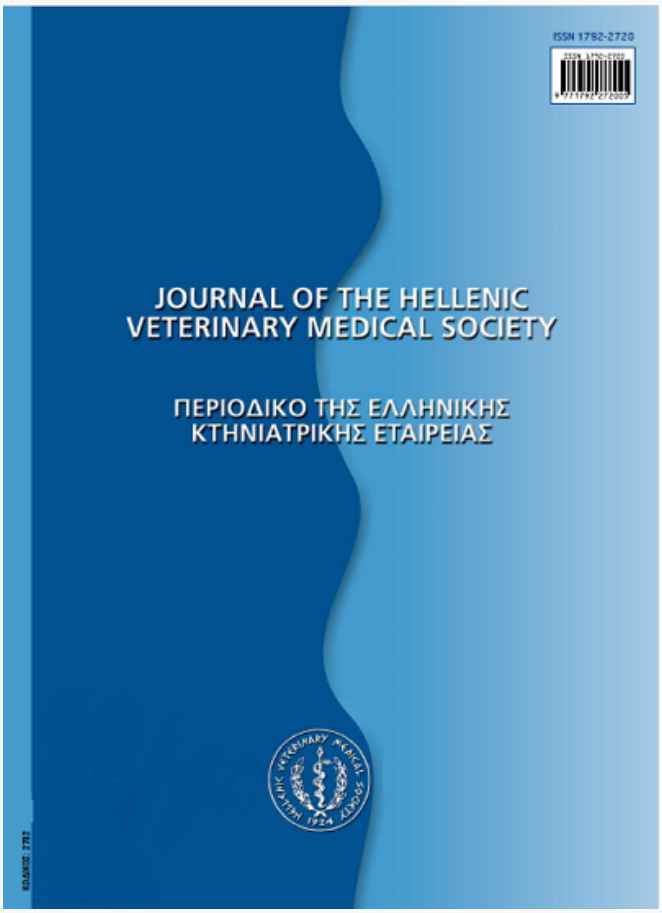
Journal of the Hellenic Veterinary Medical Society

Vol 65, No 1 (2014)



Sensitivity of preputial and vaginal exfoliative cytological examination for diagnosis of canine leishmaniosis (Leishmania infantum)

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doi: 10.12681/jhvms.15510

J HELLENIC VET MED SOC 2014, 65(1): 31-34
ΠΕΚΕ 2014, 65(1): 31-34

Research article
Ερευνητικό άρθρο

Sensitivity of preputial and vaginal exfoliative cytological examination for diagnosis of canine leishmaniosis (*Leishmania infantum*)

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Ευαισθησία της κυτταρολογικής εξέτασης δειγμάτων από την ακροποσθία και τον κόλπο για τη διάγνωση της λεϊσμανίωσης του σκύλου (*Leishmania infantum*)

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ABSTRACT. Aim of the study was to examine sensitivity of preputial and vaginal exfoliative cytological examination as a non-invasive alternative to lymph node, spleen and bone marrow cytology, for detection of *Leishmania infantum* amastigotes in dogs with leishmaniosis, as, in previous studies, the protozoa have been observed in the penis and prepuce of male dogs and in the vagina of female dogs with leishmaniosis. In total, 20 male and 18 female dogs with confirmed leishmaniosis were included in the study. Three cytology smears were prepared from different sites of the preputial cavity of males and one smear was prepared from the anterior vagina of females. *Leishmania* amastigotes were not observed in these samples after microscopic examination for 20 min at 1,000× magnification. Therefore, preputial and vaginal exfoliative cytology is not recommended for routine diagnosis of canine leishmaniosis.

