WHY *DIROFILARIA REPENS* HAS MORE ZOONOTIC IMPACT THAN *DIROFILARIA IMMITIS ?*

DE CE ESTE IMPACTUL ZOONOTIC AL LUI *DIROFILARIA REPENS* MAI MARE DECAT CEL AL LUI *DIROFILARIA IMMITIS ?*

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ABSTRACT | REZUMAT

Some major differences have to be kept in mind considering *Dirofilaria repens* and *D. immitis* infections in dogs. For heartworm infections, several rapid, easy, in clinic serological kits are available that detect the circulating antigens of female worms. It allows veterinarians to a prompt diagnosis while no serological diagnostic is available for *D. repens,* and this hamper both a rapid screening in dog population or a rapid and easy diagnosis. Blood examination for circulating microfilariae is strongly suggested for both *Dirofilaria* infections, but this is the only way of *D. repens* dagnosis. However, Knott test that allows the visualization and the identification of microfilariae is not always familiar to veterinarians, mainly in areas of recent introduction of the parasite. Melarsomine dihydrochloride, currently used against *D. immitis* infections, has no efficacy against *D. repens* and only moxidectin has shown to be able to kill most of adult worms in experimental studies. Most of macrocyclic lactones currently used for dirofilarial infections (ivermectin, milbemycin oxime and selamectin) are not fully efficacious against *D. repens* microfilariae and only moxidectin has been shown to be fully effective after four monthly administrations decreasing the risk of microfilariae transmission to mosquito vectors. Finally, while all the macrocyclic lactone preventatives available on the veterinarian market are fully effective against *D. immitis* patent infections at least in Europe (some case of resistance have been reported from the USA), not all have the same potency against *D. repens* and only moxidectin has shown a full efficacy to prevent subcutaneous *D. repens* infections. Keeping in mind all the above considerations, *D. repens* infection is much more difficult to be diagnosed and controlled in the reservoir population (microfilaraemic dogs), and its spreading will continue with the risk of increasing the public health concerns. Medical doctors and veterinarians must strictly collaborate for a better control and surveillance of this infection. Guidelines for the control and treatment *of Dirofilaria* infections can be found in ESCCAP Guideline 5.

Keywords: *Dirofilaria,* Moxidectin, zoonotic

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Trebuie sa se tina cont de cateva diferente majore cand se vorbeste despre infestatiile cu *Dirofilaria repens* si *D. immitis* la caini. Pentru infestatiile cu *Dirofilaria spp.* se gasesc kituri clinice serologice rapide si usoare, ce identified antigenele circulante ale femelei de nematod. Aceste teste ajuta veterinarii Tn diagnosticarea rapida, Tnsa nu sunt valabile si pentru *D. repens,* iar acest lucru Tmpiedica depistarea rapida a populatiei de caini infestata.

Examenul sangelui este recomandat pentru detectia microfilariilor circulante pentru ambele specii de *Dirofilaria,* fiind singura modalitate de diagnosticare a *D. repens.* Cu toate acestea, testul Knott ce permite o vizualizare si o identificare a microfilariilor nu este familiar veterinarilor, mai ales in zonele Tn care parazitul este recent introdus. Melarsomine dihydrochloride, administrat contra infestatiilor cu *D. immitis,* nu are nicio eficacitate Tmpotriva *D. repens* si doar moxidectin este eficace Tn distrugerea majoritatii adultilor paraziti, dupa cum arata studiile experimentale.

Majoritatea lactonelor macrociclice utilizate recent pentru infestatiile cu dirofilarii (ivermectin^, milbemycin oxime si selamectin) nu sunt eficace Tn totalitate Tmpotriva microfilariilor de *D. repens* si doar moxidectin este pe deplin eficace dupa patru administrari lunare, scazand riscul transmiterii microfilariilor prin vectorii tantari. In timp ce toate lactonele macrociclice ce se gasesc pe piata antiparazitarelor de uz veterinar sunt eficace Tmpotriva lui D. *immitis* eel putin Tn Europa (Tn S.U.A. a fost raportata prezenta chimiorezistentei), nu toate au aceeasi potenta contra *D. repens* si doar moxidectinul are o eficacitate crescuta si poate preveni infestatiile subcutanate cu *D. repens.*

Tinand cont de toate aceste consideratii, infestatiile cu *D. repens* sunt mult mai dificil de diagnosticat si controlat Tn rezervorul populatiei (cainii cu microfilarii), iar raspandirea lor va continua cu riscul cresterii preocuparilor de sanatate publica.

