Presence and Characterization of Zoonotic Bacterial Pathogens in Wild Boar Hunting Dogs (Canis lupus familiaris) in Tuscany (Italy)

Giovanni Cilia, Filippo Fratini, Barbara Turchi, Valentina Virginia Ebani, Luca Turini, Stefano Bilei, Teresa Bossù, Maria Laura De Marchis, Domenico Cerri and Fabrizio Bertelloni

Abstract: Domestic dogs (Canis lupus familiaris) used for wild boar (Sus scrofa) hunting may represent incidental hosts for several zoonotic pathogens. This investigation aimed to evaluate the presence of anti-Leptospira antibodies and the occurrence, antimicrobial resistance, and virulence of Salmonella spp., Yersinia enterocolitica, and Listeria monocytogenes in sera and rectal swabs collected from 42 domestic hunting dogs in the Tuscany region (Italy). Regarding Leptospira, 31 out of 42 serum samples (73.8%) were positive and serogroup Pomona was the most detected (71.4%) at titers between 1:100 and 1:400. Four Salmonella isolates (9.52%) were obtained, all belonging to serotype Infantis; two of them showed antimicrobial resistance to streptomycin, while ppB and sopE presence was assessed in all but one isolate. Concerning Yersinia enterocolitica, seven isolates (16.7%) were obtained, six belonging to biotype 1 and one to biotype 4. Resistance to amoxicillin–clavulanic acid, cephalothin, and ampicillin was detected. Biotype 4 presented three of the virulence genes searched (gstA, ystB, inv), while isolates of biotype 1 showed only one gene. No Listeria monocytogenes was isolated from dog rectal swabs. The results suggest that hunting dogs are exposed to different bacterial zoonotic agents, potentially linked to their work activity, and highlight the possible health risks for humans.

Keywords: Leptospira; Salmonella; Yersinia; Listeria; zoonoses; hunting dogs

1. Introduction

The coexistence between domestic dogs (Canis lupus familiaris) and humans has lasted for about 40,000 years. By this relationship, dogs have evolved thanks to several domestication events [1,2]. During these multiple events, dogs did not modify their complex body
language, and humans used their sophisticated forms of social cognition and communication to train them [1,2]. The training led to dividing dogs into working-class categories and employing them in jobs that help humans in several areas, including hunting.

Traditionally in Italy, domestic hunting dogs have been employed by hunters to track wildlife, as well as wild boar (Sus scrofa), during the activity known as a “drive hunt”, in in which several dogs are used [3].

Due to contact with some wild animals species and the sharing of environmental areas, domestic hunting dogs can be affected by a large variety of pathogens [4–11]. Moreover, wild boar hunting dogs during game activities or at the end of slaughtering are usually fed raw wild boar meat and/or slaughter waste, such as liver, lung, spleen, heart, kidney, and sometimes testicles; this practice can increase the risk of disease transmission [9].

Wildlife acts as a reservoir for pathogens that contribute to maintaining and/or disseminating important infectious diseases [12–17]. Regarding Tuscany (Italy), among bacterial zoonoses, different etiological agents have been detected in wild animals, in particular Leptospira, Salmonella, Yersinia, and Listeria [18–27].

Leptospirosis is a neglected and re-emerging zoonosis caused by Gram-negative bacteria belonging to the Leptospira genus [28]. Leptospira infection, which is widespread worldwide, is maintained by a large variety of domestic and wild animal species, which act as asymptomatic maintenance hosts [29,30]. The localization of renal reservoirs contributes to maintaining the infection in the environment through constant shedding of Leptospira in urine. In this way, accidental contact with Leptospira-infected urine causes an incidental infection that could lead to clinical disease, as reported in dogs [30,31].

Salmonellosis is the second most diffused zoonosis in Europe [32]. It is caused by a Gram-negative rod-shaped, flagellated, and facultative anaerobic bacteria belonging to the Salmonella enterica species, which includes more than 2600 serovars [33]. Salmonella enterica strains can cause illnesses in both humans and animals [33,34].

Yersinia enterocolitica is the etiological agent of yersiniosis, another important zoonosis [32]. Bacteria belonging to this species can survive in the environment for a long time. Yersinia enterocolitica has been divided into more than 70 serotypes, based on differences in the structure of the somatic antigen, and into 6 biotypes based on its biochemical characteristics [35].

Listeriosis is a zoonosis caused by Listeria monocytogenes, a Gram-positive and facultative intracellular bacterium [36]. Listeria infection occurs as an epidemic or sporadically, and more than 90% of human cases worldwide have generally been caused by strains belonging to serovars 1/2a, 1/2b, and 4b of 13 possible serovars [37].

All of these foodborne zoonoses are spread worldwide and diffused in several environments, such as soil, water, feces, and meat [38]. Moreover, one of the main forms of Salmonella spp. and Yersinia enterocolitica transmission is the consumption of domestic and wild swine meat [39,40].

This investigation was aimed at evaluating infection by Leptospira spp., Salmonella spp., Yersinia enterocolitica, and Listeria monocytogenes in domestic hunting dogs employed for wild boar hunting. Leptospira prevalence was analyzed using serological assay, while Salmonella spp., Yersinia enterocolitica, and Listeria monocytogenes were investigated through isolation methods. The presence of virulence genes and antimicrobial resistance of the obtained isolates was evaluated.

2. Materials and Methods

2.1. Sampling

In January 2020, at the end of the hunting season that started in November 2019, we collected radial vein blood samples and rectal swabs from domestic hunting dogs (Canis lupus familiaris) employed in wild boar hunting in the provinces of Pisa and Lucca (Tuscany, Italy). All specimens were sampled from hunting dogs belonging to hunters who collaborated with the authors during sample collection for previous research [18,22,23,41,42].
Samples were collected after authorization by the Organismo Preposto al Benessere degli Animali (OPBA) of the University of Pisa with protocol no. 21/2020. During the sampling phase, which took place where the dogs were housed by their owners, information about vaccination programs and vaccines used for leptospirosis was recorded. In addition, the occurrence of previous gastrointestinal disorders was taken into consideration, and subjects with recent or previous intestinal diseases were not included in the study.