Keywords: dog; *Leishmania infantum*; microscopy; prepuce; vagina

ΠΕΡΙΛΗΨΗ. Σκοπός της μελέτης ήταν η εκτίμηση της ευαισθησίας της κυτταρολογικής εξέτασης από δείγματα από την ακροποσθία ή τον κόλπο για διάγνωση της λεϊσμανίωσης του σκύλου, καθώς προηγούμενες μελέτες έχουν δείξει την παρουσία αμαστιγωτών μορφών *Leishmania infantum* στο πέος και την ακροποσθία των αρσενικών, καθώς και στον κόλπο των θηλυκών σκύλων με λεϊσμανίωση. Η εξέταση αυτή θα μπορούσε να αντικαταστήσει την κυτταρολογική εξέταση των λεμφογαγγλίων, του σπλήνα και του μυελού των οστών που απαιτούν περισσότερο επεμβατική δειγματοληψία. Χρησιμοποιήθηκαν 20 αρσενικοί και 18 θηλυκοί σκύλοι με επιβεβαιωμένη λεϊσμανίωση. Από κάθε αρσενικό σκύλο εξετάστηκαν τρία κυτταρολογικά επιχρίσματα από υλικό από διαφορετικά σημεία της κοιλότητας της ακροποσθίας, ενώ από κάθε θηλυκό σκύλο εξετάστηκε ένα επίχρισμα από την πρόσθια μοίρα του κόλπου. Ύστερα από μικροσκοπική εξέταση σε μεγέθυνση 1.000× επί 20 min δεν διαπιστώθηκαν

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Date of initial submission: 3 July 2013
Date of revised submission: 1 September 2013
Date of acceptance: 4 September 2013

Ημερομηνία αρχικής υποβολής: 3 Ιουλίου 2013
Ημερομηνία αναθεωρημένης υποβολής: 1 Σεπτεμβρίου 2013
Ημερομηνία αποδοχής: 4 Σεπτεμβρίου 2013

αμαστιγωτές μορφές της Leishmania spp. σε κανένα από τα παραπάνω επιχρίσματα. Κατά συνέπεια, η κυτταρολογική εξέταση δειγμάτων από την ακροποσθία ή τον κόλπο δεν αποτελεί ευαίσθητη μέθοδο για τη διάγνωση της λεϊσμανίωσης του σκύλου.

Λέξεις ευρετηρίασης: ακροποσθία, κόλπος *Leishmania infantum*, μικροσκόπηση, σκύλος.

INTRODUCTION

Canine leishmaniosis due to *Leishmania infantum* (Syn: *L. chagasi*) is an important zoonosis and one of the most common diseases in dogs in endemic areas (Saridomichlakis, 2009). Diagnosis of leishmaniosis is usually based on compatible clinical signs and laboratory abnormalities along with positive results of serological examination (Baneth, 2010). However, definitive diagnosis, especially in equivocal cases, necessitates the demonstration of the presence of the parasite parasite which, in everyday clinical practice, is usually achieved through lymph node, spleen and/or bone marrow cytology (Saridomichelakis et al., 2005; Saridomichelakis, 2009; Baneth, 2010).

In canine leishmaniosis, parasites are present, sometimes in large numbers, in the penis and prepuce of male and the vagina of female dogs (Diniz et al., 2005; Silva et al., 2008) and their venereal transmission has been demonstrated, although it has been attributed to the amastigotes present in the semen and not to the direct contact of the genital organs during mating (Silva et al., 2009; Naucke and Lorentz, 2012). Also, parasites may be present in the external genital organs because of non-specific purulent inflammation, which is very common, especially in male dogs (Feldman and Nelson, 2004) and may result in the accumulation of infected macrophages.

As there is strong evidence to support the presence of *Leishmania* amastigotes in the external genital organs of male and female dogs with leishmaniosis, this study was designed to examine the sensitivity of preputial and vaginal exfoliative cytology for the detection of *Leishmania* amastigotes in dogs with the disease.

MATERIALS AND METHODS

In total, 38 dogs with confirmed leishmaniosis were used in the study after obtaining their owners' informed consent. These included 20 intact males (12 purebreds and 8 crossbreds) and 18 intact females (10 purebreds and 8 crossbreds) with an age range of 1 to 8 years (median: 3 years). All dogs presented clinical signs and abnormal laboratory findings characteristic of the disease, e.g. peripheral lymphadenomegaly (34/38), alopecia or hypotrichosis (26/38), exfoliative dermatitis (20/38), anaemia (30/38), hyperglobulinaemia and proteinuria (22/38, each). The diagnosis of the disease was confirmed by the results of serological examiantion (IDEXX snap® *Leishmania*), which was found to be positive in 37/38 (97%) dogs and lymph node cytological examination where *Leishmania* amastigotes were observed in all dogs.

