Pathogenic *Leptospira* spp. Isolated and Detected in Reproductive Organs of Wild Boars (*Sus scrofa*) Hunted in Italy

Giovanni Cilia[1], Nicoletta M. Ponti[2], Fabrizio Bertelloni[1], Barbara Turchi[1], Ivana Piredda[2], Bruna Palmas[2], Malgorzata Noworol[2], Domenico Cerri[1], Filippo Fratini[1]

[1]Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, 56124, Pisa, Italy, [2]Istituto Zooprofilattico Sperimentale della Sardegna, Via Duca degli Abruzzi 8, 07100 Sassari, Italy

Background: Leptospirosis is a re-emerging and widespread zoonosis, caused by pathogenic serovars of *Leptospira* spp. Its worldwide distribution is favoured by a large variety of wild and domestic animal species that can play a role as natural or accidental reservoir. Wild boar (*Sus scrofa*) is a *Leptospira* host that increase everywhere in Europe and in Italy. The aim of this investigation was to evaluate the prevalence of *Leptospira* spp. in wild board hunted in 2 regions of Italy (Tuscany and Sardinia), and also, evaluate the infection in reproductive organs.

Methods: During hunting season from October 2018 to January 2019, 231 wild board have been sampled to investigate the presence of *Leptospira* spp. Serum from infra-orbital cavity, kidney, and reproductive organs (testicles and epididymis in male or uterus, ovary, vagina in female) were collected from each animal. In addition, placenta and foetuses were sampled from pregnant wild boar. From these animals, all foetuses have been pooled and considered as one samples. Sera were tested with microscopic agglutination test (MAT); Serovars of the following serogroup were used as live antigens: Australis, Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Hardjo, and Tarassovi. Titre of 1:100 was considered as threshold. Isolation was performed from sampled organs using Ellinghausen-McCullough-Johnson-Harris (EMJH) medium; cultures were incubated at 30 ± 1 °C and checked weekly, for up to 3 months, using dark field microscopy. Multilocus sequence typing (MLST) scheme 1 were used to genotype the *Leptospira* isolate, basing on the amplification of seven housekeeping genes (*mreA, pfkB, pntA, sucA, tpiA, caiB,* and *glmU*). DNA was extracted from each organ and a Taqman-based RealTime-PCR assay targeting the *lipL32* gene was used to detect pathogenic leptospires. Samples with *lipL32* Ct < 35 were considered positive.

Results: In all hunting season have been sampled 95 males and 136 females (45 of which pregnant). In total, 34 out of 231 sera (14.72%) tested positive for anti-*Leptospira* antibodies. Pomona, Tarassovi and Australis were the most often detected serovars at serological test. Ten cultures were found positive. Leptospires has been isolated from the kidneys in two females, from the testicles and epididymis in an adult male and from all organs tested in two sub-adult males. *Leptospira interrogans* serogroup Australis has been identified by MLST (Sequence Type 24) in six isolates (kidneys, testicles and epididymis of two sub-adult wild boar males) while *Leptospira kirschneri* serogroup Grippothyphosa (ST 78) in testicles and epididymis of one adult. The two Sardinian isolates have not been typed. By RealTime PCR 93 samples were positive: 33 kidneys (14.28%), 17 testicles (17.89%) and 13 epididymis (13.68%) of total

males, 10 uteri (7.35%), 3 ovaries (2.20%) and 1 vagina (0.73%) of total females, 3 placentas (6.66%) and 13 foetuses (28.88%) of total pregnant females.

Conclusion: The results of this investigation confirmed that wild boars are a potential source of pathogenic *Leptospira* spp., which can infect other animal species (domestic and wild) and humans. Pomona, Tarassovi and Australis, species-specific swine *Leptospira* serovars, resulted the most prevalent at MAT. *Leptospires serogroups* Australis and Grippothyphosa isolated from the male reproductive organs and the positive results obtained by RealTime PCR in both male and female could suggest a possible venereal transmission, as already demonstrated in pigs. Furthermore, placentas and foetuses were positive for *lipL32* target and this may be related to a possible vertical transmission of pathogenic *Leptospira.*