Blood samples were centrifuged at 10,000 rpm for 10 min to obtain serum. The sera were kept at −20 °C until use for the serological test. Rectal swabs were processed right away to obtain isolates.

### 2.2. Microscopic Agglutination Test (MAT)

To provide evidence of the vaccine’s effectiveness and to investigate the possible native response against *Leptospira* serogroups not covered by vaccines, a serological analysis was carried out. In particular, to detect *Leptospira* antibodies, sera were tested by the microscopic agglutination test (MAT) [43]. A titer of 1:100 was considered positive. The following live *Leptospira* antigens were used for the MAT: *Leptospira interrogans* serovar Icterohaemorrhagiae (serogroup Icterohaemorrhagiae, strain RGA), *L. interrogans* serovar Canicola (serogroup Canicola, strain Alarik), *L. interrogans* serovar Pomona (serogroup Pomona, strain Mezzano), *L. kirschneri* serovar Grippotyphosa (serogroup Grippotyphosa, strain Moskva V), *L. borgpetersenii* serovar Tarassovi (serogroup Tarassovi, strain Mittis Johnson), *L. interrogans* serovar Bratislava (serogroup Australis, strain Riccio 2), *L. interrogans* serovar Hardjo (serogroup Sejroe, serovar Hardjoprajitno), and *L. borgpetersenii* serovar Ballum (serogroup Ballum, strain Mus 127).

### 2.3. Bacterial Isolation and Characterization

From rectal swabs, *Salmonella* spp., *Yersinia enterocolitica*, and *Listeria monocytogenes* isolation was performed as previously described [22,44]. *Salmonella* spp. isolates were serotyped by slide agglutination test with commercial antisera (Statens Serum Institut, Copenhagen, Denmark), according to the Kauffmann–White scheme. *Yersinia enterocolitica* isolates were characterized based on biochemical tests to distinguish the biotype [45].

### 2.4. Antimicrobial Resistance

The antimicrobial susceptibility of all obtained isolates was evaluated using the disc diffusion test on Mueller Hinton Agar (Oxoid, Ltd., Basingstoke, UK) [46]. The following antibiotics (Oxoid) were employed: amoxicillin-clavulanic acid (AMC; 30 µg), ampicillin (AMP; 10 µg), aztreonam (ATM; 30 µg), cefalothin (KF; 30 µg), cefotaxime (CTX; 30 µg), cefoxitin (FOX; 30 µg), chloramphenicol (C; 30 µg), enrofloxacin (ENR; 5 µg), gentamycin (CN; 10 µg), imipenem (IPM; 10 µg), nalidixic acid (NA; 2 µg), nitrofurantoin (F; 300 µg), streptomycin (S; 10 µg), sulfamethoxazole–trimethoprim (STX; 25 µg), and tetracycline (TE; 30 µg). CLSI (Clinical and Laboratory Standards Institute) zone diameter interpretive criteria were used [47].

### 2.5. Virulence Genes

DNA was extracted from overnight bacterial cultures of each isolate using Quick-DNA Plus Kits (Zymo Research, Irvine, CA, USA) following the manufacturer’s guidelines.

In *Salmonella* spp. isolates, the presence of *mgtC*, *pipB*, *sopB*, *spvR*, *spvC*, *gipA*, *sodCI*, and *sopE* genes, linked to virulence, was evaluated using primers and protocols as previously reported [48–52].

In *Yersinia enterocolitica* isolates, the presence of the following virulence genes was evaluated using previously published primers and protocols: *ail*, *virF*, *ystA*, *ystB*, and *inv* [53–55].

Each polymerase chain reaction (PCR) was carried out in a total volume of 50 µL, including 25 µL of EconoTaq PLUS 2× Master Mix (Lucigen Corporation, Middleton, WI, USA), 0.5 µM of each primer, 3 µL of DNA, and distilled water to reach the final volume. An
automated thermal cycler Gene-Amp PCR System 2700 (PerkinElmer, Norwalk, CT, USA) was employed to perform the amplifications, consisting of initial denaturation at 95 °C for 10 min, 35 cycles of denaturation at 95 °C for 1 min, annealing for 2 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Annealing temperatures were set based on the specific primers employed. PCR products were analyzed by electrophoresis on 1.5% agarose gel at 100 V for 45 min. PCR Sizer 100 bp DNA Ladder (Norgen Biotek, Thorold, ON, Canada) was used as a DNA marker.

2.6. Statistical Analysis

Data were reported on Excel (Microsoft, Albuquerque, NM, USA) and analyzed with the chi-square ($X^2$) test. The statistical test was used to evaluate the infection rate of each pathogen in relation to sex (male or female) and hunting province (Pisa or Lucca). The statistical significance threshold was set at $p \leq 0.05$ [56], and a 95% confidence interval was calculated.

3. Results

Blood and rectal swabs were collected from 42 hunting dogs, 30 from a hunting company in Pisa and 12 from Lucca; in particular, samples were collected from dogs belonging to 10 different owners (Table 1). All dogs were housed in a single box, except during work activities or training. The box appeared clean and in good condition; owners noted that rats and mice were not present, but they did not have a regular rat-control program. All animals were fed only with commercial feed. All dogs (22 males, 20 females) were healthy and did not show clinical signs of leptospirosis or gastrointestinal disorder at sampling time. The vaccines given to the investigated dogs were Eurican L-multi® (including serovars Canicola, Icterohaemorrhagiae, and Grippotyphosa), Nobivac L-4® (including serovars Canicola, Icterohaemorrhagiae, Bratislava, and Grippotyphosa), and Canigen DHPPi/L® (including serovars Canicola and Icterohaemorrhagiae), as reported in Table 1.

