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ORIGINAL ARTICLE

Occurrence and HAT-RAPD analysis of gastrointestinal helminths in domestic chickens (*Gallus gallus domesticus*) in Phayao province, northern Thailand

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Northern Thailand

Abstract The present study determined the prevalence and distribution of gastrointestinal helminths in domestic chickens (*Gallus gallus domesticus*) between November 2012 and August 2013. One hundred and twenty domestic chickens were purchased from villages in four districts of Phayao province; Mae Chai, Dok Khamtai, Chun and Chiang Kham. Morphological differences were used to identify the helminth species, and HAT-RAPD technique was used to differentiate among closely related species. The results revealed that the total prevalence of infection was 99.2%. Cestode and nematode infections showed the highest prevalence in rainy season, while trematode infections were low and only found in hot season. The species and their prevalence were: *Ascaridia galli* (50.8%), *Heterakis gallinarum* (86.7%), *Prosthogonimus macrorchis* (1.7%), *Echinostoma revolutum* (0.8%), *Raillietina echinobothrida* (48.3%), *Raillietina tetragona* (57.5%), *Raillietina cesticillus* (12.5%), *Raillietina* sp. (35.8%), *Cotugnia chiangmaii* (14.2%) and *Cotugnia* sp. (32.5%). The prevalence of helminth infections did not differ significantly between male and female chickens. HAT-RAPD analysis, the specific fragment of 400 and 250 bp indicated that *Raillietina* sp. and *Cotugnia* sp. found, respectively, differ from other closely related species. This study has confirmed that HAT-RAPD technique can be used to differentiate among related species combined with morphological observations.

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1. Introduction

In Thailand, the occurrence of gastrointestinal helminths in domestic chickens has been studied in central, north-eastern and southern areas (Sangvaranond, 1994; Kunchara Na Ayudthaya and Sangvaranond, 1993, 1997) but few studies are available in northern area. Phayao is a province in northern Thailand. Most of the people in rural areas of Phayao have animal husbandry. Domestic chickens are a common livestock for agro-farming, and are important for food consumption and commerce in this area. Studies on the occurrence of gastrointestinal helminth parasites in domestic chickens in Phayao province have not been performed.

For species identification, morphological differences are commonly used. However, it is difficult to identify the species level based on the morphology alone. Molecular approach is the most effective and accurate method for genetic characterization of such helminths. High annealing temperature-randomly amplified polymorphic DNA (HAT-RAPD) is a useful procedure to differentiate between closely related and morphologically indistinct species because high annealing temperature gives greater polymorphisms, reproducibility, and resolution (Anuntalabhonchai et al., 2000). This technique has been used successfully for detection and identification of numerous helminths including paramphistome flukes, *Haplorchis taichui*, *Stellantchasmus falcatus* (Wongsawad et al., 2009; Wongsawad and Wongsawad, 2010; Puttalakshmamma et al., 2014). The identification of some cestodes in domestic chicken using HAT-RAPD PCR has not been reported from Thailand.

Therefore, the objective of this study was to determine the prevalence and distribution of gastrointestinal helminth infections in domestic chickens from four districts in Phayao province in northern Thailand. Additionally, molecular analysis, HAT-RAPD technique was used to identify morphologically indistinct species among closely related groups combined with morphological characters.

2. Materials and methods

2.1. Study area and parasite collection

Four districts; Mae Chai, Dok Khamtai, Chun, and Chiang Kham in Phayao province were selected for this research (Fig. 1). These districts are located at an altitude of 300–1550 m above the sea level and mean annual rainfall is 1043.9 mm (high rainfall). The mean minimum and maximum temperatures are 10.8 °C in cool season and 39.5 °C in hot season.

Domestic chickens (n = 120, 64 females and 56 males) were purchased from chicken farms in the study areas. For helminth examination, the gastrointestinal tracts were divided into 8 sections: esophagus, crop, proventriculus, duodenum, jejunum, ileum, caeca, and rectum. They were opened by a longitudinal section from the esophagus down to the rectum, rinsed several times with tap water and finally rinsed with 0.85% NaCl. The gastrointestinal helminth recovered was morphologically observed using a light microscope. The species numbers were recorded for calculation of the prevalence and mean intensity of infections. For preparing permanent slides, the specimens were flattened and fixed in 4% formalin. For molecular analysis, the specimens were frozen at −20 °C for later DNA extraction.

2.2. Permanent slide and identification of helminths

The helminth recovered was prepared for morphological investigations. Trematodes and cestodes were stained with acetocarmine or hematoxylin, dehydrated with graded alcohol

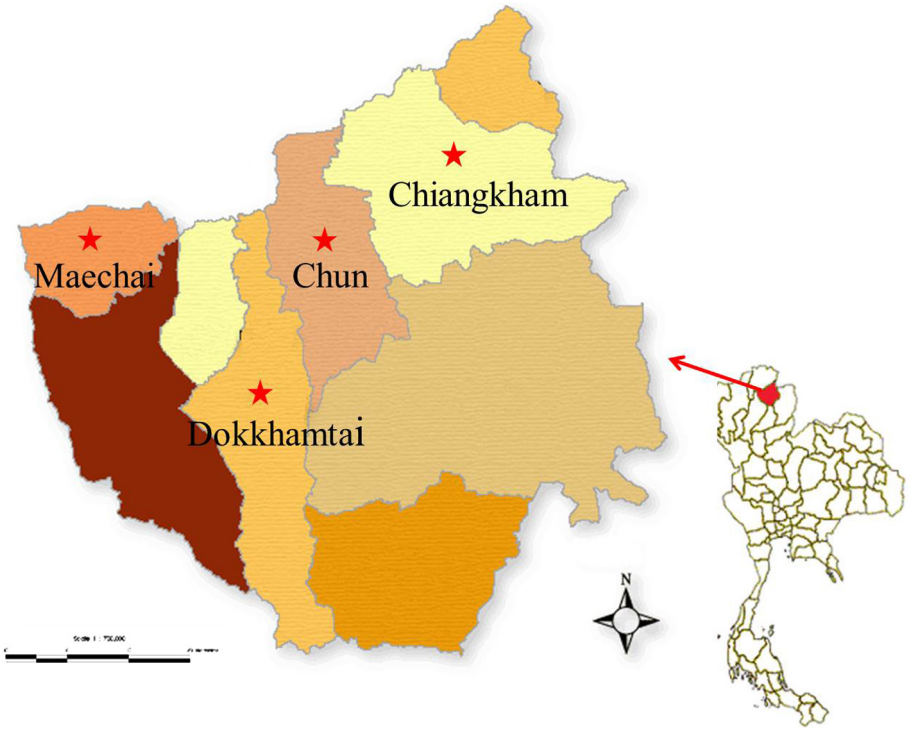

Figure 1 Four districts which were investigated for helminthic infections in domestic chickens (scale 1:700,000).