Chlamydia prevalence in sick dogs with uro-genital and/or conjunctival lesions

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SUMMARY

Chlamydiosis is an infectious disease of carnivorous, mainly associated with epithelial damage. The objective of this study was to determine the Chlamydia prevalence in sick dogs with uro-genital and/or conjunctival lesions using a direct immuno-fluorescence test on scrapings sampled from damaged areas. A total of 218 dogs were divided into 4 groups according to the localization of lesions: uro-genital signs (group A), conjunctival lesions (group B), uro-genital and conjunctival signs (group C) and other signs (group D). A high prevalence (61.9%) was recorded in the overall population of sick dogs and the proportions of positive dogs fluctuated from 54.5% in the group C to 66.7% in the group B. Although maximal infection rates were found in males (65.4% in males and 56.1% in females) and in puppies (66.7% in puppies, 58.7% in 1 year old dogs, 62.8% in 2-5 years old dogs and 63.6% in 6-10 years old dogs), the sex and the age were neither significantly associated with Chlamydia prevalence nor with the type of clinical signs. These results emphasize the strong occurrence in diseased dogs of this infection, representing a high risk for zoonosis.

Keywords: Chlamydia, dog, direct immuno-fluorescence test, prevalence, sex, age.

RÉSUMÉ

Prévalence de Chlamydia chez les chiens présentant des lésions uro-génitales et/ou conjonctivales

La chlamydiose est une maladie infectieuse chez les carnivores, associée essentiellement à des lésions épithéliales. L’objectif de cette étude était de déterminer la prévalence des chlamydiases chez des chiens malades présentant une atteinte uro-génitale et/ou conjonctivale à l’aide d’un test d’immuno-fluorescence directe réalisé sur des prélèvements des zones lésées. Au total, 218 chiens ont été répartis en 4 groupes en fonction de la localisation des lésions : signes uro-génitaux (groupe A), atteinte conjonctivale (groupe B), lésions uro-génitales et conjonctivales (groupe C), autres signes (groupe D). Une forte prévalence (61.9 %) a été observée au sein de la population totale de chiens malades et les proportions de chiens positifs ont fluctué de 54,5 % dans le groupe C à 66,7 % dans le groupe B. Bien que des taux d’infection maximaux aient été obtenus chez les mâles (65,4 % chez les mâles et 56,1 % chez les femelles) et chez les chiots (66,7 % chez les chiots, 58,7 % chez les chiens de 1 an, 62,8 % chez les chiens de 2 à 5 ans et 63,6 % chez les chiens de 6 à 10 ans), le sexe et l’âge n’ont été significativement associés ni avec la prévalence des Chlamydiases ni avec le type de signes cliniques. Ces résultats soulignent la forte présence des chlamydiases chez les chiens malades, ce qui représente un risque élevé de zoonose.

Mots clés : Chlamydia, chien, test d’immuno-fluorescence directe, prévalence, sexe, âge.

Introduction

As chlamydia is considered to be an epitheliotrope intra-cellular microorganism, chlamydia infection can be associated with various clinical signs: abortions, endometritis, pneumonia, polyarthritis, conjunctivitis, keratitis, urethritis, atherosclerosis and others [17, 21, 26].

Chlamydia has been detected in many various animals. In dogs, this bacterium was first isolated more than 50 years ago [11]. Atypical case of dog pneumonia and keratitis with a chlamydia origin, have been described for the first time in the 60s in Germany [31]. Chlamydia was also isolated and identified in the mucosal liquid of a dog suffering from conjunctivitis in South Africa [8, 22] as well as in the pleural liquid [1]. Abortions of female dogs or birth of unviable puppies have been also attributed to chlamydia infection [14, 23]. Chlamydiosis was also described as similar to the acute, semi-acute or slow syndrome of dog plaque [34].

In Lithuania also, the importance of chlamydia infection in dogs has been described by means of indirect methods for detecting anti-chlamydia antibodies. The chlamydia seroprevalence in dogs was found to be 19.5% and 38.1% using complement conjugation reaction (CCR) and enzyme - linked immunosorbent assay (ELISA), respectively [18]. No breed, among the investigated ones, exhibited a particular higher susceptibility to chlamydia [18].

In veterinary clinical practice, such indirect methods based on detection of specific antibodies only reveal a possible exposure to a previous infection. However, in sick dogs, laboratory investigations remain relevant [5, 29], since clinical and pathological signs of chlamydiosis in dogs can greatly vary and are often similar to many non infectious diseases. A method using direct immuno-fluorescence (DIF) to detect Chlamydia in an imprint-smear [29] is currently considered to be reliable and sensitive enough for chlamydiosis diagnostic in sick animals. It provides a relevant tool to assess the importance, which is not documented so far in Lithuania,
of *chlamydia* infection in the population of sick dogs, especially those exhibiting conjunctivitis and diseases of urogenital system.