Table 1. Serological reactions detected in hunting dog sera in relation to *Leptospira* serogroups, their vaccination state, and hunting company.

| Dog  | Sex | Year | Breed | Hunting Company | Leptospira Serogroup | Vaccine |
|------|-----|------|-------|-----------------|----------------------|---------|
| D1   | M   | 2    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:400 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D2   | F   | 6    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:100 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D3   | F   | 2    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:200 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D4   | M   | 2    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:200 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D5   | M   | 2    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:200 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D6   | F   | 9    | ES    | Pisa           | Ic:1:100 Ca:1:100 Po:1:100 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D7   | M   | 6    | ES    | Pisa           | Ic:1:100 Ca:1:100 Po:1:100 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D8   | F   | 6    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:200 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D9   | F   | 4    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:400 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D10  | M   | 4    | SM    | Pisa           | Ic:2:200 Ca:1:100 Po:1:100 Gr:1:200 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D11  | F   | 10   | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:100 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D12  | F   | 2    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:100 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D13  | F   | 1    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:400 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D14  | F   | 1    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:200 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D15  | F   | 2    | SM    | Pisa           | Ic:1:100 Ca:1:100 Po:1:100 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D16  | F   | 3    | SM    | Pisa           | Ic:1:100 Ca:1:200 Po:1:400 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D17  | M   | 2    | ESS   | Pisa           | Ic:1:100 Ca:1:100 Po:1:200 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
| D18  | F   | 7    | ESS   | Pisa           | Ic:1:200 Ca:1:100 Po:1:200 Gr:1:100 Ta:1:100 Au:1:100 Se:1:100 Ba:1:100 | E       |
Table 1. Cont.

| Dog | Sex | Year | Breed | Hunting Company | Leptospira Serogroup | Vaccine |
|-----|-----|------|-------|-----------------|----------------------|--------|
|     |     |      |       |                 | Ic  | Ca  | Po  | Gr  | Ta  | Au  | Se  | Ba  |
| D19 | M   | 4    | SM    | Pisa            | 1:100 | 1:200 | 1:200 | 1:100 | 1:100 | N   |
| D20 | F   | 3    | SM    | Pisa            | 1:100 |       | 1:100 |       |       |     |
| D21 | M   | 2    | SM    | Pisa            | 1:100 |       |       | 1:100 |       |     |
| D22 | M   | 9    | BS    | Pisa            | 1:100 | 1:100 | 1:400 | 1:100 | 1:100 | N   |
| D23 | F   | 3    | ESS   | Pisa            | 1:100 | 1:100 | 1:200 | 1:100 |       | E   |
| D24 | M   | 6    | BS    | Pisa            | 1:100 | 1:100 |       |       |       |     |
| D25 | M   | 3    | SM    | Pisa            | 1:200 |       |       |       |       |     |
| D26 | M   | 3    | SM    | Pisa            | 1:200 |       |       |       |       |     |
| D27 | M   | 3    | SM    | Pisa            | 1:400 |       |       |       |       |     |
| D28 | M   | 2    | SM    | Pisa            | 1:200 |       |       |       |       |     |
| D29 | F   | 3    | SM    | Pisa            | 1:200 |       |       |       |       |     |
| D30 | F   | 8    | SM    | Pisa            | 1:200 |       |       |       |       |     |
| D31 | M   | 1    | SM    | Lucca           | 1:100 | 1:100 | 1:200 | 1:200 | 1:100 | N   |
| D32 | M   | 2    | SM    | Lucca           | 1:100 | 1:100 | 1:400 | 1:100 | 1:100 | N   |
| D33 | M   | 11   | GF    | Lucca           | 1:100 | 1:100 | 1:100 | 1:100 |       | N   |
| D34 | F   | 2    | GF    | Lucca           | 1:100 | 1:100 | 1:100 | 1:100 |       | N   |
| D35 | M   | 3    | SM    | Lucca           | 1:100 | 1:100 | 1:100 | 1:200 | 1:100 | N   |
| D36 | M   | 3    | SM    | Lucca           | 1:100 | 1:100 | 1:100 | 1:100 |       | N   |
| D37 | F   | 7    | SM    | Lucca           | 1:100 | 1:100 | 1:100 | 1:100 |       | N   |
| D38 | M   | 7    | HB    | Lucca           | 1:200 |       |       |       |       |     |
| D39 | F   | 4    | SM    | Lucca           | 1:200 |       | 1:100 |       |       |     |
| D40 | F   | 4    | SM    | Lucca           | 1:100 | 1:100 |       |       |       |     |
| D41 | M   | 6    | SM    | Lucca           |       |       |       |       |       |     |
| D42 | M   | 5    | SM    | Lucca           |       |       |       |       |       |     |

Dog IDs with same symbols from same owners. SM, Segugio Maremmano; ES, English Setter; ESS, English Springer Spaniel; BS, Brittany Spaniel; GF, Griffon Bleu de Gascogne; HB, half-breed; Ic, Icterohaemorrhagiae; Ca, Canicola; Po, Pomona; Gr, Grippotyphosa; Ta, Tarassovi; Au, Australis; Se, Sejroe; Ba, Ballum; E, Eurican L-multi®, covering for serovars Canicola, Icterohaemorrhagiae, and Grippotyphosa; N, Nobivac L-4®, covering for serovars Canicola, Icterohaemorrhagiae, Bratislava, and Grippotyphosa; C, Canigen DHPPi/L®, covering for serovars Canicola and Icterohaemorrhagiae.

3.1. Leptospira spp.

Overall, all sera but one were positive by MAT (Table 1). Some of the positive reactions against Icterohaemorrhagiae, Canicola, Australis, and Grippotyphosa serogroups, 31 out of 42 serum samples (73.8%; 95% confidence interval (CI): 60.5–87.1%), probably linked to vaccination, were positive to serological analysis (shown in bold in Table 1).

