Yersinia enterocolitica in diagnostic fecal samples of European dogs and cats: Identification by FT-IR and MALDI-TOF MS

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Running title: Yersinia enterocolitica in feces of dogs and cats

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Yersinia (Y.) enterocolitica is the main cause of yersiniosis in Europe, one of the five main bacterial gastrointestinal diseases in humans. Beside pigs, companion animals, especially dogs, and cats were repeatedly discussed in the past as a possible source of pathogenic Y. enterocolitica.

To investigate the presence and types of Y. enterocolitica in companion animals, a total of 4325 diagnostic feces samples from dogs and 2624 samples from cats were tested. The isolates obtained were differentiated using MALDI-TOF mass spectrometry and Fourier transform-infrared spectroscopy (FT-IR). Isolated Y. enterocolitica strains were bioserotyped. The detection of the ail-gene by PCR and confirmation by FT-IR were used as a pathogenicity marker.

Y. enterocolitica strains were isolated from 198 (4.6%) of the dog and 8 (0.3%) of the cat feces samples investigated. 179 isolates from dogs were analyzed in detail. The virulence factor Ail was detected in 91.6% of isolates. Isolates of biotype 4 (54.7%), and to a lesser extent biotypes 2 (23.5%), 3 (11.2%), and 5 (2.2%) respectively were detected. The remaining 8.4% of strains belonged to the ail-negative biotype 1A. All of the 7 isolates from cats that were investigated in detail were ail-positive.

These results indicate that companion animals could be a relevant reservoir for a broad range of presumptively human pathogenic Y. enterocolitica types. MALDI-TOF MS and FT-IR proved to be valuable methods for the rapid identification of Y. enterocolitica, especially in regard to the large number of samples that were investigated in a short time frame.
Yersinia (Y.) is a genus of bacteria in the family Enterobacteriaceae and includes three well-known human and animal pathogens, Y. enterocolitica, Y. pestis, and Y. pseudotuberculosis. Pathogenic strains of Y. enterocolitica and Y. pseudotuberculosis cause yersiniosis, an acute enteric disease, in humans and animals. In Germany and in the European Union, yersiniosis was the third most frequently reported zoonotic infection in humans in 2008 (1, 2).

The species Y. enterocolitica is traditionally grouped by serological tests into different serotypes and phenotypically by biochemical tests in five biotypes (biotype 1 to 5) (3, 4). Different bioserotype-combinations are thought to be connected with pathogenic potential, whereas biotype 1A is deemed to be non- or less pathogenic for humans, however, some cases were attributed to this biotype as well (5, 6, 7, 8). The bioserotypes usually associated with human disease are 1B/O:8, 2/O:5,27, 2/O:9, 3/O:3, and 4/O:3, the latter being the most common in industrialized countries, including Europe (2, 9).

Pathogenic Y. enterocolitica strains are identified by detection of several gene loci, whereof the ail-gene, chromosomally coding for an attachment and invasion factor, is one of the main pathogenic markers (10, 11). The presence of the corresponding Ail-factor can be shown with a phenotypic technique by a special Fourier transform-infrared spectroscopy method (12).

The epidemiology of Y. enterocolitica infections is complex and remains poorly understood (13). It is assumed that Y. enterocolitica infections are mainly attributed to eating contaminated food, in particular contaminated pork (2, 14). Nevertheless pathogenic isolates have rarely been recovered from food samples implicated in human illness. In Europe pigs have been considered to be the primary reservoir for the main human pathogenic Y. enterocolitica bioserotype 4/O:3, or less commonly 2/O:9 (2). In 2008, 1.8% of all
investigated samples of pork and 1.8% of all pigs were reported as *Y. enterocolitica* positive in Europe (2). Objective evidence for food-borne outbreaks of *Y. enterocolitica* is indeed rare and most infections are sporadic (9, 13).