The aims of the present study were to investigate the proportions of *chlamydia*-infected animals in a population of sick dogs with mainly uro-genital and/or conjunctival signs, with the DIF method [29] and to explore the eventual gender and age influence.

### Materials and Methods

#### ANIMALS AND SAMPLES

This experiment was carried out in accordance with “The Law of Animals Protection, Keeping and Handling in the Lithuanian Republic” (State News, 1997 11 28, No. 108) and sub-law regulation – “The Order of the Head of State Veterinary Service of the Lithuanian Republic: concerning requirements for the Animals Intended for Experimental and Other Scientific Purposes Keeping, Handling, Protection and Use” (News, 22 January, 2009, No. 8, 287. P. 50-66).

A convenient population of sick male and female dogs, adults and puppies of different age (which presented recurrent clinical signs and were unresponsive to symptomatic treatments) was considered in this study. These investigations were carried out from 2004 to 2007 at the diagnostic laboratory of JSC “Jakov Veterinary Center” and the Department of Infectious Diseases of the Lithuanian Veterinary Academy. There were 82 females and 136 males and 4 age classes were considered: puppies up to 1 year (n = 24), one year old dogs (n = 75), 2 to 5 years old dogs (n = 86) and 6 to 10 years old dogs (n = 33).

The dog population was divided into 4 groups according to the type of clinical signs: dogs with uro-genital signs, for which excreta from sexual organs were observed, as well as females having experienced abortions or infertility, confirmed by preventive checking before copulation, were included in the group A (n = 127); dogs with conjunctival signs, i.e. purulent excreta from the eyes, constituted the group B (n = 57); dogs with both uro-genital and conjunctival signs were regrouped in the group C (n = 11) and finally dogs with other clinical signs were included in the group D (n = 23). Consequently, the corresponding samples (n = 218) according to the clinical signs and the possible chlamydia localization, were urethral or vaginal scrapings from dogs of the group A, conjunctival scrapings from dogs of the group B, urethral / vaginal and conjunctival scrapings for dogs of the group C and various scrapings of the injured areas (scrapings from inner ear in cases of otitis, skin scrapings in cases of dermatitis and nostril scrapings in cases of respiratory affections) in the case of dogs of the group D.

#### DIRECT IMMUNO-FLUORESCENCE TEST (DIF)

The clinical samples were analysed using a commercial kit for direct immuno-fluorescence (ChlaMonoScreen, “Nearmedic Plus”, Russia) according to the manufacturer recommendations. This test allows detection of bacteria from the *Chlamydiaceae* family on imprint-smears. Briefly, each sample was overspread on the slide, dried at room temperature and fixed by acetone. Anti-chlamydia monoclonal antibodies (40 – 50 μl) conjugated with fluoresceine isothiocyanate were poured on the fixed smear. After incubation in a damp chamber at 37°C for 20 minutes, the imprint-smear was triply washed with phosphate buffer solution, dried at room temperature, covered with special glass and studied microscopically by a luminescence microscope with 600 – 1000 magnification and under ultraviolet light (490 nm) without fluorescent immersive oil. The sample was considered to be positive when, at least, 10 elementary (a dot form) or reticular (an oval form) bodies with a green colour were detected on the smear.

#### STATISTICAL ANALYSIS

The proportions of positive samples (with their 95% Confidence Interval (CI)) were assessed among the 218 samples, as well as in the 4 dog groups (groups A, B, C and D).

Possible differences in the distribution of positive samples according to gender and age were assessed using the χ² statistics (MINITAB 14.2). Differences were considered as significant when p value was less than 0.05.

### Results

Among the 218 studied samples, 135 (61.9%) were found positive for chlamydia (Table I). This proportion slightly varied with the nature of clinical signs and ranged from 54.5% in the group C to 66.7% in the group B but the differences among groups were not statistically significant.

<table>
<thead>
<tr>
<th>Dog groups</th>
<th>n</th>
<th>Number</th>
<th>Proportions (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: uro-genital lesions</td>
<td>127</td>
<td>78</td>
<td>61.4</td>
<td>53.0 – 69.9</td>
</tr>
<tr>
<td>B: conjunctival lesions</td>
<td>57</td>
<td>38</td>
<td>66.7</td>
<td>54.4 – 78.9</td>
</tr>
<tr>
<td>C: uro-genital and conjunctival lesions</td>
<td>11</td>
<td>6</td>
<td>54.5</td>
<td>25.1 – 84.0</td>
</tr>
<tr>
<td>D: other lesions</td>
<td>23</td>
<td>13</td>
<td>56.5</td>
<td>36.3 – 76.8</td>
</tr>
<tr>
<td>Total</td>
<td>218</td>
<td>135</td>
<td>61.9</td>
<td>55.5 – 68.4</td>
</tr>
</tbody>
</table>

95% CI: 95% confidence interval.

