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MOSQUITOES (DIPTERA: CULICIDAE) AS *DIROFILARIA* VECTORS IN TULA REGION, RUSSIA

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Abstract This study was carried out to investigate the potential vectors and mosquito infection rates of *Dirofilaria immitis* and *D. repens* in Tula region which is located in central part of Russia. Mosquitoes were captured throughout three mosquito seasons (2013–2015). A total of 719 genomic DNA pools, extracted and grouped according to the species and collection site (1–5 specimens/pool) from 2877 mosquito specimens, were examined by PCR using species-specific primers for *D. immitis* and *D. repens*. DNA extraction was performed separately to thorax-heads and abdomens in order to determine infective and infected mosquito specimens, respectively. Mosquito fauna is represented by 18 species belonging to 6 genera. The most abundant species was determined as *Och. cantans* (41.8%), *Cx. pipiens* (9.5%), *Och. cataphylla* (8.9%), *Ae. geniculatus* (6.9%). The minimum infection rates (MIRs) for *Dirofilaria* infection were calculated as 2.6% (1.5% and 1.1% for *D. immitis* and *D. repens,* respectively). Filarial DNAs were found in 12 species but most frequently in Ae. *geniculatus, Och. punctor, Och. sticticus*, *Ae. cinereus, Och. cantans*. MIRs for these specimens were established as 4.0%*,* 3.9%, 3.7%, 3.5%, 3.2%, respectively. The PCR results indicated that filarial DNAs were detected mainly in mosquito abdomens: 39/719 (5.4%) and 30/719 (4.2%) pools were positive for *D. immitis* and *D. repens* infection, respectively. In thorax-heads DNAs were detected less frequently: 9/719 (1.3%) and 6/719 (0.8%) pools were positive for *D. repens* and *D. immitis* DNAs, respectively. Four species of mosquitoes (*Ae. vexans*, *Ae. geniculatus*, *Cx. pipiens*, *Och. cantans*) carried infective stages of dirofilarial nematodes and could be the main potential vector of *D. immitis* and *D. repens* in Russia.

Key words *Dirofilaria immitis, Dirofilaria repens*, potential vectors, PCR, DNA, MIR.

INTRODUCTION

Dirofilariasis is a vector-borne disease that affects canine and feline population. *Dirofilaria immitis* (Leidy) and *D. repens* (Railliet, Henry) are causative agents of dirofilariasis and parasitize in subcutaneous tissues and hearts, respectively (Simon et al., 2012). Humans are at risk of developing pulmonary and subcutaneous lesions (Cancrini et al., 2007). *D. immitis* and *D. repens* are transmitted by culicid mosquito species belonging to *Culex*, *Aedes*, *Ochlerotatus*, *Anopheles*, *Coquillettidia*, *Armigeres* and *Psorophora* genera (Yildirim et al., 2011; Mckay et al., 2013). Vectors ingest the first stage of parasite (microfilariae) during a blood meal on an infected host. In mosquito Malpighian tubules microfilariae develop to second (L2) and third stage larvae (L3). Infective L3 reach the salivary glands and proboscis where they are transferred while feeding to another host (Kartman, 1953; Montarsi et al., 2015).

In Russia, the ratio of mosquitoes with *Dirofilaria* is investigated for southern region and constitutes 1.0-14.0% (Arakeljan et al., 2008; Ermakova et al., 2014). Although dirofilariasis is a problem for Russia, many areas are not sufficiently studied. Also there are no data about mosquito species as

potential vectors of dirofalarial worms. Identification of mosquitoes in all cases was conducted only to genera. *D. immitis* and *D. repens* have been detected in *Culex*, *Aedes* and *Anopheles* genera and established as 4.0-7.0, 5.0-6.7, 0.6-3.4%, respectively (Arakeljan et al., 2008; Ermakova et al., 2014). The purposes of the current study were detecting mosquito fauna and identifying mosquito species that can transmit filarial worms in the Tula region (central part) using species specific PCR primers to estimate infection rate.

MATERIALS AND METHODS

The research was conducted in three districts in Tula (54°12′N and 37°37′E) and in five sites in Tula region (53°55′N and 37°35′E). They are typical dog accumulation areas: gardens of private houses with one or two dogs and parks where owners walk their dogs. Also there are sufficient numbers of stray dogs in parks. Mosquitoes were collected throughout three mosquito seasons (2013–2015) from May to August using exhauster. Trapping was held in the evening from 6-9 p. m. for 6-8 times a month. After trapping mosquitoes were frozen in a −19°C for 20–30 min and afterwards were identified using taxonomic keys (Gutsevich et al., 1970). The collected mosquitoes were grouped according to the species and collection site (1–5 specimens/pool).

