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GENOMIC AND TRANSCRIPTOMIC DIFFERENCES BETWEEN A VIRULENT AND ATTENUATED *LEPTOSPIRA INTERROGANS* SV POMONA BOVINE STRAIN.

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Leptospira interrogans is the main agent of leptospirosis. Pathogenic leptospires are classified in serovars with different seroprevalence according to the host and geographical location. Unlike what happens in other countries, the main serovar in cattle in Argentina is Pomona. Pomona causes a severe infection and frequent mortality in calves. The attenuation of a strain AKRFB Passage 1 (P1) that belong to Pomona was achieved by multiple subcultures in liquid medium obtaining its attenuated counterpart in the Passage 19 (P19). In order to identify genetic changes, *in vitro* genomes and transcriptomes of P1 and P19 were compared using next generation sequencing platforms. Single nucleotide polymorphisms (SNPs) analysis of non-synonymous aminoacid changes in major proteins were identified in P19. The expression analysis (RNAseq) identified 328 differential transcripts between P1 and P19, of which 180 correspond to P1 (54.88%) and 148 to P19 (45.12%). Transcriptional regulators, regulators of two-component systems and membrane transporters were identified among the up-regulated transcripts in P1. Conversely, most of the transcripts up-regulated in P19 correspond to transposases coding genes. Even though the whole attenuation process of P19 cannot be attributed to single point mutations or differentially expressed genes, the set of them identified here, could explained part of the attenuated phenotype and allows the deepen in the regulation of expression in this pathogen.

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MOLECULAR AND SEROLOGICAL PREVALENCE OF *LEPTOSPIRA SPP*. AMONG PIGS RAISED IN SOUTHERN ITALY (SICILY)

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Leptospirosis is a widespread zoonosis and has been recognized as a re-emerging infectious disease in humans and a variety of wild and domestic animal species. Swine act as both maintenance and incidental hosts of pathogenic *Leptospira spp.*. The aim of this study was to investigate the prevalence and diversity of *Leptospira spp.* by serological and molecular assays in pigs in Southern Italy (Sicily) and to characterize the strains. The serological gold standard method, Microscopic Agglutination Test (MAT), was performed on 55 pig sera collected during 2019 from autochthonous healthy pigs randomly collected in a slaughterhouse in the Sicily region. A seropositivity rate of 16.4% was determined and Australis was the most frequently identified serogroup (66.7%), followed by Pomona (33.3%) and Sejroe (11.1%). Real-time Polymerase chain reaction (PCR) to detect pathogenic species (*lipL32* gene), was carried out on 55 corresponding kidneys and blood. Pathogenic Leptospiral DNA was detected in 2 kidney samples (3.6%). Partial rpoB gene sequencing was performed for identification of *Leptospira* species. Interestingly, one of the two positive pigs by Real-time PCR, was also positive by MAT showing antibodies against the serogroup Australis. The data obtained in this study confirm that *Leptospira* could play a role in determining leptospirosis infection in pigs. It is important to use serological and molecular diagnostic techniques complementarily to identify infected individuals.The serological survey evidenced that Australis and Pomona were the most serogroups causing leptospirosis in pigs reared in Sicily.

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SCANNING & TRANSMISSION ELECTRON MICROSCOPY OF WHOLE BLOOD AND KIDNEYS OF *LEPTOSPIRA* CHALLENGED HAMSTERS