A thorough visual examination of the prepuce and vagina was followed by sampling for cytology using a cotton swab. In female dogs, the swab was inserted into the anterior of the vagina and rubbed against the mucosa. In male dogs, three separate samples were obtained, without previous cleaning of the preputial cavity: one from the opening of the prepuce, a second from the middle of the preputial cavity and the last one from the innermost site of the preputial cavity. Each swab was rolled on a separate glass slide, stained with Diff Quik and examined at 1,000× magnification for 20 min. All smears were

examined for presence of *Leishmania* amastigotes by one of the authors (LVA), whilst 10 randomly selected smears (5 from male and 5 from female dogs) were reexamined by another author (MNS). All smears from female dogs were also examined for the characterization of the ovarian cycle stage.

RESULTS

Clinical examination of the external genital organs revealed discharge (13/20 in males, 8/18 in females), erythema of the mucosa (5/20 in males, 2/18 in females), hypotrichosis (4/20 in males, 9/18 in females) and excoriations (2/20 in males, 3/18 in females) of the skin next to the mucocutaneous junction. No dog had lesions compatible with transmissible venereal tumor on the external genital organs. Based on the history and the results of vaginal exfoliative cytological examination, 1/18 female dog was in pro-oestrus, 1/18 was in oestrus and 16/18 dogs were in anoestrus.

Inflammatory cells, including neutrophils (20/20) and macrophages (14/20), and bacteria (20/20) were found in at least one of the three slides obtained from male dogs. In contrast, neutrophils and bacteria were present in 3/18 and 12/18 of the samples from female animals.

In no case, *Leishmania* amastigotes were observed.

DISCUSSION

Previous studies from Brazil have shown granulomatous inflammation and large parasitic burden in the gland penis and prepuce of male dogs with CanL (Diniz et al., 2005). In female dogs, inflammatory lesions of the vagina were much less common but parasitic DNA was detected in 4/5 dogs using PCR (Silva et al., 2008). In the present study, failure to find *Leishmania* amastigotes, especially in preputial exfoliative cytology, may be explained by the possible confinement of the parasite into the submucosa without translocation onto the surface, by the lower tropism of the local *L. infantum* strains for the external genitalia of affected dogs or by the comparatively low sensitivity of cytology to detect the parasite.

We have previously shown that the sensitivity of cytology for the detection of *Leishmania* amastigotes depends on the stage of the infection (higher in symptomatic than in asymptomatically infected dogs), on the time devoted and on the number of oil immersion fields that are examined (Saridomichlakis et al., 2005). For example, when approximately 60 min were spend in order to examine 1,000 oil immersion fields of lymph node aspiration smears, a sensitivity of 88% was achieved, whereas examination of 100 oil immersion fields (approximately 6 min microscopy time) resulted in a sensitivity of 73% (Saridomichelakis et al., 2005). Therefore, it is possible that if more time was allowed to search for amastigotes the results of the present study could have been slightly different. However, our purpose was to find a practical alternative to lymph node, spleen and bone marrow cytology, using samples that can be obtained non-invasively. A 20 min-long microscopic examination time is probably longer than the time that could be afforded in a routine laboratory setting.

A relatively low number of dogs examined could be considered as a drawback of this study. However, the 95% confidence interval (calculated using Bayesian statistics) of the 0% sensitivity is 0-7.4% for the whole study population, 0-13.3% for the male dogs and 0-14.6% for the female dogs. Therefore, it is highly unlikely that a substantially higher sensitivity could have been achieved if more dogs had been enrolled.

Results of the present study should not be interpreted as an indication of the absence of the parasite from the surface of the prepuce and the vagina of dogs with leishmaniosis. On the contrary, it is possible that more sensitive diagnostic methods, like PCR, could have resulted in a high rate of positive results

(Diniz et al., 2005; Silva et al., 2008). However, PCR from conjunctiva swab samples that are also obtained in a non-invasive way, has been previously shown to be very sensitive in CanL as well as in dogs that are asymptomatically infected by *Leishmania infantum* (Solano-Gallego et al., 2001; Strauss-Ayali et al., 2004)

CONCLUDING REMARKS

Preputial and vaginal exfoliative cytological examiantion is not a sensitive diagnostic method and cannot be recommended for the routine diagnosis of canine leishmaniosis.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

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