THE FREE-LIVING WOLF (*CANIS LUPUS*): A STUDY OF VIRAL CIRCULATION IN PHYLOGENETICALLY DIFFERENT WILD POPULATIONS

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Curriculum: ANIMAL HEALTH

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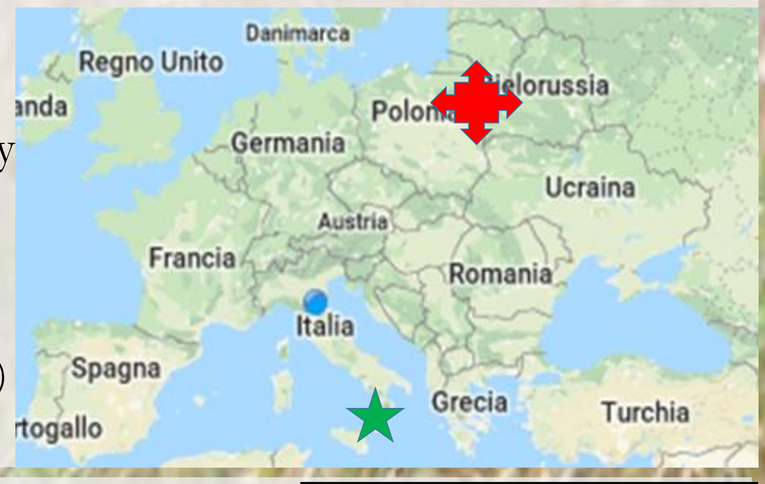
INTRODUCTION

The research proposal involves the synergistic application of genetic testing on sampled animals and virologic and serum epidemiological investigations on viruses to determine the viral circulation in wolves in three different study areas. The health survey use both non-invasive monitoring and necroscopic investigation of deceased subjects. CPV-2 Canine Parvovirus, CAdV-1 Adenovirus of the dog and CDV Canine Distemper Virus are investigate.

Virological and Ecological Target

Determination of viral prevalence at different biological moments of the species in geographically and phylogenetically remote populations[1]. The virologic study is carried out on two relict populations – the Aspromonte population (Calabria – Italy) and Białowieża National Park population (Poland) and a recent resettlement in the Tosco-Romagnolo Apennine (Regional Park of Vena del Gesso Romagnola, Regional Park of Gessi Bolognesi e Calanchi dell'Abbadessa) **[See map]**.



MATERIALS & METHODS

Materials

Sampling for genetic analysis from carcasses

The tip of the tongue was removed from the dead animals: the samples had a diameter of about 3cm and were placed in a container with 95% Ethanol respecting the proportion 1sample:3 Ethanol

Collection of duodenum, liver, tongue, spleen, lymph nodes or faeces

As for all the dead subjects we examined, the small intestine, liver, tongue, spleen and lymph nodes were sampled and stored in a freezer at –20 °C. For free-living subjects we only sampled the faeces and put them at -20°C.

Sera sampling

The blood was placed in sterile 15ml Falcon tubes. The samples were centrifuged at 1300 RPM for 10 minutes, sera were pipetted to obtain 4 aliquots for each individual and vials stored at –20 °C.

Methods

Individual genetic recognition was obtained by fingerprinting and microsatellite analysis. The identified samples were virologically processed in Real Time PCR and VI in order to obtain sequencing by phylogenetic assays. Samples were investigated for Ab using selection techniques for each pathogen. IFI to detect Ab against CAdV-1 and CDV and HI to detect Ab against CPV-2.

RESULTS

Fifty samples of excrements were collected in the 3 study areas. The samples are waiting to be attributed to the species by DNA analysis, then they will be analyzed for virological research. Twelve dead animals were examined, for each animal analyzed, we recorded age class, based on tooth wear [2] - see legend in **Tab. 1**. 50% were young of the year, 33.3% were sub-adult, 8.3% were adults, 8.3% were old animals, **Tab. 2.** 58% were male while 48% were females, **Tab. 2.** 73% of the examined animals belong to the *Canis lupus* species, while 27% were non-recent wolfxdog hybrids, **Tab. 2.** The Real time PCR investigations of the tissues are currently underway and at the time of writing this poster have not been completed. Instead, we have the partial results of the serological research on the twelve dead animals, **Tab. 2.**

AGE CLASS	AGE	MONTH OR YEARS
1	PUPPY	0 and 12 month
2	SUBADULT	1 and 2 years
3	ADULT	> 2 until 8 years
4	OLD SUBJECT	> 8 years

Tab. 1

DISCUSSION

Regarding the CPV-2, our serological results suggest an high exposure (8:12–67%) to the virus in the environment. The prevalence of Ab against CPV-2 in wolf in Italy is similar to USA, Canada and Spain. We have been able to show that the prevalence is greater in young subjects (62,50%) or sub-adults (37,5%) [3], **Tab. 2.**

Preliminary serological results for CAdV-1 and CDV demonstrate poor viral circulation in the wild. However, we expect to have more samples before drawing conclusions on the circulation of these two viruses, in fact, our working group in 2017 identified a positive wolf at CAdV-1 from tongue [4].

ID SeFSE	AGE	SEX	SPECIES	CAdV1	CDV	CPV2
2284	1	F	WOLF	NEG	NEG	1:160
2297	1	M	WOLF	NEG	NEG	1:320
2298	2	M	WOLF	NEG	NEG	1:40
2301	1	M	HYB	NEG	NEG	1:2560
2304	1	M	HYB	NEG	NEG	1:320
2311	1	F	WOLF	NEG	NEG	NEG
2312	2	M	WOLF	NEG	NEG	1:160
2321	2	M	WOLF	NEG	NEG	1:80
2323	4	F	WOLF	NEG	NEG	NEG
2324	3	M	WOLF	NEG	NEG	NEG
2327	2	F	HYB	NEG	NEG	NEG
2330	1	F	Examination	Examination	Examination	1:640

Tab. 2

References

[1] Montana L, Caniglia R, Galaverni M, Fabbri E, et al… (2017) Combining phylogenetic and demographic inferences to assess the origin of the genetic diversity in an isolated wolf population. PLoS ONE 12(5): e0176560.

[2] Gipson P. S., Ballard W. B., Nowak R. M., Mech L. D. 2000. Accuracy and precision of estimating age of gray wolves by tooth wear. Journal of Wildlife Management 64: 752–758.

[3] Nelson B., Hebblewhite M., Ezenwa V., et al…..(2012) Prevalence of antibodies to Canine Parvovirus and Distemper Virus in wolves in the Canadian Rocky Mountains. Journal of Wildlife Diseases, 48(1):68-76.

[4] GENETIC CHARACTERISATION OF CANINE ADENOVIRUS TYPE 1 DETECTED IN THE TONGUE OF A WOLF. A.Balboni, C.Musto, E.Kaehler, E.Fabbri, R.Caniglia, E.Carra, M.Battilani and M.Delogu. In: Congresso S.I.S.Vet.