Pomona was the most-recorded serogroup (71.4%; 95% CI: 57.7–85.0%), serologically detected in most of the positive sera (30/31). Among them, 8 (26.7%; 95% CI: 10.8–42.5%) showed a titer of 1:400, 17 (56.7%; 95% CI: 38.9–74.4%) a titer of 1:200, and 5 (16.7%; 95% CI: 3.3–30.0%) a titer of 1:100. One serum was also positive to serogroup Australis at a titer of 1:100. Finally, two sera (6.7%; 95% CI: 0.0–15.6%) were positive to serogroup Grippotyphosa, one at a titer of 1:100 and one at 1:200.

No statistical differences (p > 0.05) were reported for serological positivity considering hunting company, province, and dog sex.

3.2. Salmonella spp.

Four Salmonella strains (9.52%; 95% CI: 0.6–18.3%) were isolated from hunting dog rectal swabs. All isolates belonged to Salmonella enterica subspecies enterica serotype Infantis. Only two of them showed resistance to streptomycin (50.0%). All but one isolate harbored some virulence genes. Two isolates scored positive only to the pipB gene, and one to pipB and sopE (Table 2).
Table 2. Virulence genes and antimicrobial resistance profiles of analyzed and characterized Salmonella spp. strains.

| Isolate | Serotype | Dog | Virulence Gene Profile | Antimicrobial Resistance Profile |
|---------|----------|-----|------------------------|---------------------------------|
| S395    | Infantis | D2  | pipB, sopE              | Streptomycin                    |
| S396    | Infantis | D9  | -                       | -                               |
| S397    | Infantis | D13 | pipB                    | -                               |
| S398    | Infantis | D16 | pipB                    | Streptomycin                    |

No statistical differences ($p > 0.05$) were reported for the number of obtained Salmonella spp. isolates considering hunting company, province, and dog sex.

3.3. Yersinia enterocolitica

In total, seven strains of Yersinia enterocolitica (16.7%; 95% CI: 5.4–27.9%) were isolated, six of which were biochemically confirmed as biotype 1, and one as biotype 4 (Table 3).

Table 3. Virulence genes and antimicrobial resistance profiles of analyzed Yersinia enterocolitica isolates.

| Isolate | Biotype | Dog | Virulence Gene Profile | Antimicrobial Resistance Profile |
|---------|---------|-----|------------------------|---------------------------------|
| YD1     | 1       | D6  | ail                    | AMP, KF                         |
| YD2     | 1       | D7  | ystA                   | AMP, AMC, KF, FOX               |
| YD3     | 1       | D10 |                         | AMP, AMC, KF                    |
| YD4     | 4       | D13 | ystA, ystB, inv        | AMP, AMC, KF, FOX, C, S, NA     |
| YD5     | 1       | D15 | ail                    | AMP, AMC, KF                    |
| YD6     | 1       | D16 | ystB                   | AMP, AMC, KF                    |
| YD7     | 1       | D17 | virF                   | AMP, AMC, KF                    |

AMC, amoxicillin–clavulanic acid; AMP, ampicillin; KF, cephalothin; FOX, cefoxitin; S, streptomycin; NA, nalidixic acid; C, chloramphenicol.

All strains were resistant to at least two antimicrobials. In particularly, all strains (100%) were resistant to ampicillin and cephalothin. Moreover, ampicillin resistance was reported in six isolates (85.7%), cefoxitin resistance in two isolates (28.6%), and streptomycin, nalidixic acid, and chloramphenicol resistance in one isolate (14.3%).

All but one isolate presented at least one virulence gene; only one isolate, biotype 4, scored positive for more than one gene. The most detected genes were ystA, ystB, and ail in two out of the seven isolates (28.6%), followed by inv and virF, detected in one isolate (14.3%).

No statistical differences ($p > 0.05$) were reported for the number of obtained Yersinia enterocolitica isolates considering hunting company, province, and dog sex.

3.4. Listeria monocytogenes

No Listeria monocytogenes isolates were obtained from hunting dog rectal swabs.

4. Discussion

This investigation reports infection by Leptospira spp., Salmonella ser. Infantis, and Yersinia enterocolitica in a sample of hunting dogs employed in wild boar hunting in two provinces of Tuscany. To the best of the authors’ knowledge, no other investigations have been performed to research these zoonotic bacterial pathogens in Italian hunting dogs.

Concerning Leptospira serological results, all dogs but one were positive, at least for one serogroup. Some positive reactions could be related to immune response induced by vaccine administration, as previously reported [57–59]. Indeed, in all serum samples, titers of 1:100 and 1:200 were reported in vaccinated dogs for serovars included in the vaccine. In detail, antibodies for serogroup Icterohaemorrhagiae, Canicola, Grippotyphosa, and Australis were found in dogs regularly vaccinated with Nobivac L-4® (MSD Animal Health); for serogroup Icterohaemorrhagiae, Canicola, and Grippotyphosa in dogs vaccinated with Eurican L-muti® (Boehringer Ingelheim Animal Health); and for serogroup
Icterohaemorrhagiae and Canicola in dogs vaccinated with Canigen DHPPi/L® (Virbac S.r.l.). Serogroup Pomona was found in 71.4% of sampled hunting dogs, at titers from 1:100 to 1:400. The serological titers suggested chronic or very recent infection, probably mediated by vaccine status.

Pomona is a serogroup strictly connected to pigs. Indeed, *Leptospira*, which belongs to this serogroup, has been widely detected in the Tuscany region in domestic and wild swine [19,60,61], particularly in wild boar sampled in the same area and the same period [23]. Pomona was recently isolated in Tuscany from a crested porcupine [21]; even if the role of this animal as maintenance or accidental host is not still clear, this suggests a link to animals other than wild boar. Due to the rare Pomona infection reported in dogs, this serovar is not included in dog vaccines [57], even though infection causes severe disease with lethargy, fever, inappetence, diffuse hemorrhage, and renal and liver failure [62,63]. In recent years in Italy, Pomona incidence in dogs has increased [64–67], probably due to contact with wild boar, which play a role in spreading the disease as a reservoir.

