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SHORT COMMUNICATION

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Occupational transmission of an Orthopoxvirus infection during an outbreak in a colony of *Macaca tonkeana* in Lazio Region, Italy, 2015

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Summary

Orthopoxviruses spill over from animal reservoirs to accidental hosts, sometimes causing human infections. We describe the surveillance and infection control measures undertaken during an outbreak due to an Orthopoxvirus occurred in January 2015 in a colony of *Macaca tonkeana* in the province of Rieti, Latio, Italy, which caused a human asymptomatic infection. According to the epidemiological investigation, the human transmission occurred after an unprotected exposure. The contacts among wild, captive and domestic animals and humans, together with decreased immunity against Orthopoxviruses in the community, may put animal handlers at risk of infection, especially after the cessation of smallpox vaccination. To reduce these threats, standard precautions including respiratory hygiene and transmission-based precautions should be carefully applied also in veterinary medicine.

KEYWORDS

animal outbreak, *Macaca tonkeana*, occupational transmission, Orthopoxvirus, Zoonosis

1 | INTRODUCTION

Orthopoxviruses (OPV) spill over from animal reservoirs to accidental hosts, causing, in some cases, human infections. OPV, in particular Cowpox virus, is widely distributed in Europe where an increasing number of cases have been reported in the last decade (Chantrey et al., 1999; Hoffmann et al., 2015). Cases have been described in many animals, including mongooses, jaguarondis and different types of rodents used as pets (Campe et al., 2009; Kurth et al., 2009; Ninove et al., 2009; Vogel et al., 2012).

After exposure to affected animals, OPV may cause self-limited infections in humans, characterized by skin pustular lesions. Rarely, patients may report fever, malaise, lethargy, vomiting, eye complaints and sore throat, which usually last 3–10 days. Occasionally, human infections may be severe, in immunocompromised and eczematous patients, particularly children (Medscape, 2017). In Italy, few events involving OPV were previously reported, and human cases have been reported after exposure to infected llamas and cats (Cardeti et al., 2011; Carletti et al., 2009; Scagliarini et al., 2016).

In January 2015, an outbreak due to an OPV, probably part of a novel clade lying between Cowpox and Ectromelia viruses, occurred in a colony of *Macaca tonkeana* in a private nature reserve in the province of Rieti, Lazio Region (Cardeti et al., 2017). From the name of the nature reserve, this virus was called OPV Abatino. We describe the actions undertaken for the surveillance of exposed workers that led to the identification of a human asymptomatic infection.

2 | MATERIAL AND METHODS

2.1 | Epidemiological investigations

The epidemiological investigation, performed by staff members of the "L. Spallanzani" National Institute for Infectious Diseases (INMI; VP, FMF), was conducted through a site visit, and demographical and clinical data were collected by standardized structured interviews conducted on site.

2.2 | Virological and immunological investigations

Viral DNA was extracted from biological samples using QIAsymphony technology (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The detection of OPV DNA was performed using a SYBR Green I-based real-time PCR, targeting the crmB gene (Carletti et al., 2005). The differential diagnosis was performed using the Respiratory Panel for FilmArray multiplex PCR system (bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions.

Sequential serum samples and peripheral blood mononuclear cells (PBMC) from the personnel involved in macaques farming were collected and stored at −20°C and in liquid nitrogen, respectively, until use. The antibody titres were determined by both plaque-reduction neutralization test (PRNT) and Indirect Immunofluorescence Assay (IFA). PRNT was performed according to previously published methods (Cutchins, Warren, & Jones, 1960; Newman et al., 2003), against both LV and the OPV isolate causative agent of the outbreak (OPV Abatino). A control serum from a previously (4 years) vaccinated subject was used.

Slides for IFA were prepared using Vero E6 cells infected with LV and OPV Abatino, IgG and IgM were detected using standard procedures (Carletti et al., 2009). The IFA titre was established as the last serum dilution showing appreciable immunofluorescent staining, IFA reference values (titre) serum: <1:20 = negative; ≥1:20 = positive.