In the past the relevance of companion animals for zoonosis to humans was estimated to be rather low. The European Food Safety Authority stated in 2010 that "on rare occasions, transmission of *Y. enterocolitica* may occur by direct contact with infected animals" (2). This claim was substantiated by investigations, where presumptively pathogenic *Y. enterocolitica* in dog samples were found in Europe and Japan at rates of up to 30% (14, 15, 16, 17, 18, 19, 20, 21, 22). Although progress in the taxonomy of *Yersinia* makes the interpretation of data prior to 1988 difficult (4), these older findings shine a light on the diversity of results and the difficulty for generalized interpretation of older data.

Frederiksson-Ahomaa et al. (2001) expressed some doubts, whether the direct route pig-raw meat-human was the only relevant one, or whether dogs carrying *Yersinia* are more significant vectors for humans (23). Recent Chinese studies placed higher relevance on companion animals, especially dogs, as primary source of infection in humans, although dogs consumed as food were an additional source of infection, which is not a factor that needs to be considered in Europe (24, 25). It was shown that some pathogenic *Y. enterocolitica* strains isolated from ill humans and dogs had the same subtype as well as the same pattern using pulse field gel electrophoresis (25). In case control studies the contact with companion animals was shown to be a confirmed risk factor for human yersiniosis (24, 26).

In previous studies, the isolated *Y. enterocolitica* strains from dogs or cats were most frequently attributed to bioserotype 4/O:3, biotype 2/O:5,27 or to biotype 1A (16, 17, 18, 19,
Although most animals carry *Y. enterocolitica* without observable symptoms (4, 21, 28, 30), some sporadic cases of enteritis, mainly in younger dogs (27, 31), or isolated cases of hepatic or myocardial yersiniosis have been reported (32, 33).

It is therefore of special interest to investigate the extent to which companion animals might serve as sources for *Y. enterocolitica*, with special regard to *ail*-gene positive strains as a zoonotic pathogen. In this study the occurrence of potentially pathogenic *Y. enterocolitica* in feces from dogs and cats was examined in 6949 diagnostic samples received from Germany and its neighboring states within a 10 week time frame. The strains obtained were differentiated using MALDI-TOF MS for initial strain detection to genus and species level, and PCR for the detection of the *ail*-gene. Additionally, a recently developed Fourier-transform infrared-spectroscopic (FT-IR) method was used for the detection and confirmation of Ail-factor positive *Yersinia*, mirroring the phenotype (12). Bioserotyping of the strains was performed so that a comparison with older data could be made.

**MATERIALS AND METHODS**

**Fecal samples:** 4325 samples of dog feces, sent to Vet Med Labor GmbH (Ludwigsburg, Germany) for bacterial diagnostics, were examined. Although the case history was not available in many cases, the main reason for submission was gastrointestinal symptoms (diarrhea of varying quality and duration, colitis, dysbacteriosis) as indicated by the veterinarian on the submission form. The samples originated mainly from Germany (77%) as well as from 13 other European countries (Austria, Czech Republic, Denmark, France, Finland, Italy, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Sweden) and were collected on 68 consecutive days between November 2011 and January 2012.
In addition, 2624 diagnostic samples of cat feces were additionally examined in the same laboratory. The case history of the cats listed the same diseases or symptoms as given for the dog feces samples. These were collected during the same period as above and were sent from Germany (81%) and 10 other European countries (Austria, Denmark, France, Finland, Italy, Luxembourg, Netherlands, Norway, Poland, Sweden).

**Isolation of bacteria:** Feces samples were cultured according to standard procedures for the detection of enteropathogenic bacteria. Samples were plated on tryptic soy agar containing 5% sheep blood (Becton Dickinson, Heidelberg, Germany), MacConkey agar (Becton Dickinson), Campylobacter selective agar (Heipha, Eppelheim, Germany), and Salmonella selective agar (Heipha) and incubated according to standard procedures. For the detection of *Salmonella*, selenite lactose broth (Oxoid, Wesel, Germany) was used for enrichment.