Two serum samples showed a positive reaction to Grippotyphosa live antigen, at titers of 1:100 and 1:200. In Europe, this is an emerging serogroup in dogs [65,67–70] and is included in the dog leptospirosis vaccine [57]. In two serum samples, *Leptospira* co-infection was reported: one was positive to Grippotyphosa and Pomona at titers of 1:200 and 1:100, respectively, and the other to Pomona and Australis at titers of 1:200 and 1:100, respectively. It cannot be excluded that the co-infection could be related to an unspecific reaction between the serum antibody and *Leptospira* antigens [28]. Grippotyphosa and Australis infection could be related to direct or indirect contact with small rodents, lagomorphs, and hedgehogs, which act as reservoirs for these serovars and are particularly abundant among the wildlife population in this area [65]. Finally, although serology has high diagnostic value for leptospirosis and MAT is considered the gold standard test, with high sensitivity and specificity, in order to better understand the real risk for hunting dogs, it will be necessary to perform isolation or molecular investigation on urine and blood samples in the future.

Regarding rectal swabs, 9.52% were positive to *Salmonella* spp. Although no studies have been performed on hunting dogs, the prevalence found in this investigation is low, as previously reported in studies on domestic dogs [5,71,72]. All of them belonged to *Salmonella enterica* subspecies enterica serotype Infantis. This serotype is usually associated with swine [73,74] and has been isolated in free-ranging wild boar in the same investigated area [22] and in the north of Italy [75]. However, other sources of infection exist other than wild swine; indeed, *S. ser* Infantis was also detected in wild carnivores, ruminants, and birds in Italy [76]. Although no statistical differences emerged regarding *Salmonella* positivity, all infected dogs were from the same owner. For this reason, we cannot exclude that infection was not linked to work activities, but to the environment where the animals live or the management of these dogs. Only two *Salmonella* isolates were resistant to antimicrobials, and particularly only to streptomycin. Streptomycin resistance is well documented in *Salmonella*, particularly in strains isolated from dogs [77,78] and swine [76,79,80]. Concerning virulence genes, pipB and sopE were the only two detected, found only in wild boar sampled in Tuscany [22]. Both genes were found in the S395 isolates, while only pipB was found in in S397 and S398. The sopE gene is transmitted by phage and is involved in the invasion of intestinal epithelial cells. It also stimulates the inflammatory response in the host [44]. The pipB gene is associated with *Salmonella* pathogenicity island 5 (SPI-5) and plays a role in the survival of *Salmonella* in the intracellular environment [81].

During this investigation, *Yersinia enterocolitica* was isolated from 16.7% of rectal swabs from hunting dogs. As for *Salmonella*, no studies have been carried out on *Yersinia enterocolitica* infection in hunting dogs. A very close prevalence was previously detected in domestic dogs [82–84] and in wild boar hunted in the same Italian region [22]. The most diffuse is biotype 1, while only one isolate belongs to biotype 4. These data are in accordance with the prevalence of yersiniosis and biotypes distribution in wild boar [22,85,86]. As for *Salmonella*, other sources of infection cannot be definitively excluded; although
Y. enterocolitica has been isolated from resident and migratory wild fauna in Italy [87,88], no recent data on the investigated area are available to allow a robust hypothesis. Among isolates belonging to biotype 1, only one virulence gene (within ail, ystA, ystB, and virF) was detected, while in biotype 4 the ystA, ystB, and inv genes were detected. As previously reported in swine [85,89] and wild boar [22], high resistance to amoxicillin–clavulanic acid and cephalothin was also detected in isolates from investigated dogs, but this result could be linked to intrinsic resistance, as previously reported [90]. The rates of resistance to nalidixic acid, chloramphenicol, and streptomycin found in Yersinia enterocolitica isolates from dogs seem to be similar to the antimicrobial resistance associated with wild boar strains [22].

The high prevalence of Leptospira interrogans serogroup Pomona detected serologically seems to be connected to the infection circulating in free-ranging wild boar in the Tuscany region [19,23,65]. The same association could be hypothesized for Salmonella spp. and Yersinia enterocolitica, comparing the results of this investigation with the infection of wild boar sampled in the same investigated area, although other sources of infection cannot be excluded. All dogs were healthy at the time of sampling, and the owners did not report previous disease linked to the investigated pathogens; hunting dogs could potentially become asymptomatic reservoirs and shedders of these bacteria, contributing to their diffusion and representing a possible danger for the owners. This investigation highlights some of the risks hunting dogs are exposed to, presumably linked to their work activities, and the potential hazard for humans sharing the same environment for work or recreational activities.

5. Conclusions

In conclusion, the results of this investigation suggest that hunting dogs could be exposed to different pathogens, potentially during their work activities. Indeed, some of these pathogens are often associated with wildlife, in particular wild boar. To provide strong evidence on the sources of these pathogens, the number of hunting companies and dogs should be increased and a deep investigation into wildlife and the wild environment should be carried out to obtain an important number of isolates to be compared with phenotypic and molecular methods.

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References

1. Wayne, R.K.; Vonholdt, B.M. Evolutionary genomics of dog domestication. Mamm. Genome 2012, 23, 3–18. [CrossRef] [PubMed]
2. Larson, G.; Karlsson, E.K.; Perri, A.; Webster, M.T.; Ho, S.Y.W.; Peters, J.; Stahl, P.W.; Piper, P.J.; Lingaas, F.; Fredholm, M.; et al. Rethinking dog domestication by integrating genetics, archeology, and biogeography. Proc. Natl. Acad. Sci. USA 2012, 109, 8878–8883. [CrossRef] [PubMed]
3. Scillitani, L.; Monaco, A.; Toso, S. Do intensive drive hunts affect wild boar (Sus scrofa) spatial behaviour in Italy? Some evidences and management implications. Eur. J. Wildl. Res. 2010, 56, 307–318. [CrossRef]
4. Fiorello, C.V.; Straub, M.H.; Schwartz, L.M.; Liu, J.; Campbell, A.; Kownacki, A.K.; Foley, J.E. Multiple-host pathogens in domestic hunting dogs in Nicaragua’s Bosawás Biosphere Reserve. Acta Trop. 2017, 167, 183–190. [CrossRef]
Animals 2021, 11, 1139