The frequency of vaccinia virus-specific T cells was analysed by Elispot assay according to a previous report (Gioia et al., 2005). Specifically, PBMC from patients were thawed, counted by Scepter counter (Millipore) and seeded at $3x10^5$cells/well in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MS, USA) supplemented with 10% pre-tested heat inactivated FCS (Euroclone, Italy). Cells were then stimulated with LV at MOI 10 for 20 hr, and the immunological competence was evaluated by IFN-γ enzyme-linked immunospot assay (ELISpot assay, AID Diagnostika, Germany). Leucocytes from healthy donors were used as internal positive controls.

3 | RESULTS

3.1 | Infection control measures and surveillance activities among staff persons

In January 2015, an outbreak due to an OPV, probably part of a novel clade lying between Cowpox and Ectromelia viruses, occurred in a colony of *Macaca tonkeana* in a private nature reserve in the province of Rieti, Lazio Region. Details about diagnostic procedures and phylogenic characterization are described elsewhere (Cardeti et al., 2017).

Impacts

- The article describes the surveillance and infection control measures undertaken during an outbreak due to an Orthopoxvirus occurred in a colony of *Macaca tonkeana* in Italy, and causing a human asymptomatic infection;
- The human infection, suggested by marked increase of IgM, IgG by IFA, confirmed by PRNT and by specific T-cell response against orthopoxvirus, occurred in a researcher who had unprotected exposure to monkeys at the beginning of the outbreak;
- This finding highlights the importance of applying standard precautions including respiratory hygiene and transmission-based precautions in veterinary medicine.

After the identification of the outbreak among monkeys, an epidemiological investigation targeted to staff members working within the wildlife sanctuary was performed. Demographic and epidemiological data are summarized in the Table 1.

The staff of the nature reserve was composed of 11 persons, including the owner of the reserve and his wife (ID1 and 11 in Table 1). Other persons working in the sanctuary were a veterinary (ID2), 5 researchers (ID4, 5, 6, 8 and 9) and three persons working as maintenance and cleaning staff (ID3, 7 and 10). During work days, personnel share common areas for briefing, as well as eating and relaxing. All staff members underwent a structured interview about pre-existing medical conditions, level of exposure to affected monkeys, and the presence of symptoms. Four persons from the staff (the owner, his wife, and maintenance and cleaning staff born in Albania) had been vaccinated against smallpox.

From the collected data, four persons (ID1, 2, 4 and 6) reported direct contact with the affected monkeys, occurred at the beginning of the outbreak. In particular, the veterinary (ID2) performed an intubation on the first affected monkey, with no facial or respiratory protection. The other exposed persons were in contact with sick monkeys and removed the first two dead bodies with no specific Personal Protective Equipment (PPE). The remaining staff members did not report contact with affected monkeys.

After the death of the second monkey, the personnel who had contact with monkeys started to use consistently PPE, selected according to activities: in case of contacts without direct exposure (observation, food provision, cleaning of the cage) personnel wore disposable gloves and apron; while for direct contact (such as the removal of dead bodies), the following disposable PPE was worn:

TABLE 1 Demographic and clinical characteristics of staff persons, and results of serological surveillance

ID	Sex, age, country of birth	Smallpox vaccination	Role in the farm	Comorbidities	Exposure to diseased monkeys	Symptoms
1	M, 69, Italy	Yes	Coordinator	No	Yes (Direct assistance)	No
2	M, 35, Italy	No	Veterinary	No	Yes (Direct assistance including intubation)	Fever, general malaise, asthenia
3	M, 34, Albania	Yes	Maintenance staff	No	No	No
4	F, 37, Italy	No	Researcher	No	Yes (Direct assistance, removal of dead bodies)	Fever, sore throat
5	F, 37, Italy	No	Researcher	No	No	No
6	M, 38, Italy	No	Researcher	Thyroidectomy	Yes (Direct assistance, removal of dead bodies)	No
7	F, 61, Albania	Yes	Maintenance staff	Hypertension	No	No
8	F, 27, Italy	No	Researcher	No	No	No
9	F, 29, France	No	Researcher	No	No	No
10	F, 38, Italy	No	Maintenance staff	No	No	No
11	F, 67, Italy	Yes	Assistant	Rheumatoid arthritis	No	No

ND, not done.
The IFA titre was established as the last serum dilution showing appreciable immunofluorescent staining.
*The antibody titre was determined by IFA against LV and OPV Abatino.

complete tyvek suit with FFP2 mask, face shield, gloves, apron. In all cases, reusable boots were always disinfected after use with a sodium hypochlorite solution. Moreover, the cage was provided with dedicated equipment; the researchers already exposed (mainly ID4 and 6) were exclusively dedicated to the affected colony, with no contacts with other animals; they also took charge of cleaning and maintenance activities.