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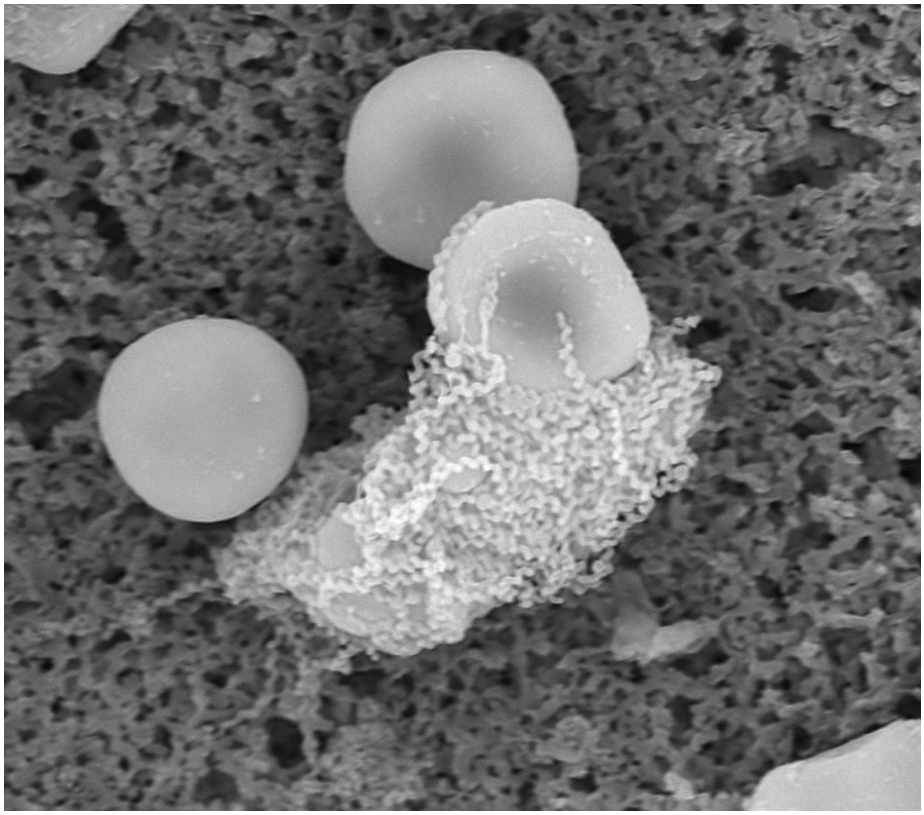
Leptospirosis is a devastating zoonotic disease affecting humans, livestock, and companion animals around the world. Leptospires can be directly or indirectly transmitted to incidental hosts and a large amount of species specificity (both pathogen and host) play an important role in symptom severity. Once an appropriate host is reached, leptospires can colonize the proximal tubules of the kidney where they chronically persist in reservoir hosts and be subsequently shed in the urine. While rats are considered the gold standard of reservoir host models of Leptospirosis, the golden Syrian hamster is wildly regarded as the primary model for clinical disease presentation, including the evaluation of bacterin vaccines for clinical and veterinary use. However, hamster models are rarely deeply phenotyped during vaccine and research trials and instead, results rely heavily on survival curves. In an effort to more expansively investigate the hamster immune interactions with infecting leptospires whole blood and kidney tissue were examined by electron microscopy after hamsters were challenged with *Leptospira borgpetersenii* or *Leptospira interrogans*. Scanning electron microscopy (SEM) of whole blood showed that while single leptospires were identifiable so were large clumps of pathogens, sometimes associated with red blood cells (see SEM Figure 1), white blood cells, or even fibrous material. Transmission electron microscopy (TEM) of kidney tissue revealed leptospires interacting with epithelial membranes or intracellular within renal cells as well as freely in blood vessels and within proximal tubules.

Figure 1: SEM of clumps of *L. borgpetersenii* in whole blood associating with red blood cells.



DETECTION OF LEPTOSPIRA GENOTYPES IN SYNANTHROPIC AND WILD CARNIVORES IN NORTH EAST ITALY: HEDGEHOG, FOX, RAT, MOUSE, VOLE AND WOLF

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Leptospirosis in dogs is an infectious disease significantly reported in clinical practice and a worldwide zoonosis. In North-East Italy, 4 serogroups are reported as most common in dogs: Icterohaemorragiae (ICT), Australis (AUS), Pomona (POM) and Sejroe (SER) (Bertasio C. et al. 2020). The aim of the study is to evaluate the *Leptospira* environmental exposure of wild and synanthropic animals and to detect the circulating genotypes in potential reservoirs. Fifty-four animal's carcasses from the Public Veterinary Service survey were enrolled and geographically mapped: 20 hedgehogs, 8 foxes, 21 rats, 4 mice, 1 vole and 1 wolf. Real-Time PCR assay was performed on kidney tissue and MLST analysis was carried out on positive samples through the 7-loci scheme proposed by Boonsilp (Bonsilp et al. 2013), using the method proposed by Weiss (Weiss et al. 2016). The Sequence Types (STs) of the strains found in wild animals in common with dogs were: AUS (ST24, ST198), ICT (ST17), SER (ST155) in hedgehogs, ICT (ST17) and AUS (ST24) in foxes, ICT (ST17) in rats, ICT (ST17), SER (ST155) in mice and POM (ST117) in wolf. Other STs detected for the first time belonged to serogroups Javanica (ST146 in hedgehogs and foxes) and Ballum (ST149 in hedgehogs). A newST was found in a vole (similar to ST100). Synanthropic and wild animals survey on *Leptospira* would be helpful to analyse environmental risk factors and to achieve epidemiological knowledge on *Leptospira* strains distribution.

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DIVERSE ENVIRONMENT RELATED (DER) PROTEIN IS A NOVEL OMP85 SUBFAMILY PRESENT IN FREE-LIVING BACTERIA AND PATHOGENIC *LEPTOSPIRA* SPP.