Samples were plated onto yersinia selective agar (CIN-agar, Becton Dickinson) and incubated for 18-24 hours at 30°C in order to isolate *Yersinia*. Additionally the samples were inoculated into phosphate buffered saline solution (Merck, Darmstadt, Germany) and incubated for 4-5 days at 4 °C. After the cold enrichment, these samples were also plated onto yersinia selective agar and incubated as above. Initial identification of suspicious colonies was performed using MALDI-TOF MS (see below). Pure cultures of isolates identified as *Y. enterocolitica* were differentiated further by FT-IR, biochemical tests and PCR.

**Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification:** Suspicious isolates were selected from the culture plates either from the primary culture or after cold enrichment and then subjected to MALDI-TOF MS according to manufacturer’s instructions (VITEK® MS, BioMérieux, Nürtingen, Germany).
Isolates were prepared using the direct smear method and analyzed using Myla® Version 2.4.0. The database (V 1.1) comprises the following *Yersinia* species: *Y. enterocolitica*, *Y. kristensenii*, *Y. aldovae*, *Y. frederiksenii*, *Y. intermedia*, *Y. pseudotuberculosis*, *Y. ruckeri*, *Y. pestis*. According to the manufacturer each database entry is based on spectra from at least 15 – 20 different isolates. As well as from the calibration control of the instrument, *Y. enterocolitica* ATCC9610 was included as an external control strain when the selected isolates were examined.

**Phenotypical and biochemical characterization:** After primary growth on sheep blood agar (Oxoid) for 24 hours at 37°C the strains were characterized by esculine hydrolysis, indole production, the ability to ferment D-xylose and D-trehalose according to Wauters et al., 1987 (3, 34). Reaction tubes were incubated at 25°C under aerobic conditions and reactions evaluated after two days. Temperature dependent motility of *Yersinia* was tested on SIT-agar (SIM-Agar, Becton Dickinson, colored with triphenyltetrazoniumchloride), incubated at 25°C and 37°C for two days (35). Serotyping was performed with a slide agglutination test using *Y. enterocolitica* O:3, O:5, O:8, O:9 and O:27 commercial antisera (Sifin, Berlin, Germany) with isolates grown at 25°C. Initial auto-agglutination was tested with a drop of 0.9% saline. Isolates without auto-agglutination were subsequently tested using all five sera.

**Molecular detection of the *ail*-gene and sequencing of the gene for 16S rRNA:** Molecular detection of the *ail*-gene by real-time PCR was performed using the protocol of Mäde et al. (2008) on a Rotor Gene 6000 Real-Time PCR Machine (Corbett Research, Australia), using the Brilliant QPCR Master Mix with Sure Start Taq DNA polymerase (Stratagene, Heidelberg, Germany). Temperature profile included 10 minutes of pre-denaturation at 95°C, followed by 45 cycles with 10 s at 95°C and 30 s at 60°C (data acquisition at the end of the 60°C step), and subsequent cooling to 40°C. Strain *Y.
enterocolitica SZ5108/01 was used as a positive control (36). For confirmation of four isolates
the 16S rDNA was partially amplified using the modified protocol of Johnson (1994) and
employing primers 27f and 1522rN (37, 38). The resulting 16S rDNA fragments were
sequenced stepwise (Microsynth AG, Balgach, Switzerland).

Identification of Yersinia isolates by FT-IR: Bacterial isolates were cultivated
independently in three replicates at 37°C for 24 hours on sheep blood agar plates (Oxoid).
Harvesting of cells, sample preparation, and the examination of the dried bacteria films by
FT-IR spectroscopy was performed as described previously for Yersinia differentiation (12,
39). The infrared spectra were recorded for each sample in a transmission mode in the 500
to 4000 cm⁻¹ wave number range with a FT-IR spectrometer (IFS 28/B, BrukerOptics,
Ettlingen, Germany). Acquisition and analysis of data were carried out using OPUS Software
(vers. 4.2, BrukerOptics) and an artificial neural network built by the NeuroDeveloper
software (Synthon, Heidelberg, Germany; 40). The differentiation was performed with an
updated version of a method described previously (12, 39). This method allows for species
differentiation of Y. enterocolitica, and independently on the presence or absence of the ail-
gene.