3.2 | Staff with symptoms and results of serological surveillance

Two staff members reported symptoms (Table 1). The veterinary (ID2) who performed the intubation without PPE developed general malaise, asthenia and fever 9 days later, without skin pustular lesions. In consideration of the exposure, he was admitted at INMI, and isolated according to contact and airborne precautions, for suspected OPV infection. An extensive diagnostic work-up was performed, including Adenovirus, Bocavirus, Parainfluenza viruses 1, 2, 3 and 4, Coronaviruses, Influenza A and B viruses, resulted negative. On the contrary, RT-PCR for rhinovirus resulted positive. In consideration of the good clinical conditions, the patient was discharged after 2 days.

The researcher (ID 4) directly exposed to affected monkeys also developed mild fever and sore throat. Symptoms appeared 12 days after the last unprotected exposure. She refused hospital admission, and the symptoms spontaneously disappeared in a few days.

All personnel underwent serological surveillance, with serum specimens taken on January 22 and after 6 weeks; IgG and IgM against OPV were investigated. Persons vaccinated for smallpox showed comparable and stable IgG IFA titres against both LV and OPV Abatino in a 6-week interval, and no IgM response. All but one person not vaccinated showed the absence of serological response to both LV and OPV Abatino; one not vaccinated researcher directly exposed to diseased monkeys (ID6) showed a serological response to both VL and OPV Abatino along a 13-week observation, with IgM seroconversion and progressive increase in IgG, suggesting a recent exposure to OPV. The response against OPV Abatino was constantly higher (2-fold) than that against LV, indicating OPV Abatino as the actual trigger of the immune response (Table 1). However, as observed by Silva-Fernandes et al. (Silva-Fernandes et al., 2009) in describing outbreaks, Orthopoxvirus infections seems to induce a limited IgM response in exposed subjects. In addition, the low IgM titres detected could be due to the poor sensitivity of the IFA test adopted.

IFA virus target	IgG* on Jan. 22, 2015	IgM* on Jan. 22, 2015	IgG*6 weeks later	IgM*6 weeks later	IgG* 12 weeks later	IgM* 12 weeks later
VL	1:80	<1:20	1:80	<1:20		
OPV Abatino	1:80	<1:20	1:40	<1:20		
VL	<1:40	<1:20	<1:40	<1:20		
OPV Abatino	<1:40	<1:20	<1:40	<1:20		
VL	Weak positive	<1:20	Weak positive	<1:20		
OPV Abatino	1:20	<1:20	1:20	<1:20		
VL	<1:40	<1:20	<1:40	<1:20		
OPV Abatino	<1:40	<1:20	<1:40	<1:20		
VL	<1:40	<1:20	<1:40	<1:20		
OPV Abatino	<1:40	<1:20	<1:40	<1:20		
VL	1:20	<1:20	1:160	1:20	1:320	1:40
OPV Abatino	1:20	<1:20	1:320	1:40	1:640	1:40
VL	Weak positive	<1:20	1:40	<1:20		
OPV Abatino	1:20	<1:20	1:20	<1:20		
VL	<1:20	<1:20	<1:40	<1:20		
OPV Abatino	<1:20	<1:20	<1:40	<1:20		
VL	<1:20	<1:20	ND	ND		
OPV Abatino	<1:20	<1:20				
VL	<1:40	<1:20	<1:40	<1:20		
OPV Abatino	<1:40	<1:20	<1:40	<1:20		
VL	1:80	<1:20	1:80	<1:20		
OPV Abatino	1:80	<1:20	1:80	<1:20		

PRNT against both VL and OPV Abatino was performed to confirm the specificity of humoral immune response of ID6. As shown in Figure 1, from week 2 onward neutralizing antibodies developed (peaking at week 6), against both LV and OPV Abatino. However, the titre of neutralizing antibodies against the outbreak isolate was 10-fold higher than against LV consistently with IFA antibodies.