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33. Jajere, S.M. A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and adaptation and antimicrobial resistance including multidrug resistance. Vet. World 2019, 12, 504–521. [CrossRef]

34. Mezal, E.H.; Sabol, A.; Khan, M.A.; Ali, N.; Stefanova, R.; Khan, A.A. Isolation and molecular characterization of Salmonella enterica serovar Enteritidis from poultry house and clinical samples during 2010. Food Microbiol. 2014, 38, 67–74. [CrossRef]

35. Tirzhiu, E.; Cumpanasoiu, C.; Gros, R.V.; Seres, M. Yersinia enterocolitica Monograph Study. J. Anim. Sci. Biotechnol. 2011, 44, 144–149.

36. Vázquez-Boland, J.A.; Kuhn, M.; Berche, P.; Chakraborty, T.; Domínguez-Bernal, G.; Goebel, W.; González-Zorn, B.; Wehland, J.; Kreft, J. Listeria pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 2001, 14, 584–640. [CrossRef] [PubMed]

37. Shamloo, E.; Hosseini, H.; Moghadam, A.Z.; Larsen, H.M.; Haslberger, A.; Alebouyeh, M. Importance of Listeria monocytogenes in food safety: A review of its prevalence, detection, and antibiotic resistance. Iran. J. Vet. Res. 2019, 20, 241–254. [PubMed]

38. EFSA, (European Food Safety Authority); ECDC, (European Centre for Disease Prevention and Control) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J. 2018, 16. [CrossRef]

39. Nesbakken, T.; Eckner, K.; Heidi, H.K.; Retterud, O.J. Occurrence of Yersinia enterocolitica and Campylobacter spp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures. Int. J. Food Microbiol. 2003, 80, 231–240. [CrossRef]

40. Hoelzer, K.; Switt, A.I.M.; Wiedmann, M. Animal contact as a source of human non-typhoidal salmonellosis. Vet. Res. 2011, 42, 1–28. [CrossRef]

41. Bertelloni, F.; Cilia, G.; Bogi, S.; Ebani, V.V.; Turini, L.; Nuvoloni, R.; Cerri, D.; Fratini, F.; Turchi, B. Pathotypes and Antimicrobial Susceptibility of Escherichia Coli Isolated from Wild Boar (Sus scrofa) in Tuscany. Animals 2020, 10, 744. [CrossRef]

42. Cilia, G.; Fratini, F.; Turchi, B.; Angelini, M.; Cerri, D.; Bertelloni, F. Genital Brucella suis Biovar 2 Infection of Wild Boar (Sus scrofa) Hunted in Tuscany (Italy). Microorganismis 2021, 9, 582. [CrossRef]

43. OIE. Leptospirosis. Man. Diagnostic Tests Vaccines Terr. Anim. 2014. Available online: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.12_LEPTO.pdf (accessed on 16 April 2021)

44. Bertelloni, F.; Tosi, G.; Massi, P.; Fiorentini, L.; Parigi, M.; Cerri, D.; Ebani, V.V.V. Some pathogenic characters of paratyphoid Salmonella enterica strains isolated from poultry. Asian Pac. J. Trop. Med. 2017, 10, 1161–1166. [CrossRef]

45. Bottone, E.J. Yersinia enterocolitica: The charisma continues. Clin. Microbiol. Rev. 1997, 10, 257–276. [CrossRef]

46. Clinical and Laboratory Standards Institute. M02-A12 Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Twelfth Edition; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2015; Volume 35.

47. Clinical and Laboratory Standard Institute. M31-A3 Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard-Third Edition; Clinical and Laboratory Standard Institute: Wayne, PA, USA, 2008; Volume 28, p. 8.

48. Skyberg, J.A.; Logue, C.M.; Nolan, L.K. Virulence Genotyping of Salmonella spp. with Multiplex PCR. Avian Dis. 2006, 50, 77–81. [CrossRef]

49. Karasova, D.; Havlickova, H.; Sisak, F.; Rychlik, I. Deletion of sodCl and spvBC in Salmonella enterica serovar Enteritidis reduced its virulence to the natural virulence of serovars Agona, Hadar and Infantis for mice but not for chickens early after infection. Vet. Microbiol. 2009, 139, 304–309. [CrossRef]

50. Huehn, S.; La Ragione, R.M.; Anjum, M.; Saunders, M.; Woodward, M.J.; Bunge, C.; Helmuth, R.; Hauser, E.; Guerra, B.; Beutlich, J.; et al. Virulotyping and antimicrobial resistance typing of Salmonella enterica serovars relevant to human health in Europe. Foodborne Pathog. Dis. 2010, 7, 523–535. [CrossRef]

51. Bottone, E.J. Virulotyping of seafood associated Salmonella enterica subsp. enterica isolated from South-west coast of India. Biotechnol. Bioinforma. Bioeng. 2011, 1, 63–69.

