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CASE REPORT

Are Parapoxvirus zoonotic diseases doomed to remain neglected?

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SUMMARY

Parapoxvirus (PPV) infections are considered neglected zoonoses because their incidence is often unknown or greatly underestimated despite being endemic globally. Here, we report the comprehensive diagnostic workflow that led to the identification of two cases of persistent PPV infections. The results obtained underline the importance of adopting a "One Health" approach and cross-sectoral collaboration between human and veterinary medicine for precise aetiological diagnosis and correct management of patients affected by zoonotic diseases.

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INTRODUCTION

Despite worldwide reports of classical and complicated forms in immunocompetent and immunocompromised patients in different regions of the world (Büttner, Rziha, 2002; Oem *et al.*, 2013; Bohelay, 2019; Kassa, 2021), it seems that parapoxvirus (PPV) infections in humans and animals are destined to remain neglected. PPVs belong to the family Poxviridae, which comprises orf virus (ORFV), pseudocowpox virus (PCPV), bovine popular stomatitis virus (BPSV), and parapoxvirus of red deer in New Zealand (PVNZ). PPVs have a worldwide distribution and a restricted host spectrum including small ruminants (ORFV), domestic and wild ruminants (BPSV and PCPV), and humans; even if they are endemic globally their true impact remains largely unknown. In addition, these infections are not included on the list of notifiable diseases compiled by the World Animal Health Organization (OIE). PPV zoonotic transmission has been reported in different parts of the world; humans can be infected by direct or indirect contact with animals during occupational, religious, or cultural practices (Hosamani *et al.*, 2009). Human PPV infections represent an occupational risk for people who handle infected animals or their products (wool, hides, meat); therefore, the most affected groups are farmers, slaughterers, milkers, shearers, veterinarians, and hunters. In recent years, cases have also been reported outside these risk categories, such as in children infected due to home or recreational exposure, making diagnosis even more difficult (Lederman *et al.*, 2007; Tack and Reynolds, 2011). In humans, PPVs are often responsible for ulcerative-crusty skin lesions on the hands, arms and face accompanied by regional lymphadenopathy, mostly with a benign and self-limiting outcome (Lederman *et al.*, 2007). Complications include erythema multiforme, bullous pemphigoid, swan-neck deformity, paresthesia, and autoimmune vesicular disorders requiring frequent dermatological consultations (White *et al.*, 2008; Hosamani *et al.*, 2009; Brinkmann, 2012; Alian *et al.*, 2015; Joseph *et al.*, 2015; Gallina *et al.*, 2016; Wu *et al.*, 2021). Localization and appearance of PPV lesions share clinical features with other zoonotic infections such as leishmaniasis, anthrax and infection caused by orthopoxviruses (OPXV), including cowpoxvirus and vaccinia virus (Lewis-Jones, 2004; Schmidt *et al.*, 2006; Scagliarini *et al.*, 2012). Failure to reach an aetiological diagnosis can lead to serious consequences, especially in certain categories of patients, such as children and

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immunocompromised subjects, who can develop atypical, persistent and recurrent forms (Hosamani *et al.*, 2009). The lack of an aetiological diagnosis also leads to the false perception that these diseases in animals and humans have no socio-economic impact. Here, we report the comprehensive diagnostic workflow that led to the identification of two cases of persistent and invalidating PPV infections.

CASE REPORTS

Case 1

A 43-year-old woman in good health presented at the Infectious Disease Unit of the University Hospital of Bologna (Bologna, Italy) due to the appearance of several skin lesions involving the right forearm and the ipsilateral hand. She had no fever, blood examination showed normal count of WBCs ($9.63x10^9$/L), and had no alteration of C reactive protein (0.36 mg/dL). Renal and hepatic functions were also normal. Skin lesions of the right hand and forearm were multiform; the lesion on the first finger appeared as serum-hematic plaque and papillomatous nodule (*Figure 1a*), lesions on the second finger and the back of the hand presented as acute exudative red plaques with a central crust (*Figure 1b*), while the lesions on the forearm showed a maculo-papular and targeting morphology.

