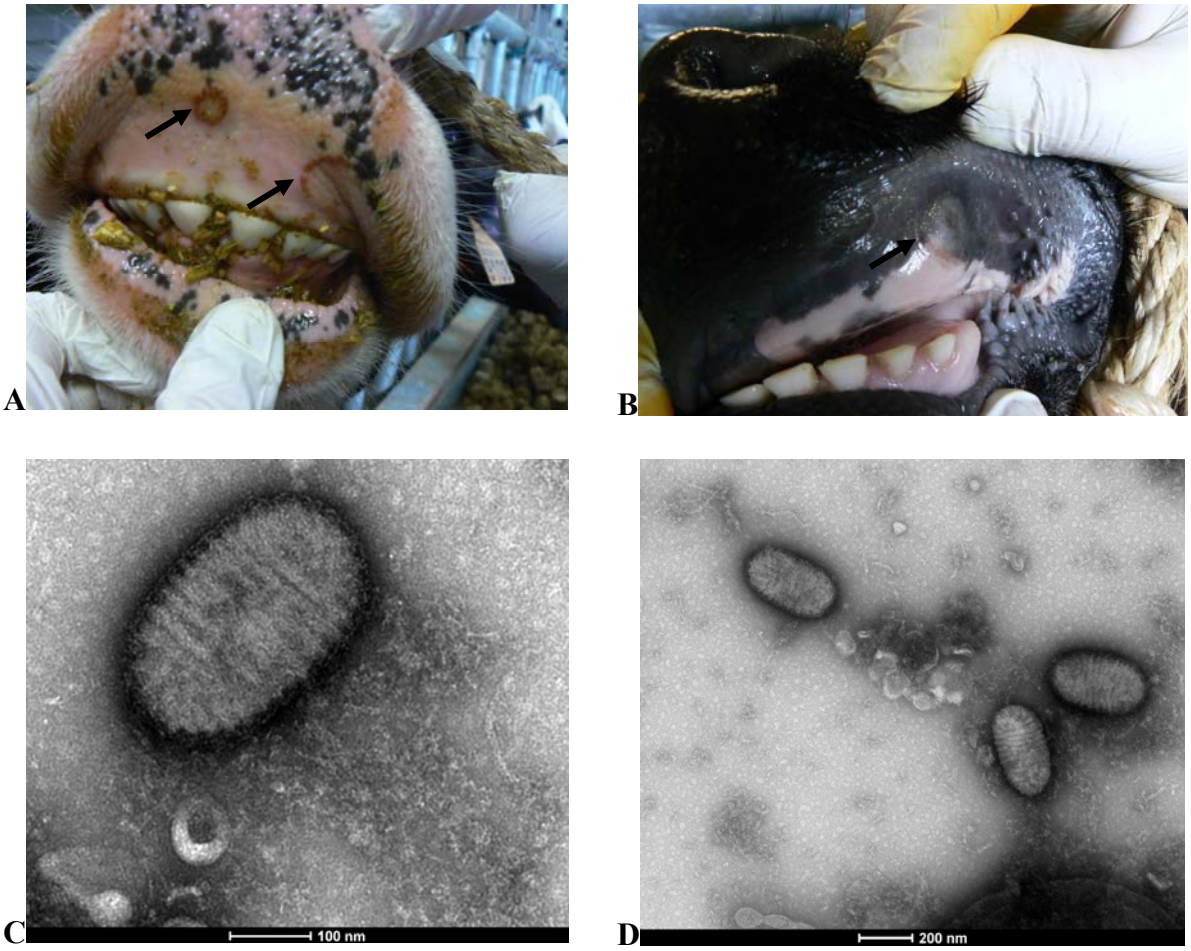
Parapoxvirus species	Geographic origin	Host	GenBank accession n.