Cluster analysis: IR spectra of all Y. enterocolitica strains were compared by cluster
analysis (cf. 38, 41). For cluster analysis the vector normalized spectra of the wave number
range 500 to 1400 cm⁻¹, and 2800 to 3000 cm⁻¹ in second derivation were used for
calculation with Ward’s algorithm (OPUS 4.2; 42). The dendrogram obtained depicts the
arrangement of isolates in groups according to their spectral differences (Fig. 1).

RESULTS
Bacterial isolates: *Y. enterocolitica* was isolated from (4.6%) of 4325 dog fecal samples. In 63.6% of these cases, *Y. enterocolitica* was isolated as the only enteropathogenic bacteria. In 36.4% of the cases other enteropathogenic bacteria were detected in parallel (mainly *Salmonella* sp. and/or *Campylobacter* sp.; data not shown). Due to various reasons (e.g., loss of culture, overgrowth by contaminants), it was possible to further characterize 179 isolates of *Y. enterocolitica*.

*Y. enterocolitica* was detected in eight (0.3%) of 2624 cat fecal samples examined for comparison. Of these, three samples also contained other enteropathogenic bacteria. Seven of the eight isolates were available for further characterization.

Identification with MALDI-TOF MS and FT-IR and determination of the bioserotype:

Suspicious isolates of *Y. enterocolitica* originating from dog fecal samples were identified to the species level using VITEK® MS (Myla™-Software 2.4.0, database V 1.1) with 99.9% level of confidence for 176 of the 179 isolates, the remaining three isolates yielding confidence levels of 99.7-99.8%. The seven isolates from cat fecal samples were all identified with 99.9% confidence level. All 186 isolates from dogs and cats were assigned to the genus *Yersinia* and specifically *Y. enterocolitica* by FT-IR analysis as well (12, 39).

The isolates were bioserotyped, and analyzed for presence of the Ail-factor by FT-IR and the *ail*-gene by PCR to obtain information about their pathogenic potential (12). Of the dog isolates, 91.6% (n=164) were analyzed as *ail*-positive by PCR and Ail-positive by FT-IR (Table 1). The *ail*-gene could also be detected in all 7 isolates available from cat feces.

All 15 *ail*-negative strains isolated from dogs feces belonged to biotype 1A. Three isolates showed bioserotype 1A/O:8, one was 1A/O:5. The remaining 11 isolates did not agglutinate with any of the five sera used. All other 164 isolates were positive for the *ail*-gene, and were
typed as biotype 2, 3, 4, or 5, respectively (Table 1). Biotype 4 predominated with 54.7% (n=98) of all isolates from dog samples, all but one combined with serotype O:3. One strain did not agglutinate with any serum used. One other bioserotype 4/O:3 strain showed no motility at 25°C. Both of these strains were confirmed as *Y. enterocolitica* by comparison with the partially sequenced 16S rDNA. Biotype 2 comprised 42 (23.5%) of the isolates. Of this group, 27 strains belonged to bioserotype 2/O:9, and 15 to bioserotype 2/O:5. Twenty strains were biotype 3 (11.1%), combined with the same serotype (O:3) as all of the biotype 4 isolates. Four isolates (2.2%) failed to ferment trehalose, therefore they were typed as biotype 5. All of these strains were of serotype O:3. Exemplary two of the four biotype 5 isolates were confirmed as *Y. enterocolitica* by partial sequencing of their 16S rDNA.

The seven available *Y. enterocolitica* isolates from cat feces samples were also bioserotyped. Four isolates were biotype 4 combined with serotype O:3, one was bioserotype 3/O:3, and the two remaining were bioserotype 2/O:9.