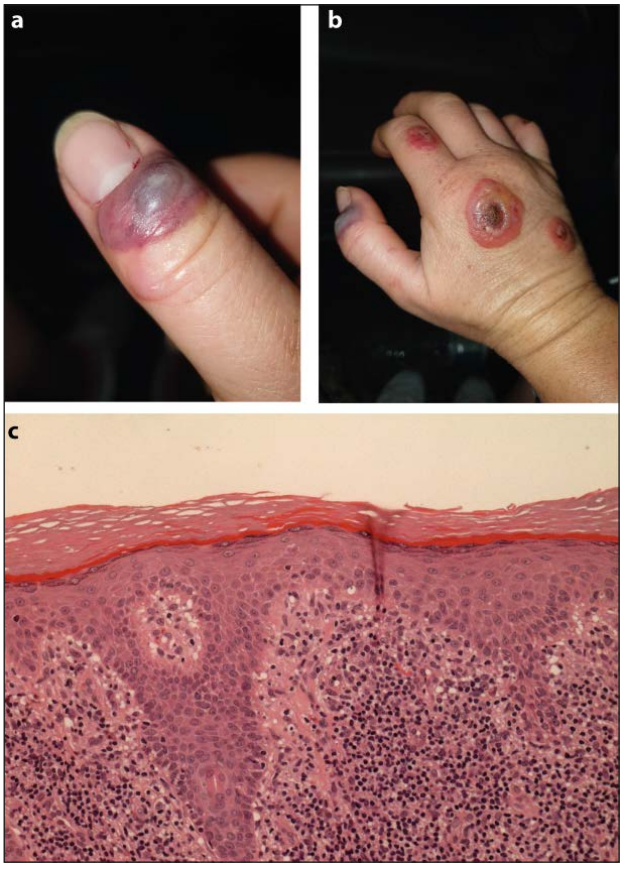

Figure 1 - *Clinical and histological features of case 1: a. serum-haematic plaque and papillomatous nodule; b. acute exudative red plaques with central crust. c. acanthotic epidermis and dense lichenoid inflammatory infiltrate in the superficial dermis consisting of lymphocytes, histiocytes and plasma cells.*

The patient, a farmer from northern Italy breeding cows and sheep, reported being bitten on the right hand while bottle feeding a calf one week before the lesion onset. Empiric antibiotic treatment with amoxicillin and ciprofloxacin was started immediately after exposure, but resulted ineffective to prevent lesion development. Therefore, the antibiotics were discontinued after one week and a topical treatment with fusidic acid was administered twice a day to prevent skin staphylococcal superinfection. A biopsy of the lesions was performed, showing acanthotic epidermis and dense lichenoid inflammatory infiltrate in the superficial dermis consisting of lymphocytes, histiocytes and plasma cells. Special stain (PAS, Grocott) did not reveal fungi. No *Leishmania* amastigotes were identified by Giemsa staining and paraffined-embedded bioptic sections tested negative for leishmanial DNA by real time PCR (Gaspari *et al.*, 2017). Given the working and environmental risk factors, a One Health approach was applied to identify the cause of skin lesions in the index patient. The DNA extracted from Formalin Fixed Paraffin Embedded (FFPE) sections was analysed at the zoonoses laboratory of the Department of Experimental, Diagnostic and Specialty Medicine (DIMES, University of Bologna) with the attempt of identifying pathogens causing look-alike diseases. A panPox Real-Time PCR was carried out to detect *Poxviridae* DNA (Luciani *et al.*, 2021) while a Loop-Mediated Isothermal Amplification (LAMP) and a Realtime PCR were performed to detect *Bacillus anthracis* (Upadhyay *et al.*, 2021; Kędrak-Jabłońska *et al.*, 2018). The positive result to the panPox Realtime PCR was subsequently confirmed by HGC Realtime PCR (Li *et al.*, 2010) that revealed the presence of PPV DNA. A qualitative heminested PCR (Inoshima *et al.*, 2000) allowed amplification of a specific product of 235 bp that was finally sequenced and identified as a BPSV (GenBank accession number MW370521). Following the diagnosis of BPSV infection, no further treatment was performed; the lesions recovered spontaneously within three months (*Figure 1c*).

Case 2

A 70-year-old woman, born in Albania but residing for years in Ravenna, northern Italy, presented to an outpatient dermatology clinic in Modena (Italy) due to the occurrence of a voluminous hypertrophic and ulcerative red mass of the second finger of the left hand that appeared six months earlier and slowly increased in size (*Figure 2a*). The lesion was swelling and itching, but not painful. Clinically, as-