PCPV			
IT1303/05	Italy	Bovine	JN171852
B035/00	Germany	Bovine	JN171853
V 619	Germany	Bovine	JN191575
00/03	Cameroon	Bovine	JN171855
1/07	Germany	Bovine	JN171856
F00.128	Finland	Reindeer	AY453653
F00.91	Finland	Reindeer	AY453658
F00.120	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	France	Bovine	JN162119
30/03	Cameroon	Bovine	JN191576
Aomori	Japan	Bovine	AB044797
Chiba	Japan	Bovine	AB044798
AR02	USA	Bovine	AY386265
V660	Japan	Bovine	AY453664



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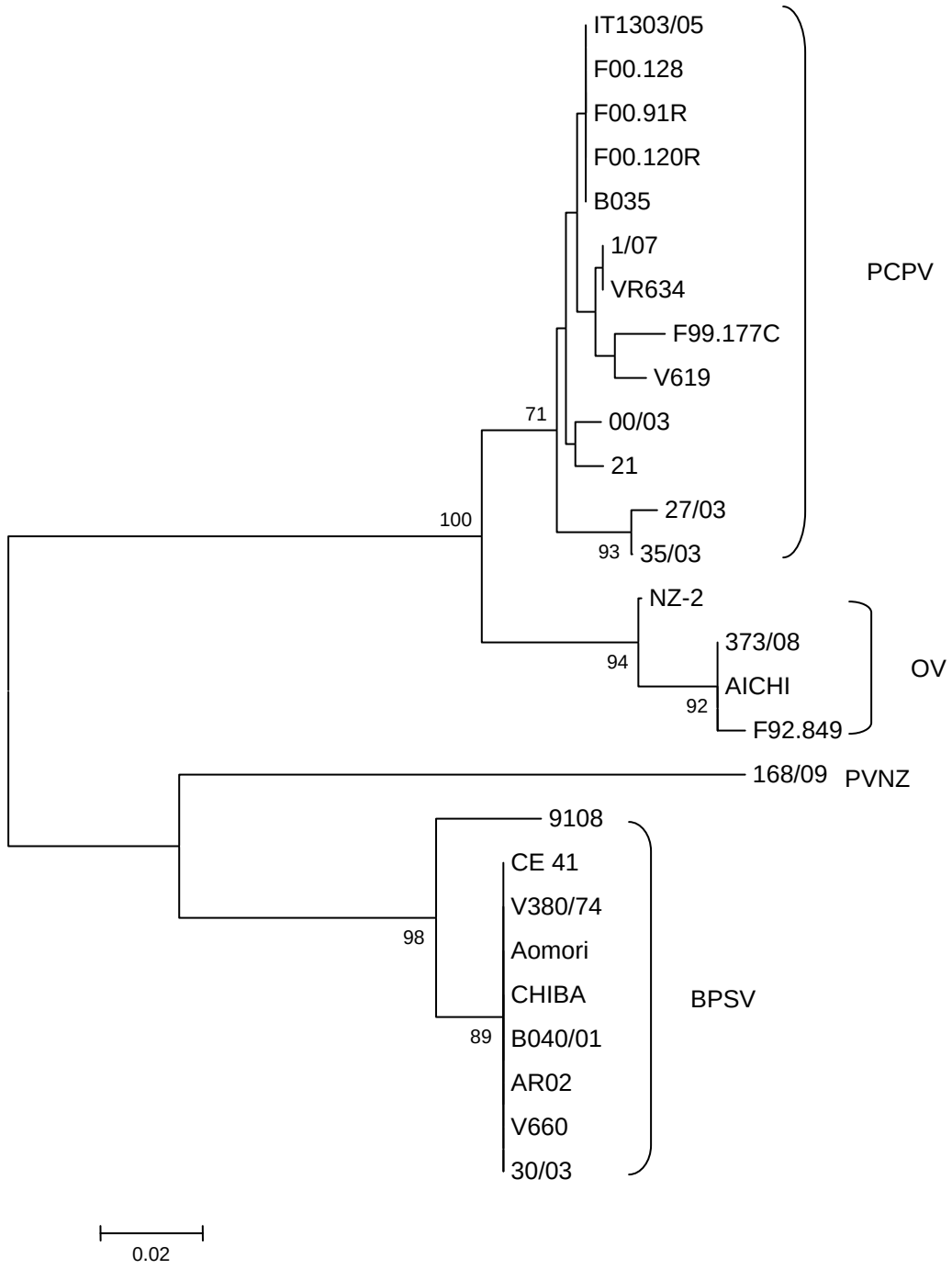

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