The infrared spectra of all canine isolates were compared by cluster analysis (Fig. 1). A clear grouping could be displayed for the spectra of the biotypes 1A and 2 and a group with biotypes 3, 4 and 5 by formation of two separate main branches. In the first main branch, distinct sub-branches were found for biotypes 1A and 2. Only two exceptions were found: The spectra of an isolate that was biotype 4, but showed no agglutination with the used sera, was similar to those of biotype 2. Close to this individual strain, the spectra of a special biotype 3/O:3-isolate was found. The second main branch was separated in a distinct biotype 4 and a close sub-branch, comprising the remaining biotype 3 and 5 isolates. The four spectra for the isolates of biotype 5 were found in a distinct branch, close to the majority of biotype 3 spectra. Except for two biotype 3/O:3 isolates found in the biotype 4 branch, all remaining 17 biotype 3 isolates clustered together.
The age of the dog was known for 99.4% (n=178) of the fecal samples. Most (144) of the 163 strains positive for the *ail*-gene were isolated from dogs younger than one year (88.3%). In contrast, 53.3% (8/15) isolates lacking the *ail*-gene were obtained from dogs, which were less than one year old (Table 1). The cat isolates, all being positive for the *ail*-gene, came from cats younger than one year in 80% (n=4) of the five animals whose age was known.

**DISCUSSION**

*Y. enterocolitica* is regarded as a significant food borne pathogen (1, 2). Beside food from animal sources, especially pork (13, 26), companion animals have been implicated as potential source for human *Yersinia* infections (4, 7, 14, 16, 23, 25, 43). Strains isolated from dogs in particular share common genotypes with pathogenic strains isolated from humans (7, 13). The contact between many dogs and cats and their owners has noticeably changed in post-industrial countries during the last decades (44, 45). Because of far closer human and companion animal contact, the transmission of microorganisms between them is facilitated (46). It is assumed that raw pork is a potential source of infection in dogs and cats (18, 23). Dogs can readily become infected by pathogenic *Y. enterocolitica* through feeding or through social interactions with other dogs and can carry and excrete the organism for more than three weeks in high cell count (28, 31, 43).

The symptoms of dogs harboring *Yersinia* range from disease of varying severity to obviously healthy (23, 27, 31, 32, 33). Asymptomatic carriage seems to be predominant in dogs, and cats (20). Thus *Y. enterocolitica* was considered as a commensal in the intestine of the animals (4). As asymptomatic carrier status of animals for *Y. enterocolitica* cannot readily be
recognized, carriers can easily transmit the infection to other animals, humans or the environment (15, 20, 21, 31, 43).

Since 1980 European investigations showed prevalence of ≤ 5% and up to 30.1% for *Y. enterocolitica* in dog feces, none of these studies included more than 250 samples (14, 15, 16, 18, 21, 29). On the other hand, the prevalence of *Y. enterocolitica* in cat feces was generally lower (14, 17).

In this study fecal samples were obtained from dogs and cats suspected of having gastrointestinal symptoms, these may include diarrhea of various degrees and durations, colitis as well as dysbacteriosis. Additionally it can be assumed that an unknown number of samples were obtained from apparently healthy animals as part of a wellness test or as therapeutic monitoring. Dogs and cats presenting with gastrointestinal symptoms, may have other enteric pathogens that might contribute to the clinical signs. These samples were not examined for viral pathogens and only infrequently tested for endoparasites.

*Y. enterocolitica* was detected in 198 of the 4325 canine feces samples. In 63.6% of these cases *Y. enterocolitica* was the only bacterial pathogen isolated, while in the remaining samples other enteropathogenic bacteria were detected along with *Y. enterocolitica*. It is not clear whether seasonality plays a role in the prevalence of *Y. enterocolitica*, however other studies have shown no increase in the isolation rate of this organism from dogs during winter months (19, 22, 28).

