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Temporal association of ambient temperature and relative humidity with *Spirocerca lupi* infection of *Onthophagus sellatus*: A 14-year longitudinal study

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ABSTRACT

The parasitic nematode *Spirocerca lupi* causes major morbidity and mortality in dogs. The scarab beetle *Onthophagus sellatus* is its major intermediate host in Israel. We investigated the prevalence of beetle infection by *S. lupi* in different years between 1994 and 2008. The average monthly maximum and minimum relative humidity (RH) and ambient temperature (AT) throughout the study period were calculated based on daily meteorological data. The infection prevalence decreased over the study period, possibly due to a chronological change resulting from increased preventive treatment of dogs against *S. lupi*, or climate change. Multivariate analysis was performed for these two hypotheses. Under the first hypothesis, chronological change was forced into the model, and environmental variables were inserted stepwise. The final model included beetle-collection date, minimum RH (RH_{min}) during the month preceding beetle collection, its interaction with maximal AT (AT_{max}) during that same month, and the interaction of maximal RH (RH_{max}) and AT_{max}, during the month of beetle collection. Under the second hypothesis, chronological change was not forced. The final model included RH_{max} during the month of beetle collection, average RH (RH_{ave}) during the month preceding beetle collection, and its interaction with AT_{max} during the latter month. The results suggest that under both hypotheses, RH and AT during the month preceding beetle collection influence *S. lupi*'s ability to develop and survive in *O. sellatus*, and may be used to predict the risk to dogs of *S. lupi* infection.

1. Introduction

Spirocercosis is a severe disease in dogs caused by the parasitic nematode *Spirocerca lupi* (Spirurida: Thelaziidae). *S. lupi* is found worldwide, especially in tropical and subtropical regions, and may be transmitted by several coprophagous beetle species or via paratenic hosts that consume the beetle vector (reviewed in van der Merwe et al., 2007). Spirocercosis is an emerging canine disease in Israel which seems to be more prevalent in urban areas, with an up to sevenfold increase in diagnosed cases in the 1990s (Mazaki-Tovi et al., 2002). In Israel, the most important arthropod host of *S. lupi* is the coprophagous beetle *Onthophagus sellatus* (Gottlieb et al., 2011).

One of the strategies for controlling vector-borne diseases is to decrease the vector's ability and opportunity to become infected with and transmit the pathogen. The capacity of arthropod vectors to actively or passively

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transmit a disease depends on intrinsic factors determining its competence, as well as extrinsic factors. The latter include climatic conditions that are favorable to vector activity, survival, and to pathogen development within the vector (Reisen, 2009). Several studies have tested the ability of dung beetles (Scarabaeidae) to serve as intermediate hosts (i.e., vectors) for passive transmission of nematodes. Those studies included surveys of the infection status of field-collected beetles at certain time points (e.g., Du Toit et al., 2008; Mowlavi et al., 2009), and experimental laboratory infection of certain beetles and other arthropods with *S. lupi* (e.g., Mukaratirwa et al., 2010). However, to our knowledge, specific climatic parameters that may influence the probability of the intermediate beetle host's to become infected with *S. lupi*, and its development in the beetle, have never been tested.

In this study, we analyzed the correlation between infection prevalence (IP) of *S. lupi* in *O. sellatus* and relative humidity (RH) and ambient temperature (AT) over a 14-year period.

2. Materials and methods

2.1. O. sellatus collection and determination of S. lupi infection prevalence

Four annual field collections of *O. sellatus* were performed between 1994 and 2008 as follows: February 1994 to January 1995, January 1998 to December 1998, July 2004 to June 2005 and June 2007 to August 2008. Data from the third collection had been analyzed previously for a different purpose (Gottlieb et al., 2011). All surveys and collections were performed at the same location, where *S. lupi* is known to be prevalent (Mazaki-Tovi et al., 2002): a public park in the city of Ramat Gan, on a typical urban, irrigated lawn shaded with trees, in the coastal plain of Central Israel (32°4′13.8″ N, 34°49′36.12″ E). Up to 20 dung pats, estimated to be 2–3 days old, with evidence of scarab beetle activity were collected every 1 to 2 months into ventilated plastic vials, and brought to the laboratory. In the laboratory, beetles were separated from the dung, identified to the species level and their number recorded. Each *O. sellatus* beetle was dissected under a stereoscope and screened for *S. lupi* larvae. The proportion of *S. lupi*-infected beetles (IP) was recorded.

2.2. Meteorological data

The daily minimum and maximum ambient temperature (AT_{min} and AT_{max}, respectively) and minimum and maximum and average relative humidity (RH_{min}, RH_{max} and RH_{ave}, respectively) were recorded at 50 cm height during the study period by a nearby meteorological station (Tel-Mond; 32°15′10.43″ N, 34°55′8.76″ E).

2.3. Statistical analysis

The average daily AT_{min}, AT_{max}, RH_{min}, RH_{max} and RH_{ave} were calculated for each beetle-collection month and for the preceding month. Collection periods were numbered chronologically. To detect trends in AT and RH during the study period, we first analyzed the correlation between the order of the collection period and the climatic variables, while controlling for the month of collection, using a linear regression model. Then, each of the above variables was inserted separately as an explanatory variable into a logistic regression model, to analyze its association with the prevalence of beetle infection by *S. lupi*. The climatic variables, as well as the interactions between AT and RH variables in the same month, were then inserted by the stepwise method into two multivariate logistic regression models. This analysis was performed under two hypotheses: the first assumed a chronological change in IP, possibly due to an increase in the preventive treatment of dogs against *S. lupi* by veterinarians in Israel, and was therefore forced to include the consecutive collection period number; the second assumed that the change in IP occurred due to environmental (e.g., climatic) changes, and therefore excluded the consecutive collection period number. For each model, the odds ratio for one unit increase in each explanatory variable was calculated. A P-value ≤ 0.05 was considered statistically significant. The models' goodness of fit was estimated by calculating the R^2 for the models' predicted IP versus the observed IP. All analyses were performed by SPSS 19.0 for Windows.

3. Results

Overall, 10,935 beetles were dissected for detection of *S. lupi* infection. Monthly adjusted IP of *S. lupi* larvae in *O. sellatus* and the average monthly RH_{max}, RH_{min}, RH_{ave}, AT_{max} and AT_{min} are depicted in Fig. 1. There were statistically significant ($P < 0.001$) decreases in IP, RH_{min}, RH_{max}, RH_{ave}, AT_{min} and a decrease in AT_{max} ($P = 0.058$) during the study period (February 1994 to August 2008).

The final logistic regression model under the first hypothesis (chronological change) included the consecutive collection time, RH_{min} during the month preceding beetle collection, its interaction with AT_{max} during that month, and the interaction of RH_{max} and AT_{max} during the month of beetle collection (Table 1). The R^2 of the correlation between the predicted IP based on the model and observed IP was 0.811 (Fig. 2a).

The final logistic regression model under the second hypothesis (climate change) included RH_{max} in the month of beetle collection, RH_{ave} in the month preceding beetle collection, and its interaction with AT_{max} in the latter month (Table 1). The R^2 of the correlation between the predicted IP based on the model and observed IP was 0.779 (Fig. 2b).

4. Discussion

This is the first longitudinal study testing the infection prevalence of *S. lupi* infection in one of its scarab beetle vectors. The results of this long-term observational analysis show that the IP of *S. lupi* in *O. sellatus* changes both monthly and annually. This change was found to be significantly associated with RH and AT variables. The IP change may have resulted from changes in the arthropod's activity which may not be optimal for *S. lupi* ingestion and development, and may be significantly associated with the possible