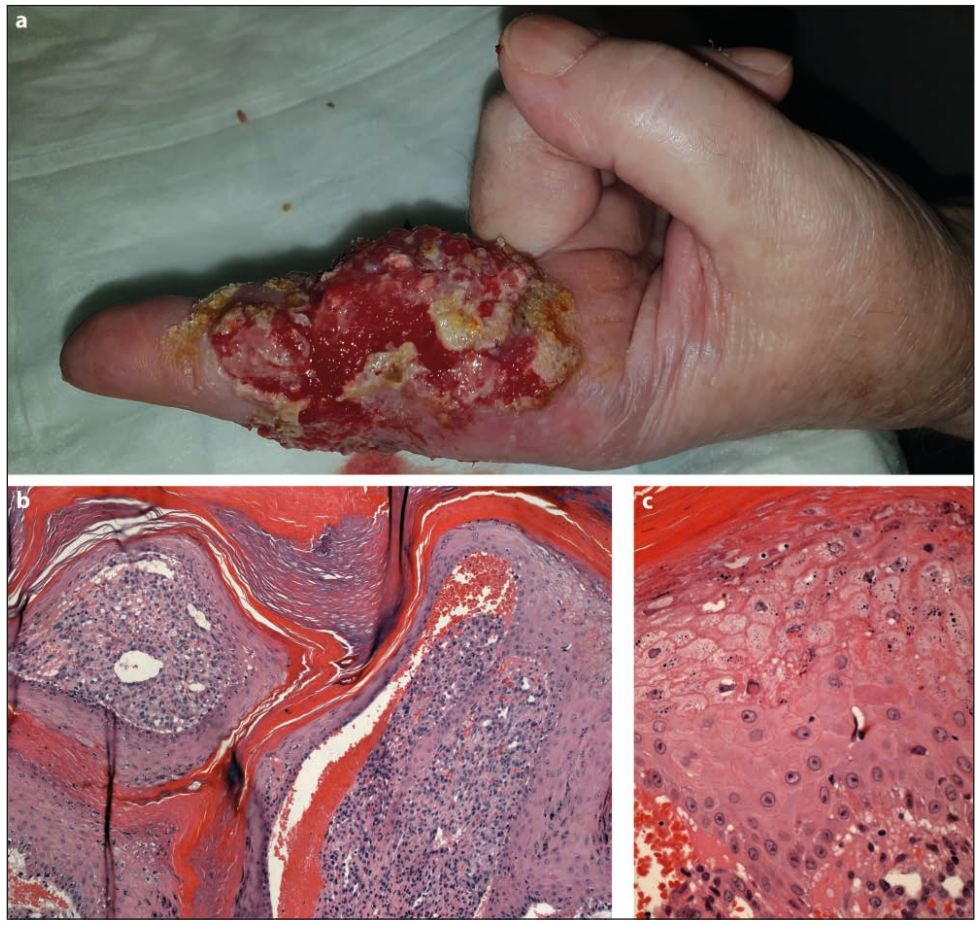

Figure 2 - *Clinical and histological features of case 2: a. voluminous ulcerative swelling and bleeding mass of the second finger of the left hand; b.-c. necrotic epidermis with hyper-parakeratosis, papillomatous features and scattered eosinophilic cytoplasmic inclusions.*

pects of tenosynovitis and panniculitis, with stiffness in extension and limited flexion were present. The patient reported handling raw animal entrails with bare hands for food preparation during a trip to Albania one week before the onset of the lesion. Complete blood count, renal and hepatic function, blood glucose, serological findings for HIV, HCV, HBV, C reactive protein, VES, and paraneoplastic markers were normal. Quantiferon TB test for tuberculosis was positive. ANA Reflex tests for antinuclear antibodies was weakly positive with a titre of 1:80. Five months after the onset of the lesion, shaving plus diatermocoagulation of the lesion was carried out due to suspicion of a hypertrophic pyogenic granuloma. The patient was also treated with several antibacterial and antifungal drugs (penicillin, cotrimoxazole, fluconazole, azithromycin, levofloxacin) without benefit.

Seven months after onset of the lesions, multiple biopsies of the lesion were performed at the Complicated Wounds Cure Unit of the dermatology clinic of Modena to exclude skin cancer and cutaneous mycobacteriosis. Histologic examination of the skin showed partly necrotic epidermis, with hyper-parakeratosis, papillomatous features, and scattered eosinophilic cytoplasmic inclusions. The dermis appeared edematous, with vascular proliferation and inflammatory infiltrate that was constituted of B and T lymphocytes and scattered activated CD30+ lymphoid elements (*Figure 2b-2c*). Mycobacterial culture from the biopsy was negative. Several swabs were collected from the lesion, resulting in detection of *Staphylococcus epidermidis*. Imaging was also performed: the X-ray of the left hand showed soft tissue swelling of the second finger, degenerative aspects of the distal phalanx with multiple areas of osteolysis, and rhizoarthrosis. The Magnetic Resonance Imaging of the left hand showed irregularities on the second and third finger, oedema of the soft parts of the second finger with inflammation of the flexor tendon and involvement of the cortical bone. The patient was treated with doxycycline 200 mg/day for three weeks for the *Staphylococcus epidermidis* infection; and the lesion was treated locally with cryotherapy twice a week for two weeks with poor results. Silver nitrate was subsequently employed twice a week with complete resolution of the lesion after 6 months. After seeking veterinary advise on the case, the DNA extracted from FFPE sections obtained from the pathological material was analysed to identify pathogens causing look-alike diseases as in the previous case. PanPox and HGC Realtime PCR showed a positive

result and the heminested PCR and subsequent sequencing led to identification of the infectious agent as ORFV (GenBank accession n.MW370520).

