Haematophagous mites on poultry farms in the Republic of the Union of Myanmar

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Abstract

Haematophagous ectoparasites of poultry, such as Ornithonyssus sylviarum, northern fowl mites (NFMs), Dermanyssus gallinae, poultry red mites (PRMs), and Ornithonyssus bursa, tropical fowl mites (TFMs) are prevalent worldwide. Although poultry farming is a major industry in Southeast Asia, there are only a few reports concerning the prevalence of avian mites in this region. In this study, we sampled twenty farms in four major poultry farming areas in Myanmar. We detected the mites on six farms, and they showed morphological similarities to NFMs and TFMs. The nucleotide sequences of cytochrome c oxidase subunit I indicated that some mites were NFMs. This is the first report confirming the presence of NFMs and TFMs among the hematophagous mites infesting chickens on Myanmar poultry farms.

Keyword: Agriculture

1. Introduction

Infestation of haematophagous ectoparasites in the poultry industry is a global problem that leads to decreased productivity. Ornithonyssus sylviarum, northern fowl
mites (NFM) (Canestrini and Fanzago), *Dermanyssus gallinae*, poultry red mites (PRM) (De Geer), and *Ornithonyssus bursa*, tropical fowl mites (TFM) (Berlese) are harmful ectoparasites affecting poultry farming. Infestation by these mites induces stress in chickens, such as reduction in egg production and egg quality, anaemia, and diminished immunity; severe infestation often causes death by blood loss (Arce et al., 2018; Denmark and Cromroy, 2003; Lemke and Kissam, 1986; Sparagano et al., 2014). NFM are the most common and most damaging ectoparasites of poultry in North America (Mullens et al., 2009) and TFM are prevalent in tropical and temperate zones (Denmark and Cromroy, 2003). PRM are endemic to European countries, Japan, and Brazil (Roy et al., 2010; Sparagano et al., 2014), and are prevalent in North America, particularly in the western region although they are not as important as NFM. Although these avian mites are globally distributed, the predominant species varies per region.

The Republic of the Union of Myanmar (Myanmar) lies in the western region of mainland Southeast Asia. Poultry farms in Myanmar occur on the outskirts of urban areas, and the produce is mainly consumed in the cities. Although poultry farming is a major industry in Southeast Asia, including Myanmar, there are few reports concerning the distribution of avian mites. In this study, we captured mites from poultry farms at the outskirts of four large cities in Myanmar. We detected the hematophagous mites and classified the collected mite species, based on the morphological observations and nucleotide sequence analysis.

2. Materials and methods

2.1. Sample collection

We collected mites from twenty poultry farms around four large cities in Myanmar. iTraps-2 (Kondo Electronics Industry Co. Ltd., Osaka, Japan) were set up to capture chicken mites from the poultry houses. One to three days later, the iTraps-2 were collected and transferred to the laboratory at room temperature. The mites were captured from the poultry houses on twenty farms around the cities Mandalay (Ma 1–3), Pyin Oo Lwin (Py 1–5), Taunggyi (Ta 1–3), and Yangon (Ya 1–9).

2.2. Morphological observation

The mites were stored in 70% ethanol for a few days, followed by overnight incubation in 60% lactic acid at 45 °C. The mites were then gently washed with distilled water and transferred onto a small drop of Hoyer’s medium on microscope slides. The prepared specimens were observed using a light microscope.

Upon morphological observation, we focused on differences in the anal plates to distinguish the PRMs from NFM (Di Palma et al., 2012). The anal plate of NFM
is oval shaped, whereas PRMs have a triangle-shaped anal plate. In addition, NFM
have an open anus in front of the anal plate, whereas PRMs have the anus behind
the anal plate. NFM and TFM were distinguished based on the shape of dorsal plates
and the number of setae on the sternal plates (Denmark and Cromroy, 2003). The dor-
sal plate gets narrow toward the posterior end, and the shape of NFM is indicated
more sharply than the TFM. The setae on the sternal plate of NFM are present in
two pairs, while those on TFM are present in three pairs.

2.3. Nucleic acid extraction and cDNA synthesis

The mites were homogenized using a mortar and pestle in 200 μL of phosphate-
buffered saline, and total cellular DNA was extracted using QIAamp DNA Mini
Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions.
Samples were eluted in 200 μL of elution buffer and stored at -20 °C. For RNA
extraction, FastGene RNA Premium Kit (NIPPO Genetics Co. Ltd., Tokyo, Japan)
was used. After the chicken mites were homogenized in 350 μL of RL buffer, total
 cellular RNA was extracted according to the manufacturer’s instructions. cDNA was
synthesized using PrimeScriptTM Reverse Transcriptase (Takara Bio Inc., Shiga,
Japan), as directed by the manufacturer.