Moreover, to evaluate the poxvirus-specific T-cell response, PBMC were stimulated with vaccinia virus as previously described (Gioia et al., 2005) and analysed by Elispot assay. A marked increase of specific T-cell response, from 25 spot forming cells (SFC)/10^6 PBMC to 89 SCF/10^6 PBMC at week 6, was observed in the same researcher (ID6) showing serological response, supporting the hypothesis of a recent asymptomatic infection with OPV. All other staff members showed no sign of specific T-cell response, consistent with lack of serological response.

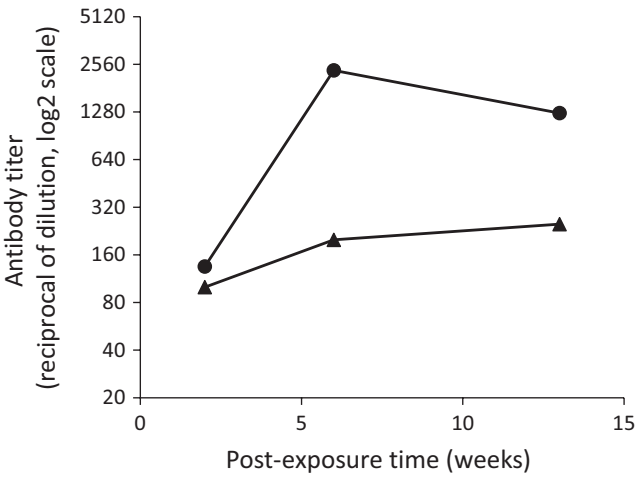

FIGURE 1 Humoral immune response. Serum samples collected at different time points (weeks 2, 6 and 13) were tested using Plaque-Reduction Neutralization Test (PRNT). Each serum sample was tested with either the Orthopoxviruses (OPV) Abatino (circle) or the LV reference vaccinia strain (triangle). The PRNT titre was assessed as the serum dilution causing 50% PFU reduction compared with the PFU of virus controls, using the Reed and Muench method for calculation. Serial twofold dilutions of sera were used

4 | DISCUSSION

OPV spill over is increasing and, despite it is described as an extremely rare zoonotic disease among humans, many reports

among animals, with or without involvement of humans, have been recently described (Campe et al., 2009; Kurth et al., 2009; Ninove et al., 2009; Vogel et al., 2012). Monkeys belonging to *Macaca* genus have been already involved in a cowpox outbreak, in the Netherlands (Martina et al., 2006). Data about lethality among *Macaca* were not reported in that study; thus, it is not possible to compare the two outbreaks. In the Italian outbreak among *Macaca tonkeana*, the clinical attack rate among animals was 89%, and lethality 67% (Cardeti et al., 2017).

Reports of human cases are increasing in recent years, also. This increase may reflect the fact that a continuously decreasing number of persons have immunity against OPV after the cessation of smallpox vaccination. Even the asymptomatic infection case occurred in an unvaccinated person.

The occurrence of an asymptomatic case suggests that the transmission occurred after an unprotected exposure. Similarly, to existing recommendations for patient caring, standard precautions, respiratory hygiene and transmission-based precautions should be applied in veterinary medicine, too, as suggested by specific guidelines (CDC, 2015; OIE, 2016). Furthermore, two staff members developed symptoms after unprotected exposure: even if OPV infection had been excluded, the clinical management and diagnostic procedures, at least in the patient admitted in hospital, involved a considerable use of resources that would not be used in case of protected exposure.

The close contact among wild, captive and domestic animals, and humans, together with decreased immunity against OPV in the community, underscore the need for physicians and veterinarians and animal handlers to become aware of the risk for OPV zoonoses.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

No formal ethics approval is required in this particular case as it is a descriptive study; all procedures described followed the normal good standard of care and have not been used experimental/innovative treatments and/or approaches; patients have been anonymized; patients have given their written consent for the publication of their clinical histories. Written informed consent is available in case of request.

CONFLICT OF INTEREST

All authors declare that no competing interests exist.

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