DNA extraction was performed separately to thorax-heads and abdomens in order to determine infective and infected mosquito specimens, respectively. For the PCR analysis, we used 2877 female mosquitoes which were divided into 719 pools. Each pool was tested separately for identifying of *D. immitis* and *D. repens*. PCR amplification was performed with two sets of primers: DIR-3: F-5’ – CCGGTAGACCATGGCATTAT - 3’ и DIR-4: R – 5’ - CGGTCTTGGACGTTTGGTTA - 3’ for detection of *D. repens* (Vakalis et al., 1999); COI intF — 5’ -TGATTGGTGGTTTTGGTAA - 3’ and COI intR — 5’ - ATAAGTACGAGTATCAATATC -3’ for detection of *D. immitis* (Murata et al., 2003). The presence of filarial DNA was examined by agarose gel electrophoresis.
Minimum infection rates (MIRs) were calculated by the standard formula: (number of positive mosquito pools)/(total number of mosquitoes tested)×100 (Cancrini et al., 2003).

RESULTS

Totally, 2877 mosquito specimens belonging to *Aedes, Ochlerotatus, Culex, Culiseta, Coquillettidia* and *Anopheles* genera were caught during three mosquito seasons (2013–2015) in the studied area. The most abundant species was *Och. cantans* with the ratio of 41.8% and this rate was followed by *Cx. pipiens* (9.5%), *Och. cataphylla* (8.9%), *Ae. geniculatus* (6.9%) (Table 1).

Table 1. Numbers of wild-caught mosquitoes in Tula region, 2013-2015

Species	May	June	July	August	Total
1. *Anopheles maculipennis*	0	0	4	19	23
2. *Culiseta alaskaensis*	3	5	28	15	51
3. *Cs. annulata*	0	0	7	3	10
4. *Coquillettidia richiardii*	3	5	41	8	57
5. *Aedes cinereus*	4	14	86	40	144
6. *Ae. vexans*	8	13	78	32	131
7. *Ae. geniculatus*	0	7	151	42	200
8. *Ochlerotatus cantans*	409	248	446	100	1203
9. *Och. excrucians*	5	22	10	4	41
10. *Och. communis*	32	34	12	5	83
11. *Och*. punctor	20	14	12	5	51
12. *Och. sticticus*	23	23	4	4	54
13. *Och. diantaeus*	24	35	0	1	60
14. *Och. intrudens*	54	55	41	6	156
15. *Och. cataphylla*	87	111	47	13	258
16. *Och. leucomelas*	26	20	8	3	57
17. *Culex modestus*	0	0	12	13	25
18. *Cx. pipiens*	3	32	112	126	273

The minimum infection rates (MIRs) for *Dirofilaria* infection were calculated as 2.6% (1.5% and 1.1% for *D. immitis* and *D. repens,* respectively). Filarial DNAs were found in 12 species but most frequently in *Ae. geniculatus, Och. punctor, Och. sticticus*, *Ae. cinereus, Och. cantans*. MIRs for these specimens were established as 4.0%, 3.9%, 3.7%, 3.5%, 3.2%, respectively (Table 2). Six species, which included Oc*h. cantans, Ae. qcinereus, Ae. geniculatus, Ae. vexans,* Oc*h. cataphylla* and four species (Oc*h. cantans, Ae. geniculatus,* Oc*h. cataphylla, Och. punctor)* had more than one positive pool for *D. immitis and D. repens* infection, respectively (Table 2). No specimen of mosquito showed a mixed infection.

D. immitis was found in 5.4% and 0.8% abdomen and thorax-head pools, respectively. *D. repens* DNAs were detected in 4.2% and 1.3% abdomen and thorax-head pools. Only four species of mosquitoes (*Ae. vexans*, *Ae. geniculatus*, *Cx. pipiens*, *Och. cantans*) carried the infective stage of dirofilarial nematodes. The number of *D. repens* infective pools established for *Och. cantans*, *Ae. geniculatus* and *Cx. pipiens* as seven, one, one, respectively. Concerning *D. immitis* (L3) pools *Och. cantans, Ae. geniculatus* and *Ae. vexans* had three, two and one positive pools, respectively. Regarding *Och. cantans*, three pools with *D.immitis* and seven pools with *D. repens* contained filarial DNA both in abdomen and thorax-head.