**Table I:** *Chlamydia* prevalence in scrapings from sick dogs (n = 218) with recurrent and various clinical signs (mainly, uro-genital and conjunctival lesions).

As shown in Table II, 89/136 males (65.4%) were positive for chlamydia, equivalent to 65.9% (89/135) of the total positive samples, whereas a slightly lower proportion of females (46/82 or 56.1%) gave a positive response to the DIF test, representing 34.1% of the total positive samples, but the distribution of positive samples according to dog gender did not significantly differ ($P > 0.05$). In males, the proportions of positive samples ranged from 57.1% for the group C (dogs with uro-genital and conjunctival lesions) to 73.0% for the group B (dogs with conjunctival lesions alone) whereas the highest chlamydia prevalence in females (59.2%) was observed in the group A (dogs with uro-genital signs alone) and the lowest (44.4%) in the group D (dogs with other clinical signs). Nevertheless, the distributions of positive samples from males or from females according to the nature of clinical signs were not significantly different ($P > 0.05$).

The proportions of positive samples according to the age classes did not vary significantly ($P > 0.05$) and fluctuated from 58.7% (one year old dogs) to 66.7% in puppies (Table III). Moreover, there was no significant association between the nature of the clinical signs occurring during chlamydiosis and the age classes ($P > 0.05$).

### Discussion

The distribution of chlamydia into the population of carnivorous, especially in dogs, is poorly documented, although humans can be infected by bacteria from Chlamydaceae family carried by carnivorous [10, 28, 30]. It is often considered that chlamydia infection is widely spread in cats, and less in dogs [9]. This study showed however that, in Lithuania, chlamydia is very frequent in sick domestic dogs with uro-genital and/or conjunctival signs, particularly in those with conjunctivitis (66.7%). This finding confirmed previous estimations of seroprevalence carried out in Lithuania (19.5 to 38.1%; [18]), in Japan and Germany (9 to 50% [9, 32, 33]).

Animals can contract chlamydia infection throughout direct contacts with sick people, wild and domestic animals, and mites [4, 16, 30]. Birds are often recognized as a main source, as more than 130 species of birds are susceptible to chlamydia [7, 29]. A large proportion of migratory birds, as well as pigeons in big cities and exotic birds kept in cages (up to 90%) are often infected with Chlamydophila psittaci, Chlamydophila pneumoniae or Chlamydophila abortus [3, 15, 27]. Consequently, the sources of contamination for domestic dogs are numerous. In our study, the importance of exotic birds as chlamydia reservoir would be minor because only few dog owners kept such bird species while the role of other birds and vagabond carnivores was probably great, as already shown in a previous study [9]. Mites could be also partly responsible for infection, as they can carry viable Chlamydia in their organism up to 45 days and can transmit the infection to other animals [20]. In addition, chlamydia is known to be particularly resistant in the environment. In faeces, the bacteria remain viable during 6 months at 5-10°C and during 10-15 days at room temperature. They also can survive in dry faeces from birds for several months and in snow for several weeks [19].
On the other hand, there was no significant association between the type of clinical signs and the proportions of positive chlamydia samples in the studied population of sick dogs (the chlamydia prevalence varied from 54.5% to 66.7%). This bacterium was already detected in clinical samples taken from abortive foetuses, and from conjunctive of sick cats [25]. Furthermore, it is often difficult to diagnose chlamydiosis as a separate nozologic unit [36]: the infection with chlamydia alone is not always associated with clinical signs and this pathogen is known to interact with some other bacteria, viruses and mycoplasma or to emerge in cases of metabolic disturbances or stress, by aggravating the failure of various organs.

In agreement with a previous serological study [18], the proportions of positive chlamydia samples did not differ according to the sex, although females tended to be less susceptible to the infection in the present study (56.1% in females vs. 65.4% in males). In addition, the age did not appear as a predisposing factor since no significant difference for chlamydia prevalence was evidenced according to the age classes. The absence of significant effects due to age or sex was also reported in a seroprevalence study for chlamydia in cats [13]. However, in the present study, the highest infection rate (66.7%) was found in puppies (< 1 year old) and such a tendency was also described in young cats [24, 35]. The high proportion of positive puppies could be related to a possible transmission of bacteria from the female, during parturition [2]. Besides, the type of clinical signs associated with chlamydiosis appeared to be independent from the sex or the age.

In conclusion, this study shows, in agreement with previous results, that bacteria of Chlamydiaceae family are probably widely spread in the population of domestic dogs in Lithuania. Such knowledge is particularly important, as bacteria from the Chlamydiaceae family can be transmitted to humans by carnivorous pets [6, 12] and can induce zoonosis.

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References


