Infezione da Cowpoxvirus negli animali: studi sulla presenza e diffusione del virus in Italia Centrale

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SUMMARY

The Cowpox virus (CPXV) is one of the 10 species belonging to the genus Orthopoxvirus, and like other 4 in the genus it is pathogenic to humans. The main feature of CPXV species is to have a broad spectrum of host among domestic mammals, wild and exotic kept in captivity (circuses, zoos and recovery centers). The virus is endemic in northern Europe and western Russia, but in the last 20 years the cases described in animals are increasing in central and southern Europe. Furthermore, in the last decade an increase in human cases has been observed, related to the abandonment of the vaccination against Smallpox since 1980, to the increase in receptive animal species such as horses, dogs and circus animals and zoos, and for incorrect use of antiviral drugs. The ability of the CPXV to overcome species barriers and to move between domestic, wild and exotic animals in captivity, to dispose of small wild rodents as a natural reservoir, and to induce serious forms in immunocompromised patients, makes it necessary to carry out targeted studies on the virus.

In compliance with theese elements and considering the low number of investigations carried out in Italy, this research has conducted specific studies to detect the presence and spread of Cowpoxvirus in domestic and wild animals, allochthonous and/or synanthropic, and exotic both in Rome and province and in the territory of the Istituto Zooprofilattico Sperimentale of Lazio and Tuscany (IZSLT). The objectives to be achieved by the project are: to sensitize human and veterinary doctors to the zoonosis; to collect data on the diffusion of CPXV in zoos and recovery centers for exotic animals; to obtain information on the spread of CPXV in cats in the province of Rome; to gather information on the spread of CPXV in people in contact with infected animals; to find seropositivity in domestic and wild animal species, alien and/or sinantrope, and exotic; to analyze the risk factors associated with the prevalence of the aforementioned infection. **Phase 1** consisted in organizing three workshops on Cowpoxvirus infection at the IZSLT, Rome. During the last year of the project, six seminars were organized for free professional veterinarians and held at the headquarters of the OMV of three provinces of Lazio and three provinces of Tuscany.

During phase 2, the animal populations at risk were defined (owned and colony cats of the province of Rome, exotic animals hosted in different structures of central Italy) on which to carry out clinical, virological and serological investigations against Cowpoxvirus.

In the following phase 3, the veterinarians of ASL, Zoo and Centers for the recovery and recovery of wild and exotic animals in the Lazio and Tuscany regions were contacted for the request for collaboration and collection of material.

In the meantime (phase 4) two informative brochures have been developed on the etiological agent and on how to collect and send samples. Two questionnaires were formulated to identify possible environmental and behavioral risk factors to be completed in all suspect. Brochures and questionnaires were distributed to the provincial sections of the IZSLT, to ASL, veterinary clinics, zoos and centers for the recovery of wild and exotic animals, to be filled out and sent together with the samples to be analyzed.

During the project period (phase 5) samples were collected for the detection of the virus: 32 between cutaneous and mucosal lesions, 550 samples of organs and tissues from 164 suspected and unidentified animals, of alien and/or sinantrope species, wild and exotic. The material collected was stored frozen and/or in formalin.

A total of 1234 blood samples (phase 6) were taken from cats (free ranging and domestc, symptomatic or not) and exotic animals anesthetized for surgery or deceased. This phase has been prolonged beyond the expected and has led to the one year extension of the project deadline.

On the received organs, tissues and blood samples from animals (phase 7), the following laboratory tests were performed: anatomo-istopathological examination; virological tests such as negative

staining-electron microscopy, isolation on two cell lines; biomolecular techniques (Real Time PCR and Sequencing); serological tests for antibody detection by serum neutralization (SN) test and indirect immunofluorescence test (IFA). All the pathological material subjected to virological investigations was negative for Cowpox virus, with the exception of samples taken from the outbreak in Abatino Park. All the sera examined with the two tests, including the 441 colony cat sera sampled in the 4 districts of the ASL Roma 3, did not reveal any specific antibodies against the virus.
Phase 8 provided for a series of actions when a CPXV outbreak was diagnosed, an event that occurred in January 2015 with fatal outcome in 12 of the 18 Tonkean macaques of the affected social group. In this circumstance we proceeded as follows:
- the health rules laid down by the Veterinary Police Regulations have been applied; being present other receptive animal species, epidemiological investigation, clinical examination and sampling for both virological and serological tests were conducted;
- clinical examination and blood sampling were carried out by the 11 operators of the Center for serological investigations. A subject never vaccinated and came into contact with the infected social group, he highlighted a conversion serum.
- small circulating wild rodents were captured, both during disinfection and deratting operations and free in the surrounding forest, for virological and serological investigations.
In the last months of the project, the epidemiological analysis of the sampling results among the colony cats of the ASL Roma 3 was conducted, to evaluate the spread of Cowpoxvirus in the urban and extra-urban areas of the Province of Rome according to a risk assessment for humans. Given the complete negativity of the sample examined, considering the assumed and the sampling design adopted and assuming sensitivity and specificity of the SN test equal to 100%, the theoretical maximum prevalence of the infection in the target population was equal to 0.67%.
The histological, virological and serological investigations carried out on the samples received during the project, with the exception of the OPXV outbreak among Tonkean macaques in the recovery center in the province of Rieti, did not reveal any virus circulation because all the exams have given negative outcome (no typical lesion, no isolated CPXV strain or detected DNA, no specific antibody response).
From the results obtained using the SN test, a low sensitivity of the method was highlighted. Therefore, an IFA was developed for those species of which an anti-gammaglobulin conjugated serum with FITC was available with the consequent adoption of the IF as a definitive test for the detection of anti-CPXV antibodies in primates and in rodents. It was not possible to use it instead for all other species, including cats, for which the SN was continued to be used.
The viral strain isolated in Tonkean macaques at the Parco dell'Abatino Wildlife Park was characterized as a new Orthopoxvirus strain and its sequences were deposited in GenBank, (Accession Number: XY123456).
From the results obtained in 4 years of activity, during which a new case of Orthopoxvirus infection practically identical to the strain isolated in macaques, was isolated and typed by a cat from Siena with symptoms related to CPXV infection, it would be interesting to expand the survey area extending the withdrawals to the whole territory of IZSLT competence and activating collaborations with ASL and veterinary clinics and human dermatologists.