Table 2. Mosquito pools examined by PCR for filarial DNA

			PCR (*D. immitis*) +		PCR (*D. repens*) +		MIRs
	Tested	**Pools**	**Head-thorax**	**Abdomens**	**Head-thorax**	**Abdomens**	**%**
Cs.alaskaensis	51	12	0	1	0	0	1.9
Ae. cinereus	144	36	0	4	0	1	3.5
Ae. vexans	131	32	1	2	0	0	2.3
Ae. geniculatus	200	50	2	2	1	3	4.0
Och. cantans	1203	300	3	21	7	17	3.2
Och. communis	83	20	0	1	0	1	2.4
Och. punctor	51	12	0	0	0	2	3.9
Och. sticticus	54	13	0	1	0	1	3.7
Och. intrudens	156	39	0	2	0	1	1.9
Och. cataphylla	258	64	0	3	0	4	2.7
Och. leucomelas	57	14	0	1	0	0	1.8
Cx. pipiens	273	68	0	1	1	0	0.7

DISCUSSION

In the current study mosquito fauna is represented by 18 species belonging to 6 genera (Table 1). After comparing the fauna of mosquitoes in Tula with the studies of the neighboring regions of European Russia, similar data were noted (Gornostaeva and Danilov, 2000). There are no data concerning the species composition of mosquitoes in Tula region.

Och. cantans and *Och. cataphylla* were the dominant mosquito species in this study in May and June. These species are associated with intermittent floodwaters which are numerous in spring. Other species, *Ae. geniculatus* and *Cx. pipiens*, became abundant from July to August. They reach the maximum number in the second part of summer. At the same time *Och. cantans* was dominant from July to August too due to their ability to give several generations per year (Gutcevich et al., 1970).

Russia is endemic for *Dirofilaria* infection. Two species of *Dirofilaria* (*D. immitis* and *D. repens*) have been identified in mosquitoes, dogs and humans in other reseaches (Arakeljan et al., 2008; Ermakova et al., 2014). Both types of worms were found in Tula mosquitoes in the present study. Several cases of *D. repens* infection in humans were detected in Tula region previously (Derzhavina et al., 2010). However, *D. immitis* have not been identified in humans in Tula (Ermakova et al., 2014). The first case of *D. immitis* was detected in 2015 in Moscow region. Immature female was removed from 14-month-old child (Tumolskaya et al., 2016).

The rates of MIRs are studied in other countries. Thus MIRs for *Cx. pipiens* varies from 0.05 to 0.54% for European countries (Cancrini et al., 2007; Yildirim et al., 2011; Latrofa et al., 2012; Capelli et al., 2013), for *Ae. vexans* it conducted 0.03-1.6% for European countries (Yildirim et al., 2011; Latrofa et al., 2012; Bockova et al., 2013; Sulesco et al., 2016) and 9.6% for USA (McKay et al., 2013). In Moldova MIRs for *Ae. geniculatus*, *Ae. cantans* and *Och. sticticus* established as 7.7; 13.3 and 4.2%, respectively (Sulesco et al., 2016). Our data are partially agreed with results of other studies. Differences of infection rates in the same mosquito species from different regions could be linked with ecological factors such as season, climate and geographical features which are specific for each region (Genchi et al., 2009).

Positive pools with *D. immitis* and *D. repens* were detected for the worm observing season. Moreover, according to other surveys infective stage (L3) of *Dirofilaria* were also determined in *Ae. vexans*, *Ae. geniculatus* and *Cx. pipiens* (Petruschke et al., 2001; Cancrini et al., 2007; Yildirim et al., 2011, McKay et al., 2013, Bockova et al., 2013).

This is the first study in Russia to examine the mosquito species as potential vectors of *D. immitis* and *D. repens*. In this study filarial DNA was revealed in 12 species of mosquitoes, only four of them contained infective stage (L3) in the head or thorax. A location exclusively reached by the L3 demonstrating that these two mosquitoes are able of supporting the development of *Dirofilaria* species up to the infective stage. Thus *Ae. vexans*, *Ae. geniculatus*, *Cx. pipiens*, *Och. cantans* can be considered as important vectors in Russia. However, more studies are needed to establish the vector competence of *Och. punctor*, *Och. sticticus* and *Ae. cinereus* as high level of positive pools (16.7, 7.7, 13.9%, respectively) were identified only in the abdomen.

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