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## BACKGROUND:

- *Wolbachia* was first reported in Serbia in dogs naturally infected with *Dirofilaria* spp. using a *wsp* gene PCR (Kosić et al., 2018).
- Different genes (*wsp*, 16S rRNA, *dnaA*, *ftsZ*, *groE*, *hcpA*, *gltA*, *fbpA*, *gatB* and *coxA*) can be used in PCR detection of *Wolbachia* (Sarwar et al., 2018).
- The aim of this study is to analyze whether the usage of PCR for two genes (*wsp*, 16S rRNA) improves the detection of *Wolbachia* in dogs, especially in those treated with antibiotics for heartworm disease (HWD).

**Table 1.** The alternative therapy protocol

Drug and dose	Weeks
<b>Doxycycline</b>	0 – 6 .weeks
10 mg/kg p/o s.i.d.	11. and 12. weeks
	17. and 18. weeks
	23. and 24. weeks
	29. and 30. weeks
	35. and 36. weeks (end of therapy)
<b>Ivermectine</b>	every two weeks
6-14 µg/kg p/o s.i.d.	

## RESULTS:

- The prevalence of *Wolbachia* in *Dirofilaria* spp. infected dogs was 53.85%.
- The effectiveness of the alternative therapy for *D.immitis* and *Wolbachia* infection was 88.89% (n.8/9) and 83.33% (n.5/6), respectively.

**Table 2.** Results of *Wolbachia* genes detection by PCR in dogs infected and non-infected with *Dirofilaria* spp.

<i>Dirofilaria</i> spp. infected dogs	<i>Wolbachia</i> <i>wsp</i>	<i>Wolbachia</i> 16S rRNA	<i>Wolbachia</i> <i>wsp</i> and 16S rRNA	<i>Wolbachia</i> non detected
<i>Dirofilaria immitis</i>	0	5	5	12
<i>Dirofilaria repens</i>	0	1	0	0
Coinfection <i>D.immitis</i> and <i>D. repens</i>	1	0	2	0
<i>Dirofilaria</i> spp. non-infected dogs	0	0	0	10

## MATERIALS AND METHODS:

- The presence of *Wolbachia* was investigated in blood samples of dogs infected (N1=26) and non-infected (N2=10) with *Dirofilaria* spp. by PCR detection of *wsp* and 16S rRNA genes (Simsek and Turcan, 2016; Foster et al., 2008).
- Parasitological examinations (modified Knott test for circulating microfilariae and SNAP Test IDEXX for circulating *Dirofilaria immitis* antigen) was used for diagnosing and differentiating *Dirofilaria* spp..
- Nine dogs infected with *D.immitis* (9/26 dogs) and treated for HWD with an alternative therapy were analyzed again for *Wolbachia* at the end of therapy.
- Alternative therapy protocol were used until antigen negativization, but not longer than 9 months (Bazzocchi et al., 2008).

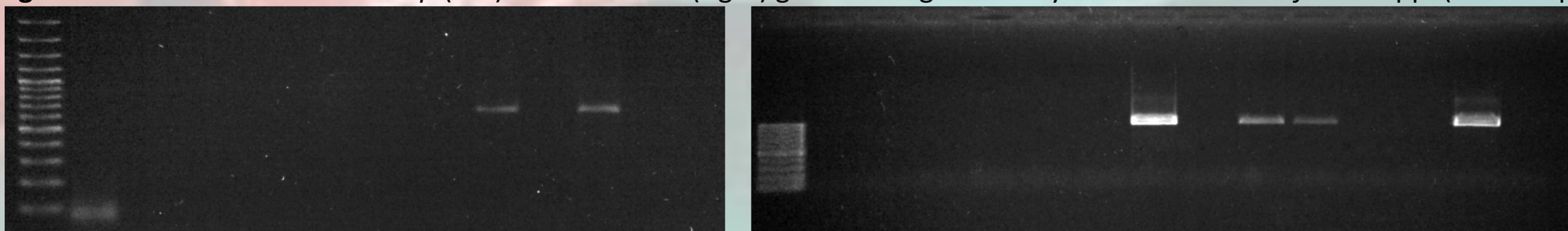


**Figure 1.** Microfilariae of *D. immitis* and *D. repens* detected by modified Knott test (left) and high level of *D. immitis* antigen detected by Snapp HTWM, Idexx, USA (right) in dog 2

**Table 3.** The results of parasitological examinations for *D. immitis* and PCR for *Wolbachia* genes in dogs treated with the alternative therapy for HWD

The beginning of the therapy (D+W+ dogs)					The end of the therapy			
Dogs	Ag	mf/ml	<i>wsp</i>	16S rRNA	Ag	mf/ml	<i>wsp</i>	16S rRNA
Dog 1	+	0	+	+	ND	0	-	-
Dog 2	++	3360	+	+	ND	0	-	-
Dog 3	++	750	-	+	ND	0	-	-
Dog 4	++	500	+	+	ND	0	-	-
Dog 5	+	400	+	+	ND	0	-	-
Dog 6	ND	100	+	+	ND	0	+	-
The beginning of the therapy (D+W- dogs)					The end of the therapy			
Dogs	Ag	mf/ml	<i>wsp</i>	16S rRNA	Ag	mf	<i>wsp</i>	16S rRNA
Dog 7	+	1920	-	-	ND	0	+	-
Dog 8	++	0	-	-	++	0	-	-
Dog 9	++	0	-	-	ND	0	-	-

**Figure 2.** Conventional PCR for *wsp* (left) and 16S rRNA (right) genes in dogs naturally infected with *Dirofilaria* spp. (M-100 bp)



## CONCLUSIONS:

- A higher number of dogs infected with *Wolbachia* were detected when PCR for two genes were used.
- The effectiveness of the alternative therapy for *D. immitis* and for *Wolbachia* infection was similar.

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