2.4. Polymerase chain reaction and sequence analysis

The DNA or cDNA samples were used as templates for polymerase chain reaction
(PCR). A part of the mitochondrial gene cytochrome c oxidase subunit I (COI) was
amplified by using the primers, RhipiCOIF (5’-CGA ATA AAT AAT ATA AGA
TTT TGA-3’) and TyphloCOIR (5’-GCT AAT CAA GAA AAA ATT TTA AT-
3’) (Roy et al., 2009). The PCR mixture contained 10 pmol of each primer, 1 U
of Takara Ex Taq (Takara Bio Inc.) and, 200 μM of each deoxynucleotide. The
PCR was performed with 1 cycle of 94 °C for 5 min for initial denaturation, followed
by 40 cycles of 94 °C for 30 sec, 52 °C for 30 sec, and, 72 °C for 1 min each. The
amplified fragments were separated on agarose gels (2.0%) and visualized under ul-
traviolet light after staining with ethidium bromide.

2.5. Sequence analysis

For sequencing analysis, the amplicons were purified using FastGene gel/PCR
extraction kit (NIPPO Genetics Co. Ltd.), cloned into T-vector pMD20 (Takara
Bio Inc.), and sequenced using GenomeLabTM GeXP Genetic Analysis System
(Beckman Coulter, Fullerton, CA, USA). The resulting nucleotide sequence of the
COI gene was aligned using MEGA software, version 7.0 (Kumar et al., 2016)
and the phylogenetic tree was constructed with the same software, using the neigh-
bour -joining method (Saitou and Nei, 1987).
3. Results

3.1. Detection of chicken mites in Myanmar poultry houses

The mites were detected from the poultry houses on six farms around Taunggyi (Ta-3), Mandalay (Ma-1), and Pyin Oo Lwin (Py-1,2,3,5) (Table 1). No mites were found in the poultry houses around Yangon (Table 1). In Py-1, village chickens were bred outside the poultry houses, and mites similar to those in the poultry houses of Py-1 were confirmed on the village chickens.

3.2. Classification of the mites by morphological observation

Only one specimen each was observed from Ma-1 and Py-3. These mites were lost during morphological analysis. Therefore, we analysed the morphology of chicken mites from four farms: Ta-3, Py-1,2,5. We prepared the mite specimens by sealing them in Hoyera’s medium after treatment with 60% lactic acid for taxonomic classification. NFMs/TFMs and PRMs were differentiated based on anal plate shape and anus location (Di Palma et al., 2012). The anal plates of NFMs/TFMs are oval, and PRMs have a distinct shape.

Table 1. Chicken mites detected in the poultry houses in Myanmar.

| Area and year                     | Farm ID | Capture by traps | Number of mites captured | Mite species¹) |
|-----------------------------------|---------|------------------|--------------------------|---------------|
| Taunggyi (December 2017)          | Ta-1    | -                | -                        | -             |
|                                   | Ta-2    | -                | -                        | -             |
|                                   | Ta-3    | +                | >30                      | TFM           |
| Mandalay (February 2018)          | Ma-1    | +                | 1                        | N.D.²)        |
|                                   | Ma-2    | -                | -                        | -             |
|                                   | Ma-3    | -                | -                        | -             |
| Pyin Oo Lwin (February 2018)      | Py-1    | +                | >30                      | NFM           |
|                                   | Py-2    | +                | 20–30                    | NFM           |
|                                   | Py-3    | +                | 1                        | N.D.²)        |
|                                   | Py-4    | -                | -                        | -             |
|                                   | Py-5    | +                | 1                        | NFM/TFM³)     |
| Yangon (May 2018)                 | Ya-1    | -                | -                        | -             |
|                                   | Ya-2    | -                | -                        | -             |
|                                   | Ya-3    | -                | -                        | -             |
|                                   | Ya-4    | -                | -                        | -             |
|                                   | Ya-5    | -                | -                        | -             |
|                                   | Ya-6    | -                | -                        | -             |
|                                   | Ya-7    | -                | -                        | -             |
|                                   | Ya-8    | -                | -                        | -             |
|                                   | Ya-9    | -                | -                        | -             |

NFM: Northern fowl mite; TFM: Tropical fowl mite.

¹) Mite species were determined based on morphological features and cytochrome c oxidase subunit I (COI) gene sequence.

²) N.D.: not determined. One mite was collected. However, the species was not determined because the mite was lost during analysis.

³) Mite morphology in Py-5 resembled NFMs and TFM, based on the features of the anal plate and the anus. However, the COI gene sequence was not determined because there were too few mites for the analysis.
whereas PRMs have a triangular anal plate. In addition, NFM/TFMs have an open anus in front of the anal plate, whereas the PRM anus is behind the anal plate. The anal plates of the mites from Py-1,2,5 were oval, and their anuses opened in front of the anal plates, suggesting that the mites from these three farms were NFM or TFM (Fig. 1A).

3.3. Comparison of the COI gene

Next, we determined the genetic characteristics COI, because the partial sequences of the COI genes of NFM and PRM are available in GenBank. We amplified and sequenced the COI gene from the mites from Ta-3, Py-1,2, because we were able to obtain approximately 10–30 chicken mites from each farm for DNA extraction. The COI gene from Py-1,2 showed 100% and 99% similarities, respectively, to NFM, whereas it showed a lower similarity to PRM (Table 2). However, the similarities in the COI gene from Ta-3 and NFM/PRM were lower. The phylogenetic analysis revealed that the mites from Ta-3 were relatively closer to NFM (Fig. 1B).

