

PROGETTI DI “RICERCA CORRENTE 2013”

RELAZIONE FINALE

N. identificativo progetto: IZS LT 02/13 RC

SUMMARY OF THE PROJECT

Title: Multidisciplinary investigation of zoonotic pathogens in wild boar populations of Lazio and Tuscany.

Project ID: IZS LT 02/13 RC

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Keywords: zoonoses, wild boar, Public Health, diagnosis.

Aims

The overall objective of the project was to conduct a study on some important zoonotic agents in wild boar populations of Lazio and Tuscany shoot down/captured during the 2013-2016 hunting seasons. Sampling was carried out at slaughterhouses and/or at collection centres.

In particular the objectives of the project were:

- To evaluate the presence and estimate the prevalence of some zoonotic agents in wild boar populations of Lazio and Tuscany shoot down/captured during the study period, taking several samples (biological samples of various types from the slaughtered animals, meat).
- To acquire information to assess the epidemiological significance of this species, for the various pathogens under study, both in terms of risk for the Public Health and for the populations of domestic animals.
- To provide the Competent Authority with information useful for the elaboration of practical proposals for the correct management of wild boar populations in the studied areas.

Methodology and Results

The bibliographic knowledge regarding the epidemiology, the diagnostic and pathogenic aspects of the zoonotic agents under study have been updated. Information regarding the wild boar population in the sampled area was collected, the sample size and the sampling time-schedule were defined. Sampling forms were prepared and the operational procedures of sampling and samples delivery established.

From the main study area (province of Viterbo), 188 wild boars were sampled, including 89 males (47.3%), 90 females (47.3%) and 9 animals of not specified sex (4.8%). Other 43 animals, including 26 males (60.5%), 14 females (32.6%) and 3 animals in which sex was not specified (7%) were sampled from three provinces of the Tuscany region (FI, PO, PT). Overall, of the 231 animal sampled, the majority (53.7%) were adult animals with an estimated age > 24 months and a weight >50 kg.

The samples to be taken for each subject were the following: nasal swabs (n = 2), serum from intracardiac clot, and/or alternatively a portion of the lung (10 gr.), Lymph nodes of the head (mandibular and retropharyngeal), spleen, large intestine content, ileocaecal lymph nodes, deep skeletal muscle, diaphragmatic muscle, gall bladder, any other organs with macroscopic lesions.

The zoonotic agents searched and the analyses performed were as follows:

- Direct research of *Staphylococcus aureus* from nasal swabs.
- Serological research of *Brucella spp.* antibodies from the serum obtained from the intracardiac clot, and/or from a portion of the lung (10 gr.).
- Direct research of *Brucella spp.* from lymph nodes of the head and from the spleen.
- Anatomico-histopathological and culture analyses for *Mycobacterium spp.* from lymph nodes/organs with macroscopic lesions related to tuberculosis.
- Direct research of *Salmonella spp.*, *Campylobacter spp.*, *Yersinia enterocolitica* and *Escherichia coli* from the intestinal content (large intestine) and the skeletal muscle.
- Direct research of *Salmonella spp.* from the ileum-caecal lymph nodes.
- Direct research of *Trichinella spp.* from the diaphragmatic muscle.

All samples were tested individually. For bacteriological and parasitological examinations, accredited standard methods were used, following the ISO methods when available, or international standard methods (e.g. OIE Manual). For *Mycobacterium spp.* an "in series" diagnostic scheme was implemented. The presence of macroscopic anatomical-pathological lesions attributable to tuberculosis were first assessed, and only if present, anatomico-histopathological and culture analyses were performed.

The isolated/identified agents were phenotypically and genotypically characterized by the use of qualitative microbiological techniques and molecular methods (e.g. PCR, sequencing and analysis of the obtained sequences).

In tables 1, 2 and 3 are reported the results of the zoonotic agents detected in the different areas of the study.

Discussion and Conclusions

The number of collected samples is conform to what predetermined in the objectives of the project (numerosity able to detect at least one "positive", if the minimum prevalence of the various agents in the population is at least 2.0%, with 95% CI).

In most cases all the provided samples were properly collected and the material was considered adequate for the execution of the analyses. Taking into account that the sampling was performed on wild animals and carried out in field conditions this testify the excellent work done by the Veterinary Services (U.O. 8), with the precious collaboration of the involved hunting teams.