DISCUSSION

Human PPV infections by sheep, goats and cattle, commonly known as "Farmyardpox", cause skin lesions and histopathological findings that are clinically indistinguishable (Barraviera, 2005; Andreani *et al.*, 2019). Therefore, a correct diagnosis must be based on a detailed epidemiological history and subsequent laboratory confirmation (Scagliarini *et al.*, 2004; MacNeil *et al.*, 2010). In rural areas and in at-risk categories in contact with infected animals (breeders, veterinarians, milkers), these infections are considered a frequent event, but their real incidence remains unknown (Scagliarini *et al.*, 2004). Worldwide, PPV transmission has been frequently reported in connection with religious or cultural practices (Kassa, 2021).

BPSV human infection is rarely identified, with 5 cases reported globally in scientific papers between 2000 and 2022. One such case is an Italian farmer with lesions on his hands resulting from contact with infected animals (Bianchi *et al.*, 2019). Our result further demonstrates that BPSV infection in humans is circulating in the country and is indeed under-diagnosed.

ORFV is known to occur in rural as well as urban areas. In the case reported here, PPV infection occurred due to lack of awareness when handling contaminated material from infected animals. In recent years, an increasing number of ORFV cases have been reported in Muslim communities in which sheep are slaughtered with bare hands during religious festivals (Khan *et al.*, 2005; Gallina *et al.*, 2016; Veraldi *et al.*, 2019). Our report underlines the importance of having standardized diagnostic protocols to rapidly identify zoonotic agents currently not included in routine diagnostics of skin lesions. In the two cases reported here, accurate anamnestic data and information on risk factors were collected through an interdisciplinary diagnostic approach, thus orienting the diagnosis and identifying the cause of the disabling skin lesions affecting the patients. In fact, missed or mistaken diagnosis lead to inappropriate treatment (Lewis-Jones, 2004; Santiago *et al.*, 2019). The two reported cases clearly demonstrate the consequences of delayed diagnosis: both patients underwent several clinical diagnostic investigations, complementary investigations and empirical treatment protocols that also involved the use of unnecessary antimicrobials, potentially contributing to the emergence of antimicrobial resistance. A proper diagnosis of PPV infections can easily be reached through a detailed patient history and subsequent virological confirmation (MacNeil *et al.*, 2010; Gallina *et al.*, 2016; Bianchi *et al.*, 2019), so that physicians may formulate an appropriate therapy (Hosamani *et al.*, 2009; Caravaglio, 2017). Given the persistent invalidating nature of the lesions, a topical therapy with acyclic phosphonated nucleosides (HPMC, Cidofovir) would have been feasible for the two reported cases, as this antiviral has proved effective in cases of atypical and recurrent PPV lesions (Lederman *et al.*, 2007). PPV infections do not confer long-lasting immunity, thus a missed aetiological diagnosis represents a missed opportunity to prevent further disease transmission. Indeed, diagnostics also play a key role in zoonotic disease surveillance (Cross *et al.*, 2019). The absence of specific diagnostic protocols or commercial tests is one of the main causes of the absence of information on the prevalence and social and economic impact of many neglected zoonoses. The lack of epidemiological data gives the false perception that prevalence and social impact of neglected zoonoses is low; thus, these diseases do not attract the resources and research efforts needed for their control (WHO, 2007). In the two described cases, the persistent scabby-ulcerative lesions were shown to be the result of two different viruses belonging to the PPV genus, whose clinical features may easily be mistaken for other neglected zoonotic diseases such as leishmaniasis, cowpox, vaccinia, and anthrax. According to the WHO, leishmaniasis and anthrax are included on the list of neglected zoonotic diseases (NZDs) causing infections in humans and domestic animals in different countries across the world (Okello *et al.*, 2015). On the other hand, OPXV and PPV infections are not even mentioned on any official NZD lists. Furthermore, if we compare the scientific attention paid to NZD and their aetiological agents by searching in the PUBMED database between 2000 and April 2022, more than 12,000 publications can be found for leishmaniasis, and more than 4,000 for anthrax, while only around 300 scientific articles were released concerning OPXV and PPV human infections. Thus, we can say that *Poxviridae* zoonoses are twice neglected: first, due to the absence of official surveillance plans, being excluded from the list of priority diseases according to WHO and OIE, as opposed to leishmaniasis and anthrax, and second, due to the small number of research groups dealing with and publishing on these diseases.

Maintaining reliable information on international prevalence and detailed case histories for infection incidence is of paramount importance to the One Health collaborative surveillance approach (Cross *et al.*, 2019). Our results underline the importance of adopting cross-sectoral collaboration when dealing with zoonoses, so as to focus on health assessment and disease prevention rather than exclusively on treatment. The adoption of a One-Health perspec-

tive in clinical case management and diagnostics will contribute to bringing many endemic infections out of neglected status and reduce their impact on human and animal health and welfare.

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