Medicii umani si cei veterinari trebuie sa colaboreze strans pentru un mai bun control si supraveghere ale acestei infestatii. Instructiunile pentru controlul si tratamentul infestatiilor cu *Dirofilaria* pot fi gasite in ESCCAP Guideline 5.

Cuvinte cheie: *Dirofilaria,* Moxidectin, zoonotic

Dirofilarial diseases are vector-borne parasitic diseases mainly of dogs and cats (and wild carnivores) caused by *Dirofilaria repens* and D. *immitis. D. repens* is endemic in many countries of the Old World but has not yet been found in the Americas while *D. immitis* infections have a worldwide distribution (25) For long time the two species were confused and it was assumed that *Dirofilaria* worms could be found both in the pulmonary artery and in subcutaneous tissues. In 1910, Bonvicini an Italian scientist, studying the effect of essential turpentine oil against that he supposed to be heartworms, recovered some adult worms from subcutaneous tissues of a necropsied dog and sent them to Raillet and Henry in Lyon (France) who subsequently described and named the species as *D. repens* (32). Nonetheless, the first observation of D. *repens* was probably done in a human being.

In 1566, a Portugase medical doctor, known as Amato Lusitano, in his *Curationum Medicinalium Centuriae* reported *"puella trima... per oculi internam partem, quam angulum magnum appellamus, a jumbrici cuiusdamcaput appere coepis ..."* (in a three-year-old girl, suddenly it started to appear in the area we call big angle of the eye the tip of one worm which sometimes are sited in the eye making its opacity) (23).

Infected dogs are the main reservoir as they frequently have microfilariae in the peripheral blood. To note that in several eastern countries *D. repens* prevalence is higher than D. *immitis* such as, for example, in Romania where heartworm prevalence range 2%-0.2% and D. repens prevalence 7%-20% (8; 18; 19).

D. repens adult worms are permanently located in subcutaneous tissues (subcutaneous dirofilariosis) while *D. immitis* are located in pulmonary arteries and the right heart cavities (heartworm infection) and humans can be an accidental hosts for both species.

However, comparing the data of zoonotic infections caused by *D. immitis* in the United States and Japan with that observed in Europe in the last years, the figures are surprising different. In the US, in the last 60 years about 110 human cases have been reported (21; 38) (roughly 1.8 cases/year) and 277 cases in the last 39 yeas in Japan (1) (roughly 7.1 cases/year) while in Europe less than 30 cases have been reported in the last 37 years (4; 11; 12). To note that the prevalence of canine heartworm infection in endemic areas of the US, Japan and Europe are quite the same (12% to 60% in not preventively treated dogs). About *D. repens,* more than 3,500 cases of human infections have been reported in Europe from 1977 until 2016 (13; 15; 28; 29; 30; 34; 37) roughly about 89 cases /year in the last 39 years.

However, three cases have been described in the US in individuals who previously acquired the infection during staying or travelling in Italy, Greece and Africa (14; 24; 26; 27).

Considering these data, Pampiglione (29) hypothesized that "perhaps twin *D. immitis* populations exist with different genotypes". However, this hypothesis is likely not valid, as it has been demonstrated that most isolates have genetic identity among *D. immitis* worms from different geographical locations around the world (5) even recently some differences have been found both in Europe (33) and Americas (9).

Both *D. immitis* and *D. repens* are able to grow into the same mosquito species and at the same temperature and humidity under laboratory conditions, and they have the same developmental time from the microfilaria! stage to the infective larva (16; 40). Recently, *D. repens* larvae have been identified in *Anopheles maculipennis* and *Aedes vexans* from Slovakia and Austria, respectively (7; 36) and both *D. immitis* and *D. repens* in *Culex pipiens* from Germany (20). These mosquitos species are well known to be competent vector for *Dirofilaria* infections (7). Then, different factors should play a critical role in such a difference in the risk of zoonotic infections.

On the contrary of *D. immitis* larvae, that once transmitted by the infected mosquito undergone migrations in deep tissues of the host before the final localization in the pulmonary arteries and right heart, *D. repens* is a parasite permanently located in the subcutaneous connective tissues. Once infective larvae are transmitted by an infected mosquito, they migrate throughout the subcutis until they develop to adult stages. Furthermore, a frequent localization in humans is the ocular conjunctiva. We can hypothesize that it is much more easy for a parasite located in subcutaneous tissues to escape the natural resistance (immune response) of unusual hosts, such humans, than for *D. immitis* larvae that have a long migration also in humans and can be frequently found in deep localizations such as lungs (coin lesions) or mesentery.