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The bacterial outer membrane has a fundamental role in the interplay between an organism and its environments. Omp85s are transmembrane β-barrels with functions in protein translocation and outer-membrane biogenesis. Besides their conserved C-terminal domain, OMP85 superfamily can be divided into 10 subfamilies based on domain architecture of their periplasmic regions. *L. interrogans* proteome describes four proteins containing the conserved Omp85 domain (PF01103). LIC11623 contains canonical N-terminal POTRAs (PF07244) and can be considered the leptospiral BamA homolog. Other three proteins (LIC12252, LIC12254 and LIC12258) contain no previously defined periplasmic domains. Using ThreaDomEx server we predicted boundaries for an extra domain of unknown function (DUF) in their N-terminal regions. As identified by RT-PCR, the three novel OMP85 are upregulated in infected mice urine compared to *in vitro* and DMCs. LIC12258 presented the highest transcriptomic changes, with a urine-upregulation of 4.67- and 6.58-fold compared to DMCs and *in vitro* cultures, respectively. Using HMMER web server and DUF sequences as queries, we identified nineteen common hits outside *Leptospira* genera with high residue similarity (54.4-67.6%). All matches contain an OMP85 domain in their C-terminal regions. Most of orthologs found are from free-living species from Bacteroidetes phyla, isolated from harsh environments. Phylogenetic analysis demonstrate the existence of five Der orthologs in *Leptospira* spp., with only one present in pathogenic and saprophytic species, suggesting evolutionary adaptations for each bacteria-environment interaction. Therefore, we named the novel OMP85 as Diverse Environment Related (Der) proteins. Leptospiral mutants are under development towards solving the intriguing leptospiral Der protein function.

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MOLECULAR TYPING OF PATHOGENIC *LEPTOSPIRA* SPECIES ISOLATED FROM WILD MAMMAL RESERVOIRS IN SARDINIA

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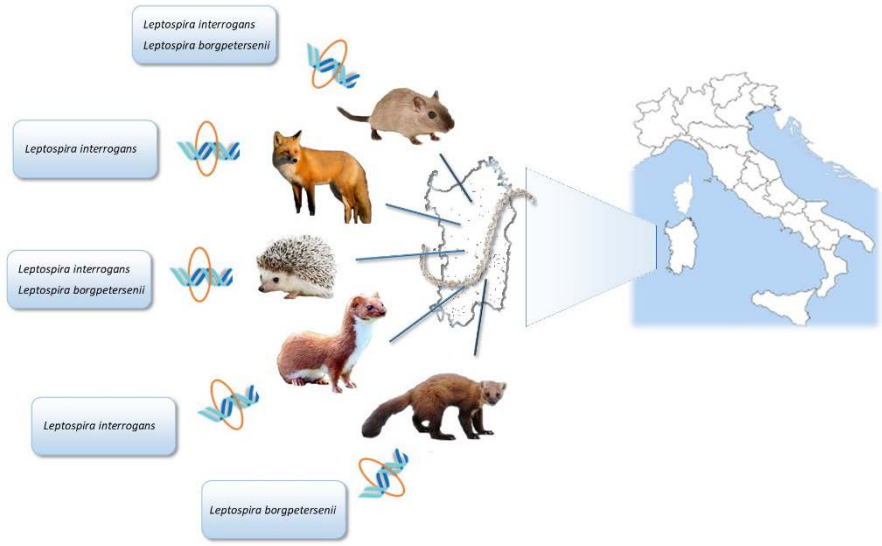
Leptospirosis is a global zoonosis caused by pathogenic species of *Leptospira* that infect a large spectrum of domestic and wild animals. This study is the first molecular identification, characterization, and phylogeny of *Leptospira* strains with veterinary and zoonotic impact in Sardinian wild hosts. All samples collected were cultured and analyzed by multiplex real time polymerase chain reaction (qPCR). Sequencing, phylogenetic analyses (based on *rrs* and *secY* sequences), and Multilocus Sequence Typing (MLST) based on the analysis of seven concatenated loci were also performed. Results revealed the detection of *Leptospira* DNA and cultured isolates in 21% and 4% of the samples examined, respectively. Sequence analysis of *Leptospira* positive samples highlighted the presence of the *interrogans* and *borgpetersenii* genospecies that grouped in strongly supported monophyletic clades. MLST analyses identified six different Sequence Types (STs) that clustered in two monophyletic groups specific for *Leptospira interrogans*, and *L. borgpetersenii*. This study provided about the prevalence of leptospires in wild mammals in Sardinia, and increased our knowledge of this pathogen on the island. Monitoring *Leptospira* strains circulating in Sardinia will help clinicians and veterinarians develop strategic plans for the prevention and control of leptospiral infections.



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