3.4. Sequencing and phylogenetic tree analysis

Since the mites in Ta-3 were not NFM or PRM, we asked if the mites in Ta-3 were TFM. By closely examining their morphology, we reconfirmed that the features of the anal plate were similar to those of NFM/TFM (Fig. 1C, b). Although the morphological features of TFM are similar to NFM, they can be distinguished based on dorsal plate shape and the number of setae on the sternal plates. The dorsal plate narrows posteriorly, and that of NFM narrows more sharply. The setae on the sternal plate of NFM are present in two pairs, while those on TFM occur in three pairs. The dorsal plates of the Ta-3 mites narrowed posteriorly (Fig. 1C, arrow), and three pairs of setae were present on the sternal plates (Fig. 1C, a). Thus, the Ta-3 mites is TFM.

4. Discussion

Hematophagous chicken mites are a major threat to the poultry industry. The prevalence of chicken mites varies from region to region. However, despite poultry farming being an important industry in Southeast Asia, the classification of mites affecting poultry in this region is lacking. Therefore, we sought to detect the mite species in Myanmar, which is in mainland Southeast Asia.

We found that NFM were observed in the poultry houses in and around of Py-1,2, and TFM were identified from Ta-3. PRM, however, were not collected from any of the farms. Thus, NFM and TFM may be the predominant species in Myanmar. TFM are prevalent in warm areas, such as tropical and temperate zones. (Denmark and Cromroy, 2003), whereas NFM are widely distributed in various areas (Murillo
Fig. 1. A. Morphological observation of mites from four farms in Myanmar: Pyin Oo Lwin (Py-1, Py-2, and Py-5) and Taunggyi (Ta-3). The mites were sealed in Hoyer’s medium after treatment with 60% lactic acid. The middle and lower panels represent the magnified images of the squares from the upper panel. The anal plate and the anus are indicated by the dotted lines on the lower panels. B. Phylogenetic analysis of the COI gene from the mite samples. The tree was constructed with MEGA software, version 7.0 (Kumar et al., 2016), using the neighbour-joining method (Saitou and Nei, 1987). The numbers next to the branches indicate the percentage of 1,000 bootstrap replicates. The scale bar indicates the number of nucleotide substitutions per site. C. Morphology of mites captured from Ta-3. The mites captured from...
The climate in Pyin Oo Lwin is relatively cool throughout the year, which may affect the prevalence of chicken mites.

Myanmar farmers often raise village chickens outside the poultry houses. We found that the mites on cage-free village chickens in Py-1 were similar to those in the Py-1 poultry houses. Therefore, mites that usually infest village chickens may get transmitted from village chickens to farmed chickens via equipment or humans. In addition, the mites may enter the poultry houses via birds and rodents, since the poultry houses in Myanmar are usually open-sided houses and there are no bird nets on most of the farms. Therefore, to prevent mite infestations, biosecurity should be improved by enacting safety measures like excluding wildlife from poultry farms.

Declarations

Author contribution statement

Masaki Takehara: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Shiro Murata: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ken Katakura, Saw Bawm: Conceived and designed the experiments; Performed the experiments.

Myint Myint Hmoon, Shwe Yee Win, Lat Lat Htun, Ye Htut Aung, Mar Mar Win: Performed the experiments.

Ta-3 were sealed in Hoyer’s medium after treatment with 60% lactic acid. The whole-body image of a mite captured from Ta-3. The dorsal plate gently narrowed posteriorly. The arrow indicates the dorsal plate. The centre and right panels represent the magnified images of the squares from the left panel. (a) The circles represent the pores of setae on the sternal plate. (b) The anal plate and anus are indicated by the dotted lines.

Table 2. Homology between the cytochrome c oxidase subunit I (COI) gene among the mites from each farm with Ornithonyssus sylviarum, northern fowl mites (NFM) and Dermanyssus gallinae, poultry red mites (PRM).

| Areas          | Farm ID/species | Homology with COI (%) |  |
|----------------|-----------------|------------------------|---|
|                | Py-1            | Py-2                   | NFM | PRM |
| Taunggyi       | Ta-3            | 84.0                   | 84.2 | 84.2 | 76.7 |
| Pyin Oo Lwin   | Py-1            | -                      | 99.8 | 100  | 78.0 |
| Pyin Oo Lwin   | Py-2            | -                      | -    | 99.8 | 77.8 |
| -              | NFM             | -                      | -    | -    | 78.0 |

NFM and PRM nucleotide sequences obtained from GenBank (NFM: KF218580; PRM: FN 650615).
Masayoshi Isezaki, Naoya Maekawa, Tomohiro Okagawa, Satoru Konnai, Sotaro Fujisawa: Analyzed and interpreted the data.

Kazuhiko Ohashi: Conceived and designed the experiments; Analyzed and interpreted the data.

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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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