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Original Article

Molecular methods indicate lack of spread of *Acarapis woodi* introduced to honey bees in western Norway

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ABSTRACT

The tracheal mite, *Acarapis woodi*, may be one of many factors contributing to the decline in honey bee (*Apis mellifera*) populations. Databases on the widespread distribution of *A. woodi* exist, but the data seem patchy. Norway is not listed as being infested, although there have been at least two separate introductions of the parasite. Investigations in 2003, 2006, and 2009 using standard microscopy methods indicated persistence of *A. woodi* in honey bees in this region.

In 2013, we conducted another survey. Samples were sent in from 335 beehives belonging to 39 apiaries, and all were asked to complete a questionnaire. Analysis for *A. woodi* in the submitted samples was by PCR, with sequencing of positive results.

The results described in this article indicate that this parasite still persists in some apiaries in this region, but at a low, and possibly decreasing, level, with positive results obtained from just two (5.1%) of the apiaries. Of the 17 beekeepers that answered the questionnaire, none reported symptoms of infestation with *A. woodi.*

Sequencing of PCR products indicated a difference between the two *A. woodi* isolates.

Our results were generally encouraging regarding the apparent lack of spread of *A. woodi*, within the County. Furthermore, the sequencing results may indicate two separate introductions rather than spread. Nevertheless, the scarcity of data, the vulnerability of honey bee populations globally, and the potential contribution of this parasite to reduced survival, indicate that the situation should be continued to be monitored. In addition, Norwegian beekeepers should be made aware of, and follow, restrictions regarding import and transport of bees, both nationally and internationally.

1. Introduction

Acarapis woodi (Rennie) the tracheal mite of honey bees (*Apis* spp.), reside in the trachea of the bees, where they reproduce. Female mites attach their eggs to the tracheal walls, and here the larvae hatch and develop. Transmission occurs by gravid females of the next generation moving to the tip of a bee hair and moving to a new host, usually a bee less than 24 h old, via the first thoracic spiracle.

Within the trachea, the mites pierce the tracheal walls to feed on bee haemolymph. Although bees are weakened by infestation with *A. woodi* (acarapisosis), with lesions in the tracheal walls and depletion of haemolymph, along with reduced pollen collection and honey production, infestations do not tend to cause acute disease; in Europe, *A. woodi* is not usually associated with massive die-offs or colony collapse disorder.

However, bees infested with *A. woodi* may have limited capacity for activities requiring high metabolic rates, such as flying in cool weather, due to the mites decreasing tracheal gas-exchange (Harrison et al., 2001). In addition, acarapisosis has been associated with reduced capacity for winter survival, due to the bees' inability to thermoregulate (McMullan and Brown, 2009). *A. woodi* has also been noted as a contributor to the reduction in honeybees globally (Kadowaki, 2010).

The distribution of *A. woodi* is often described as “global”, with the exception of Sweden, Norway, Denmark, New Zealand, Australia, and Hawaii (e.g., Garrido-Bailón et al., 2012), and on the EPPO global database (https://gd.eppo.int/taxon/ACASWO/distribution updated 2015) and the CABI invasive species compendium (http://www.cabi.

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org/isc/datasheet/2527; updated 2015) these countries are not listed as reporting *A. woodi*. However, the information in these databases does not seem to be complete. For example, *A. woodi* was apparently first reported in Japan in 2010 (Maeda and Sakamoto, 2016), but Japan also does not appear on either database. Thus, information on the global distribution of *A. woodi* seems patchy.

In Norway, which is considered free of *A. woodi* according to the international databases and the published literature, this parasite was first reported as being introduced in 1982 (Bipestnemnda, 1982), when it was imported with some "Buckfast" queens from UK. Exposed bees were destroyed and spread of *A. woodi* was not found to occur. Beekeeping regulations in Norway at this time meant that apiaries were screened (for both *A. woodi* and varroa mites) every two years, and movement of bees from infested areas was restricted.

In 2002, *A. woodi* was detected in three commercial apiaries in Sogn and Fjordane County in western Norway (Kirkevold, 2002). Import of queens was, again, suggested as the possible route by which the mite had been introduced, as had occurred in Denmark and Finland (Helland, 2002); beekeepers were instructed to follow the regulations to prevent further spread. In 2003, seven apiaries in the same area were found to be infested (Helland, 2003; Gjessing, 2003), and in 2005 and 2006 tracheal mites were again found in one of the previous locations (Unpublished data; Helland, 2006).

In 2008, the regulations were changed, and only apiaries in municipalities bordering the county in which *A. woodi* had been identified were subject to compulsory testing, and only when hives were to be moved or sold. Movement of colonies out of the Sogn and Fjordane County remained prohibited.

In 2009, a project on the occurrence of *A. woodi* in Sogn and Fjordane was conducted in which apiaries (defined here as all the colonies/hives belonging to an individual beekeeper) were invited to participate voluntarily. Only 22 apiaries participated of a possible 48 (registered as members of the Norwegian Beekeepers Association in the county at this time) (46% participation), and for each 65 bees were examined by dissection and microscopy; *A. woodi* was detected at two apiaries (approximately 9%), and within apiary infestation was 1.5% and 12.3% (unpublished data).