52. Parvathi, A.; Vijayan, J.; Murali, G.; Chandran, P. Comparative virulence genotyping and antimicrobial susceptibility profiling of environmental and clinical Salmonella enterica from Cochin, India. Curr. Microbiol. 2011, 62, 21–26. [CrossRef]

53. Thoerner, P.; Kingombe, C.I.B.; Bögli-Stuber, K.; Bissig-Choisat, B.; Wassenaar, T.M.; Frey, J.; Jemmi, T. PCR detection of virulence genes in Yersinia enterocolitica and Yersinia pseudotuberculosis and investigation of virulence gene distribution. Appl. Environ. Microbiol. 2003, 69, 1810–1816. [CrossRef]

54. Thisted Lambertz, S.; Danielsson-Tham, M.L. Identification and characterization of pathogenic Yersinia enterocolitica isolates by PCR and pulsed-field gel electrophoresis. Appl. Environ. Microbiol. 2005, 71, 3674–3681. [CrossRef]

55. Falcão, J.P.; Falcão, D.P.; Pitondo-Silva, A.; Malaspina, A.C.; Broochi, M. Molecular typing and virulence markers of Yersinia enterocolitica strains from human, animal and food origins isolated between 1968 and 2000 in Brazil. J. Med. Microbiol. 2006, 55, 1539–1548. [CrossRef]

56. R Core Team, R. A Language and Environment for Statistical Computing; R Found. Stat. Comput: Vienna, Austria, 2015.

57. Klaasen, H.L.B.M.; van der Veen, M.; Sutton, D.; Molkenboer, M.J.C.H. A new tetravalent canine leptospirosis vaccine provides at least 12 months immunity against infection. Vet. Immunol. Immunopathol. 2014, 158, 26–29. [CrossRef]

58. Martin, L.E.R.; Wiggins, K.T.; Wengnole, S.A.; Curtis, K.; Chandrashekar, R.; Lappin, M.R. Vaccine-Associated Leptospira Antibodies in Client-Owned Dogs. J. Vet. Intern. Med. 2014, 28, 789–792. [CrossRef]

59. Miller, M.D.; Annis, K.M.; Lappin, M.R.; Lunn, K.F. Variability in Results of the Microscopic Agglutination Test in Dogs with Clinical Leptospirosis and Dogs Vaccinated against Leptospirosis. J. Vet. Intern. Med. 2011, 25, 426–432. [CrossRef]
Animals 2021, 11, 1139

60. Bertelloni, F.; Turchi, B.; Vattiata, E.; Viola, P.; Pardini, S.; Cerri, D.; Fratini, F. Serological survey on Leptospira infection in slaughtered swine in North-Central Italy. *Epidemiol. Infect.* 2018, 1–6. [CrossRef] [PubMed]

61. Ebani, V.V.; Cerri, D.; Poli, A.; Andreani, E. Prevalence of *Leptospira* and *Brucella* Antibodies in Wild Boars (*Sus scrofa*) in Tuscany, Italy. *J. Wildl. Dis.* 2003, 39, 718–722. [CrossRef] [PubMed]

62. Greenlee, J.J.; Alt, D.P.; Bolin, C.A.; Zuerner, R.L.; Andreassen, C.B. Experimental canine leptospirosis caused by *Leptospira interrogans* serovars pomona and bratislava. *Am. J. Vet. Res.* 2005, 66, 1816–1822. [CrossRef]

63. Goldstein, R.E.; Lin, R.C.; Langston, C.E.; Scrivani, P.V.; Erb, H.N.; Barr, S.C. Influence of infecting serogroup on clinical features of leptospirosis in dogs. *J. Vet. Intern. Med.* 2006, 20, 489–494. [CrossRef] [PubMed]

64. Cerri, D.; Ebani, V.V.; Fratini, F.; Pinzauti, P.; Andreani, E. Epidemiology of leptospirosis: Observations on serological data obtained by a “diagnostic laboratory for leptospirosis” from 1995 to 2001. *New Microbiol.* 2003, 26, 383–389. [PubMed]

65. Bertelloni, F.; Cilia, G.; Turchi, B.; Pinzauti, P.; Cerri, D.; Fratini, F. Epidemiology of leptospirosis in North-Central Italy: Fifteen years of serological data (2002–2016). *Comp. Immunol. Microbiol. Infect. Dis.* 2019, 65, 14–22. [CrossRef]

66. Scanziani, E.; Origgi, F.; Giusti, A.M.; Iacchia, G.; Vasino, A.; Pirovano, G.; Scarpa, P.; Tagliabue, S. Serological survey of leptospiral infection in kennelled dogs in Italy. *J. Small Anim. Pract.* 2002, 43, 154–157. [CrossRef]

67. Ayral, F.C.; Bicout, D.J.; Pereira, H.; Artois, M.; Kodjo, A. Distribution of *Leptospira* with different suspected serogroups in cattle herds and dogs in France. *Am. J. Trop. Med. Hyg.* 2014, 91, 756–759. [CrossRef]

68. Renaud, C.; Andrews, S.; Djelouadji, Z.; Lecheval, S.; Corrao-Revol, N.; Buff, S.; Demont, P.; Kodjo, A. Prevalence of the *Leptospira* serovars bratislava, grippotyphosa, mozok and pomona in French dogs. *Vet. J.* 2013, 196, 126–127. [CrossRef]

69. Geisen, V.; Stengel, C.; Brem, S.; Müller, W.; Greene, C.; Hartmann, K. Canine leptospirosis infections? clinical signs and outcome with different suspected *Leptospira* serogroups (42 cases). *J. Small Anim. Pract.* 2007, 48, 324–328. [CrossRef]

70. Tsai, H.J.; Huang, H.C.; Lin, C.M.; Lien, Y.Y.; Chou, C.H. Salmonellae and campylobacters in household and stray dogs in Northern Taiwan. *Vet. Res. Commun.* 2007, 31, 931–939. [CrossRef]

71. Procter, T.D.; Pearl, D.L.; Finley, R.L.; Leonard, E.K.; Janecko, N.; Reid-Smith, R.J.; Weese, J.S.; Peregrine, A.S.; Sargeant, J.M. A Cross-Sectional Study Examining *Campylobacter* and Other Zoonotic Enteric Pathogens in Dogs that Frequent Dog Parks in Three Cities in South-Western Ontario and Risk Factors for Shedding of *Campylobacter* spp. *Zoonoses Public Health 2014, 61*, 208–218. [CrossRef]

72. Noda, T.; Murakami, K.; Ishiguro, Y.; Asai, T. Chicken Meat Is an Infection Source of *Salmonella* Serovar Infantis for Humans in Japan. *Foodborne Pathog. Dis.* 2010, 7, 727–735. [CrossRef]