The overall prevalence of 4.6% for *Y. enterocolitica* is an indication of the possible virulence of this organism for dogs. Particularly because most (91.6%) were *ail*-positive bioserotypes which is remarkably high for this kind of sample. A clear preponderance was shown for biotype 4 isolates (54.7%), followed by biotype 2 (23.5%), biotype 3 (11.2%), and in low
numbers biotype 5 strains (2.2%) (Table 1). The reported ratios of pathogenic bioserotypes isolated from dog samples, varied in a broad range from <0.5% (15, 29) up to more than 75%, in which bioserotype 4/O:3 also predominated (19, 30). Apart from the *ail*-positive bioserotype combinations most frequently isolated from human cases and pigs, such as 4/O:3, 2/O:9 and 2/O:5,27 (9, 13), only a few other bioserotype combinations were observed. In 98 out of 179 samples of dog feces (54.7%) a biotype 4 profile was found and only one of these isolates did not possess the classical 4/O:3 combination and was not serotypeable with the sera used. The frequent appearance of the bioserotype 4/O:3 could be a direct or indirect consequence of contaminated feed (especially raw pork; 23), or a result of a higher susceptibility for this bioserotype, which is assumed for younger dogs and humans (28, 5).

Biotype 2 was the second most frequently detected biotype in this set of strains. In earlier reports biotype 2 isolates ranked second in frequency, occasionally first, in dog studies (7, 19, 28, 30). This mirrors the presence of these bioserotypes in human cases, as well as in pigs, or pork (1, 2, 9, 47). All of the 20 biotype 3 isolates were combined with serotype O:3. This bioserotype combination has occasionally been reported in Japanese dog studies before, but not for Europe (28). Biotype 5 isolates have so far only been recovered from infections in animals (4, 9). Bioserotype 5/O:2,3 is recognized as an animal pathogen, commonly associated with diseased sheep and goats or hares (4, 7, 48). In our study, four strains isolated from dog feces showed biotype 5, and all were combined with serotype O:3. The relevance of this, obviously rare biotype for dogs remains unclear.

The ratio of *ail*-negative biotype 1A isolates in samples from dog feces was 8.4% and was therefore relatively low compared to the data of Murphy et al. (2010), who found 100% of the isolated *Y. enterocolitica* from canine samples to be biotype 1A (29). This could reflect our
focus on samples from diseased animals, as can be assumed for the majority of the samples in this study. Hence, a generalized conclusion in regard to the pathogenicity of biotype 1A for dogs is not possible based on this biased data set.

For humans biotype 1A Y. enterocolitica strains were originally considered to be non-pathogenic. This biotype has recently been discussed as a possible opportunistic pathogen (5, 8, 49).

In this study, the majority of Y. enterocolitica strains isolated from dogs younger than one year were positive for the ail-gene (94.7%) and typed as suspected pathogenic biotypes 2, 3, 4, or 5. The few isolates lacking the ail-gene were more often found in samples of older dogs (46.7%). This probably reflects the higher susceptibility of young animals by various enteropathogenic microorganisms, with Yersinia being either a main or a contributing etiologic factor for clinical symptoms.

In 2624 cat fecal samples a significantly lower prevalence for Y. enterocolitica of 0.3% was found, compared to the dog samples. The seven isolates investigated were the known pathogenic bioserotypes 4/O:3, 2/O:9, and 3/O:3, which were also found most frequently in samples from dogs (Table 1). Previous studies in cats showed low prevalence (≤ 5%) of pathogenic bioserotypes of Y. enterocolitica as well (17, 30).

The identification methods used (MALDI-TOF MS and/or FT-IR) proved to be effective tools for the detection of Y. enterocolitica in routine samples (12, 50). The combination of these methods presented a valid base for rapid and detailed diagnostic information. FT-IR not only could be used for species determination but was also successfully applied for the detection of the Ail-factor in this study. The obtained IR-cluster shows a clear hierarchical grouping for Y. enterocolitica according the biotype in general, with marginal exceptions (Fig. 1). In FT-IR
cluster analysis biotype 2 strains represent a compact branch, close to the branch of the 1A isolates. Biotypes 3 and 5 were related to biotype 4 in observed bioserotype combinations and in the main branches of IR-cluster analysis. Some spectra of isolates from different dogs were nearly indistinguishable (Fig. 1). An epidemiological approach by comparison by the IR-spectra, as shown for Bacillus cereus, Corynebacterium ulcerans or Staphylococcus aureus, seems to be attainable (38, 51, 52).