Here we describe a follow-up project conducted in Sogn and Fjordane County during 2013, in which the occurrence and spread of *A. woodi* was investigated using PCR for detection.

2. Materials and methods

2.1. Sampling area and sampling strategy

Samples were collected in the county of Sogn and Fjordane, Norway where the parasite had previously been detected. Sogn and Fjordane is a large county in the middle of western Norway, known for its long fjords and surrounding mountains creating natural geographical barriers (Fig. 1).

Participation in the project was voluntary, and not all beekeepers in the area participated. Samples were obtained from 335 beehives belonging to 39 beekeepers, and sampling was organized by the Norwegian Beekeepers Association with help of the local beekeepers. Thus, based on membership in the Norwegian Beekeepers Association in Sogn and Fjordane County at this time (55 beekeepers with around 400 colonies), participation was approximately 70%, including over 80% of bee colonies. It is clear from Fig. 2 that the majority of apiaries participating in the project were situated along the fjords.

Samples were collected during spring (April–May) 2013, with the exception of two samples collected during October 2013.

Beekeepers were asked to collect between 50 and 100 bees from each colony. The bees were killed by freezing and sent by post to the laboratory at Norwegian University of Life Sciences (NMBU), Oslo, where they were stored in a freezer at − 18 °C until analysis. As the rate of infestation was expected to be low, based on the data from previous years, it was decided that a minimum of 60 bees should be included in each analysis.

Each beekeeper was asked to complete a brief questionnaire on the condition of the beehive and send it with the samples (see Table 1).

2.2. Laboratory analyses

2.2.1. Microscopy

Detection of *A. woodi* mites by microscopy for control samples from which DNA was subsequently extracted was conducted according to the dissection description protocol by Shimanuki and Knox (1991). In brief, the head and first pair of legs were removed using a sharp blade, then, thin transverse disc-sections were cut from the anterior face of the thorax by cutting at the same angle as had been used to remove the head and first pair of legs. The discs were placed in glass Petri dishes containing 10% potassium hydroxide (KOH), and incubated at 37 °C for 16–24 h. The KOH dissolves the muscle and fat tissue, leaving the trachea exposed. The tracheal tubes were checked for transparency, and then examined at 200–400 × magnification.

2.2.2. Molecular analyses

DNA was isolated from pooled samples consisting of between 60 and 100 bees; for example 10 bees from six hives. From small apiaries (1–10 colonies) one pooled sample was taken, from bigger apiaries (over 10 colonies) or where the hives were placed in separate different locations, more pooled samples were taken. Thus, single samples were analyzed from small apiaries, whereas several samples were analyzed from larger apiaries. In total, 4188 bees were used for DNA isolation.

Abdomens of the bees were removed, and the heads and thoraxes placed in a stomacher bag with filter (Seward, UK), laboratory-grade water was added to the bag, in the ratio 0.5 ml water per bee, and the sample homogenized using a paddle blender (Stomacher 400, Colworth, UK) for 5 min. One ml of the homogenate was transferred into 1.5 ml microcentrifuge tube (12 tubes per apiary) that was centrifuged at 13000 rpm for 3 min. The supernatant was discarded and the pellet either used directly for DNA isolation, or stored frozen at − 18 °C. DNA was extracted using the QIAamp® DNA Mini Kit and or QIAamp DNA Blood Mini Kit – tissue protocol (Qiagen GmbH, 140 Hamburg, Germany), following the manufacturer's instructions. The eluted DNA was frozen at − 18 °C until used as a template for PCR.

A PCR protocol and primers developed for screening apiaries in Spain for *A woodi* were used (Garrido-Bailón et al., 2012), with the mitochondrial gene, cytochrome oxidase subunit 1, as the target. Primers were purchased from Invitrogen, Life Technologies A/S (Norway). The expected size of the amplicon was 162 bp. For each reaction, DNA isolated from *A. woodi* from infested hives from 2009 identified by microscopy (see Section 2.2.1) was used as positive control and nanopure water used as negative control.

The products were separated and visualized by electrophoresis on 2% agarose gels using SYBRsafe® DNA gel stain under UV radiation, and appropriate ladders for estimating the size of products. Positive samples were purified using High Pure PCR product purification kit (Roche Diagnostics, GmbH, Mannheim, Germany), and sequenced on both strands at GATC Biotech, Germany. Sequences were examined and contiguous sequences made. The sequences obtained were submitted to GenBank™, and comparisons were conducted using NCBI BLAST.

Nucleotide sequence data reported in this paper are available in the GenBank™ database under the accession numbers: **MF405076** and **MF405077**.

3. Results

3.1. Questionnaire

Of the 39 beekeepers that sent in samples for analysis, 17 (44%) also sent the completed questionnaire. Of these, 14 (82%) reported that they