73. Borowiak, M.; Szabo, I.; Baumann, B.; Junker, E.; Hammerl, J.A.; Kaesbohrer, A.; Malorny, B.; Fischer, J. VIM-1-producing *Salmonella* Infantis isolated from swine and minced pork meat in Germany. *J. Antimicrob. Chemother.* 2017, 72, 2131–2133. [CrossRef]

74. Chiari, M.; Zanoni, M.; Tagliabue, S.; Lavazza, A.; Alborali, L.G. *Salmonella* serotypes in wild boars (*Sus scrofa*) hunted in northern Italy. *Acta Vet. Scand.* 2013, 55, 42. [CrossRef]

75. Botti, V.; Valérie Navillold, F.; Domenis, L.; Orusa, R.; Pepe, E.; Robetto, S.; Guidetti, C. *Salmonella* spp. and antibiotic-resistant strains in wild mammals and birds in north-western Italy from 2002 to 2010. *Vet. Ital.* 2013, 49, 195–202. [CrossRef]

76. Philbey, A.W.; Mather, H.A.; Gibbons, J.F.; Thompson, H.; Taylor, D.J.; Coia, J.E. Serovars, bacteriophage types and antimicrobial sensitivities associated with salmonellosis in dogs in the UK (1954–2012). *Vet. Rec.* 2014, 174, 94. [CrossRef]

77. Seepersad Singh, N.; Adesiyun, A.A.; Seebarsingh, R. Prevalence and antimicrobial resistance of *Salmonella* spp. in diarrhoeic dogs in Trinidad. *Vet. J.* 2004, 170, 375–378. [CrossRef]

78. Caleja, C.; de Toro, M.; Gonçalves, A.; Themudo, P.; Vieira-Pinto, M.; Monteiro, D.; Rodrigues, J.; Sáenz, Y.; Carvalho, C.; Igrejas, G.; et al. Antimicrobial resistance and class I integrons in *Salmonella enterica* isolates from wild boars and Bisaro pigs. *Int. Microbiol.* 2011, 14, 19–24. [CrossRef] [PubMed]

79. Zottola, T.; Montagnaro, S.; Magnapera, C.; Sasso, S.; De Martino, L.; Bragagnolo, A.; D’Amici, L.; Condoleo, R.; Pisanelli, G.; Iovane, G.; et al. Prevalence and antimicrobial susceptibility of *Salmonella enterica* in European wild boar (*Sus scrofa*); Latium Region –Italy. *Comp. Immunol. Microbiol. Infect. Dis.* 2013, 36, 161–168. [CrossRef] [PubMed]

80. Knodler, L.A.; Vallance, B.A.; Hensel, M.; Jäckel, D.; Finlay, B.B.; Steele-Mortimer, O. *Salmonella* type III effectors PipB and PipB2 are targeted to detergent-resistant microdomains on internal host cell membranes. *Mol. Microbiol.* 2003, 49, 685–704. [CrossRef] [PubMed]

81. Wang, X.; Cui, Z.; Wang, H.; Tang, L.; Yang, J.; Gu, L.; Jin, D.; Luo, L.; Qiu, H.; Xiao, Y.; et al. Pathogenic strains of *Yersinia enterocolitica* isolated from domestic dogs (*Canis familiaris*) belonging to farmers are of the same subtype as pathogenic *Y. enterocolitica* strains isolated from humans and may be a source of human infection in Jiangsu Province, China. *J. Clin. Microbiol.* 2010, 48, 1604–1610. [CrossRef] [PubMed]

82. Wang, X.; Cui, Z.; Wang, H.; Tang, L.; Yang, J.; Gu, L.; Jin, D.; Luo, L.; Qiu, H.; Xiao, Y.; et al. Transmission of *Yersinia enterocolitica* 4/O:3 to pets via contaminated pork. *Lett. Appl. Microbiol.* 2001, 32, 375–378. [CrossRef] [PubMed]
85. Takahashi, T.; Kabeya, H.; Sato, S.; Yamazaki, A.; Kamata, Y.; Taira, K.; Asakura, H.; Sugiyama, H.; Takai, S.; Maruyama, S. Prevalence of yersinia among wild sika deer (Cervus nippon) and boars (Sus scrofa) in Japan. *J. Wildl. Dis.* 2020, 56, 270–277. [CrossRef]

86. Syczyło, K.; Platt-Samoraj, A.; Bancerz-Kisiel, A.; Szczera-Turek, A.; Pajda-Czau, J.; Labuč, S.; Procajło, Z.; Socha, P.; Chuzhebayeva, G.; Szweda, W. The prevalence of *Yersinia enterocolitica* in game animals in Poland. *PLoS ONE* 2018, 13, e0195136. [CrossRef]

87. Foti, M.; Rinaldo, D.; Guercio, A.; Giacopello, C.; Aleo, A.; De Leo, F.; Fisichella, V.; Mammina, C. Pathogenic microorganisms carried by migratory birds passing through the territory of the island of Ustica, Sicily (Italy). *Avian Pathol.* 2011, 40, 405–409. [CrossRef]

88. Foti, M.; Siclari, A.; Mascetti, A.; Fisichella, V. Study of the spread of antimicrobial-resistant Enterobacteriaceae from wild mammals in the National Park of Aspromonte (Calabria, Italy). *Environ. Toxicol. Pharmacol.* 2018, 63, 69–73. [CrossRef]

89. von Altrock, A.; Seinige, D.; Kehrenberg, C. *Yersinia enterocolitica* isolates from wild boars hunted in Lower Saxony, Germany. *Appl. Environ. Microbiol.* 2015, 81, 4835–4840. [CrossRef]

90. Clinical and Laboratory Standards Institute (CLSI). *M100 Performance Standards for Antimicrobial Susceptibility Testing A CLSI Supplement for Global Application*, 28th ed.; CLSI supplement M100; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.