The relevance of Y. enterocolitica in companion animals is not yet known. The unexpected high ratio of ail-positive, and therefore presumptive human-pathogenic Y. enterocolitica presence in dog feces, with broad variety of bioserotypes, should expand the focus on the possibility of transmission of this organism from pigs to companion animals. Interestingly, while the occurrence seems to be relatively high in dogs as shown by the prevalence of 4.6% for Y. enterocolitica in dog feces, the samples from cats revealed a much lower prevalence (0.3%). Further studies need to be conducted to assess the potential risk of transmission of this zoonotic pathogen to the animal owners.

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PCR, and infrared spectroscopy in consideration of thermotolerant isolates [German].

Table 1: Bioserotype and *ail*-gene of *Y. enterocolitica* isolates from companion animal feces

Fig. 1. Dendrogram of FT-IR spectra of canine *Y. enterocolitica* strains. Cluster analysis was performed using the second derivatives of the spectra considering the spectral ranges 500–1400 cm⁻¹, 2800–3000 cm⁻¹. Ward’s algorithm was applied (42). Bioserotype and number of isolates were given. *amotil* bioserotype 4/O:3 isolate.
Table 1: Bioserotype and *ail*-gene of *Y. enterocolitica* isolates from companion animal feces

<table>
<thead>
<tr>
<th><em>ail</em> -gene</th>
<th>bioserotype</th>
<th>dog ≤1yr</th>
<th>dog &gt;1yr</th>
<th>dog age unknown</th>
<th>cat</th>
<th>n=7 (100%)</th>
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<tbody>
<tr>
<td><em>ail</em> neg</td>
<td></td>
<td>179 (100%)</td>
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<tr>
<td>1A/O:5</td>
<td>1</td>
<td>152 (84.9%)</td>
<td>26 (14.5%)</td>
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<td>1A/O:8</td>
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<td>2 (14.5%)</td>
<td>2 (14.5%)</td>
<td>1 (0.6%)</td>
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<td>1A/nag</td>
<td>11</td>
<td>6 (3.4%)</td>
<td>5 (2.9%)</td>
<td>1 (0.6%)</td>
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<td>1A</td>
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<td>14 (8.2%)</td>
<td>1 (0.6%)</td>
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<td>2/O:9</td>
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<td>20 (73.1%)</td>
<td>7 (26.9%)</td>
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<td>2/O:5,27</td>
<td>15</td>
<td>14 (93.3%)</td>
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<td>1 (0.4%)</td>
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<td>20</td>
<td>18 (90.0%)</td>
<td>2 (10.0%)</td>
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<td>1</td>
<td>1 (28.6%)</td>
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<td>20</td>
<td>18 (90.0%)</td>
<td>2 (10.0%)</td>
<td>1 (0.5%)</td>
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<td>1 (28.6%)</td>
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<tr>
<td>4/O:3</td>
<td>97</td>
<td>87 (90.3%)</td>
<td>10 (9.7%)</td>
<td>1 (0.5%)</td>
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<tr>
<td>4/nag</td>
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<td>1 (100%)</td>
<td>0 (0.0%)</td>
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<td>4</td>
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<td>94 (96.9%)</td>
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<td>5</td>
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<td>0 (0.0%)</td>
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<td></td>
</tr>
<tr>
<td><em>ail</em> neg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8 (53.3%)</td>
<td>7 (46.7%)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ail</em> pos</td>
<td>164 (100%)</td>
<td>144 (88.5%)</td>
<td>19 (11.5%)</td>
<td>1 (0.6%)</td>
<td>7</td>
<td>7 (100%)</td>
</tr>
</tbody>
</table>

*nag* = no agglutination with specific sera for O:3, O:5, O:8, O:9, or O:27