From the 231 examined animals no evidence of microbiological infection with *Mycobacterium spp.* was observed. In only one case microbiological evidence (direct diagnosis) of *Brucella spp.* infection was observed, even though other positive serological results were detected (24 animals positive at the complement fixation test (CFT), although generally with a low titre (≤ 160). The *Brucella spp.* isolate belonged to the species *Brucella suis* biovar 2. All animals were negative for *Trichinella spp.*

The most frequently reported zoonotic agents were:

-*Campylobacter spp.* The intestinal content of 30.3% of the animals from the province of Viterbo were positive. Only in rare cases *Campylobacter jejuni* (1.1%) and *Campylobacter coli* (3.7%) were isolated. In some cases, after excluding the presence of *Campylobacter jejuni/coli*, species considered relevant for the public health, diagnosis stopped after the genus typing. No *Campylobacter spp.* was isolated from any muscle samples, or from any samples collected in Tuscany.

-*Staphylococcus aureus* methicillin susceptible (MSSA). Nasal swabs of 12.8% of the sampled animals in the province of Viterbo and of 27.9% of the sampled animals in Tuscany tested positive. Methicillin-resistant *Staphylococcus aureus* (MRSA) was not detected, while the presence of the *mecA* gene, responsible for the resistance to beta-lactams, was detected in two out of the eight

isolates of *Staphylococcus pseudintermedius* identified.

-Pathogenic *Escherichia coli*. From the intestinal contents of 6.4% of the animals from the province of Viterbo and from 4.7% of the animals from Tuscany, *Escherichia coli* with the adhesion factor defined intimina (*eae* gene) were isolated. None of them possessed verocytotoxin genes (*stx-1/stx-2*), typical of *Escherichia coli* VTEC or STEC. Some muscle samples were positive for *eae* genes (3.7%), but also for *stx-1/stx-2* (8%). These genes, however, were identified only in the broth cultures and not from individual colonies, which is indicative of a presumed presence.

-*Salmonella spp.* The presence of *Salmonella spp.* was detected in 4.1% of the animals (intestinal contents and/or ilieocecali lymph nodes) from the province of Viterbo. The isolates detected belonged to different serotypes: *Salmonella enterica subsp. houtenae*, *Salmonella enterica subsp. kottbus*, *Salmonella enterica subsp. diarizonae* and one strain being R, serologically untypable. The only positive isolate from Tuscany belonged to the serotype *Salmonella typhimurium* monophasic variant. No *Salmonella* isolates were detected from skeletal muscle samples.

-*Yersinia enterocolitica*. The presence of *Yersinia enterocolitica* was detected in the intestinal contents of only two animals from the province of Viterbo (1.1%) and in 14% of the animals from Tuscany. The percentage of positivity for *Yersinia enterocolitica* in skeletal muscle samples was of 3.2%. In the latter case serotyping ruled out the presence of *Yersinia enterocolitica* belonging to groups O1, O2, O3, O5, O8 and O9.

In general, the results obtained showed low positivity rates for the sought zoonotic agents. Negative results for major zoonotic agents such as *Mycobacterium spp.* is reassuring even in an indirect way: in our regions wild boar cannot be considered a reservoir of *Mycobacterium tuberculosis*-complex (e.g. *M. bovis* and *M. tuberculosis*), and the occasional identification of *M. bovis* encountered over the years can be considered an epiphenomenon of the resurgence of tuberculosis in cattle grazing (associated *M. bovis* reservoir). The constant absence of *Trichinella spp.* in samples of our study is also in line with expected prevalence in wild boar, usually extremely low on the Italian territory, despite some reported cases with infection and disease in humans..

Of relevance the detection in a subject of a strain of *Brucella suis* biovar 2, known to be circulating in wild boar populations in the territory of the Lazio Region and in other regions of central Italy.

The percentage of isolation of *Salmonella*, with the detection in one case of a strain of *Salmonella Typhimurium* monophasic variant, confirms the role of the species *Sus scrofa* (both wild and domestic populations) as reservoir of *Salmonella enterica subsp. enterica*, including serovars most relevant for the zoonotic impact in Man.

On the other hand, the absence of *Salmonella spp.*, *Campylobacter spp.* and of positive colonies related to *E. coli* VTEC in the muscle of the animals under study, as well as the absence of *Yersinia enterocolitica* belonging to groups O1, O2, O3, O5, O8 and O9, suggests an adequate level of hygiene of the meat at slaughtering, demonstrating that the hunting and the subsequent slaughtering procedures had been executed had been properly carried out and performed in compliance with common hygienic practices.

Investigations to detect the presence and circulation of zoonotic pathogens are, however, essential in order to estimate the prevalence in function of a reduction of the risks for the Public Health, considering that the marketing and consumption meat from wild populations, when not controlled, can be a source of exposure to a risk difficult to assess for the consumers.

More targeted studies may serve in the future to better understand the epidemiological significance of the wild boar in relation to different zoonotic agents, and to provide the Competent Authority of additional information necessary for the elaboration of practical proposals for a correct management of the the health status of the species, even in function of disease Human exposure in relation to hunting, consumption, and production and distribution of its meat.