While *D.immitis* causes a severe/very severe condition in infected dogs (and even more severe in cats) and if not cured, the disease can be fatal, most D. *repens* infections are asymptomatic.

Seldom, not pathognomonic signs such as pruritus, alopecia or dermatitis, and even less frequently nodules containing microfilariae and/or adult worms, can be observed. As a consequence, most infections run undiagnosed by veterinarians.

DIAGNOSIS OF CANINE DIROFILARIOSIS

For heartworm infections, several rapid, easy, in clinic serological test kits are available able to detect the circulating antigens of adult female worms.

It allows veterinarians to a prompt diagnosis while no serological diagnostic is available for *D. repens,* and this hamper both a rapid screening in dog population or a rapid and easy diagnosis in infected ones.

To note that cross-reactions have been observed in some case of other parasitic infections (e.g. *Angiostrongylus vasorum* and *Spirocerca lupi;* 3; 35) and the lack of cross-reactions between *D. repens* and *D. immitis* is due to the localisation of the two parasites (subcutis and blood stream, respectively) rather than the specificity of the tests. Blood examination for circulating microfilariae is strongly suggested for both *Dirofilaria* infections, but this is the only way of *D. repens* dagnosis. However, Knott test, that allows the visualization and the identification of microfilariae, is not familiar to veterinarians, mainly in areas of recent introduction of the parasite.

PREVENTION AND ADULTICIDE TREATMENT

The macrocyclic lactones currently available on the veterinarian market (ivermectin, milbemycin, oxime, moxidectin and selamectin) are fully effective to prevent *D. immitis* patent infections, at least in Europe (some case of resistance have been reported from the USA), not all have shown the same potency against D. *repens* and only moxidectin has shown a full efficacy to prevent subcutaneous dirofilariosis (16). Furthermore, most of macrocyclic lactones currently used for dirofilarial infections (ivermectin, milbemycin oxime and selamectin) are not fully efficacious against *D. repens* microfilariae and only moxidectin has approved as a microfilaricidal agent after four monthly administrations (10). Melarsomine dihydrochloride, currently used against *D. immitis* infections, has no efficacy against *D. repens* and only moxidectin has shown able to kill most of adult worms in experimental studies (31).

The reason of such a differences between avermectins and milbemycins are as a consequence of several factors: moxidectin is more lipophilic in nature than ivermectin and it is stored in the fat, which may act as a drug reservoir. Compared to ivermectin, moxidectin has a higher distribution volume and a longer half-life elimination. This may facilitate its distribution from the bloodstream to different tissues and longer residence time for the drug in the body (2; 39).

Furthermore, the moxidectin minimum dosage effective against *D. immitis* infective larvae is 3 meg/kg *vs6* mcg/kg of ivermectin.

D. repens larval stages and the adult worms are permanently resident in subcutaneous tissues, which are rich of connective and fat tissues. Therefore, the high lipophilic nature of moxidectin is probably the reason of the full efficacy observed in the studies.

P-glycoprotein is a membrane protein belonging to the superfamily of the ATP-binding cassette (ABC) transporters and exerts a potent action *vs* drug disposition. Moxidectin toxicity depends on P-gp activity in a less extent than that of ivermectin [moxidectin seems to be a weaker substrate inhibitor of P-gp compared with ivermectin, 22]. This may explain the possibility to increase the dosage of milbemycins in companion animals, increasing the broad spectrum of efficacy of these macrocyclic lactones.

CONCLUSIONS

In conclusion, keeping in mind all the above considerations, *D. repens* infection is much more difficult to be diagnosed and controlled in the reservoir population (microfilaraemic dogs), and its spreading will continue with the risk of increasing of public health concerns. On the other hand, we can not exclude that the high zoonotic impact of *D. repens* should be as the consequence of more "visible" clinical signs in humans (e.g.: subcutaneus nodules and conjuctival and subconjunctival infections), more easy to be etiologically diagnosed than, for instance, *D. immitis* lung infections. Nonetheless, medical doctors and veterinarians must strictly collaborate for a better control and surveillance of this parasitic infection. For instance, in Ukraine reporting cases of dirofilariosis has been mandatory since 1975, and the disease was included in the national surveillance system for notifiable diseases (Salamatin et al, 2013). Guidelines for the control and treatment of Dirofilaria infections can be found in ESCCAP Guideline 5 (41).

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