

SEROPREVALENCE OF TRICHINELLOSIS IN PIGS IN NORTHERN STATES OF MALAYSIA

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ABSTRACT. A seroprevalence study on the status of *Trichinella* infection in pigs was conducted by the Veterinary Research Institute, Malaysia. Of 442 pigs tested from Penang and Perak states, 2% were found to be positive by an ELISA test for antibodies to *Trichinella*. Further studies are required to determine the significance of these findings and the status of *Trichinella* infection in pigs across Malaysia. This includes epidemiological investigations where positive pigs were found, recovery of the parasite and identification to species or genotype, and increased sampling to represent the national population and distribution of pigs.

INTRODUCTION

Trichinella is a zoonotic nematode parasite comprised of 12 genotypes, 7 of which have been named as species (Pozio and Murrell, 2006). All species and genotypes can infect humans causing disease (trichinellosis, also known as trichinosis) ranging from asymptomatic to life-threatening. Although *Trichinella* can infect most mammals and a

few members of other classes of animals, pigs are considered the primary source of infection for humans, and *Trichinella spiralis* is most commonly implicated. The parasite spends almost its entire life as a first stage larva within muscle cells of hosts and must be eaten by a new host in order to continue its life cycle. After ingestion of infected meat, the larvae are released in the stomach and rapidly develop into adult worms in the intestine. Newborn larvae are produced for about a month and migrate to muscle cells, particularly in tissues such as the tongue, masseter and diaphragm, where they develop and remain infective for years. Determination of the species or genotype of *Trichinella* as well as local husbandry and abattoir practices, and culinary habits are important in elucidating the epidemiology of *Trichinella* and subsequently, the risk, if any, to consumers.

The outbreak of trichinellosis, presence of species of *Trichinella* as well as the food and eating habits of people affected in Asia are reviewed with emphasis on Japan, China and Thailand. *Trichinella* seems to be prevalent throughout this

region although outbreaks of trichinellosis have not been reported in some areas. Major outbreaks of the disease have been reported primarily in China and Thailand. This epidemic is the result of three factors. Firstly, China and Thailand are endemic areas for this particular parasite. Secondly, the two countries are well-organized and there is a public health system that enables precise reporting of disease outbreaks and finally, culinary habits provide many opportunities to eat undercooked meats. *Trichinella* found in Asia includes both encapsulated species (*Trichinella spiralis*, *Trichinella britovi*, *Trichinella nativa*) and noncapsulated species (*Trichinella pseudospiralis*, *Trichinella papuae*) (Poizio and Murrell, 2006). *T. britovi*, isolated in Japan, is a different genotype from the European strain. Therefore, the Japanese strain of *T. britovi* is designated as *Trichinella* T9. Economic losses due to *Trichinella* infection are not negligible in China, where there have been more than 500 outbreaks of human trichinellosis, affecting more than 20,000 people and causing more than 200 deaths. In Thailand, over the past 27 years, 120 outbreaks were reported involving nearly 6700 patients and 97 deaths. Japan has had fewer outbreaks and some sporadic cases have been attributed to imported infection (Gajadhar and Gamble, 2000).

Microscopy of compressed tissues and enzymatic digestion of muscle samples are the only practical methods available for the detection of *Trichinella* infection in meat. Several serological methods are

commercially available for the detection of antibodies to *Trichinella* in infected pigs or humans. The ELISA (Enzyme-Linked ImmunoSorbent Assay) is widely used for serological survey of pig populations and has also been adapted for use on meat juice. A review of direct and indirect methods for *Trichinella* detection, including molecular methods for genotyping has been recently published (Gajadhar *et al.*, 2009). A pilot study was performed in northern Malaysian pigs to determine if there was any serological evidence of porcine trichinellosis in the country. This report suggests a low prevalence which needs further investigation.

MATERIALS AND METHODS

The ELISA test (PrioCHECK®*Trichinella* Ab) detects antibodies against secretory (E/S) antigens of *Trichinella* spp in serum and meat juice samples of pigs. This test requires, serum samples from pigs and control reagents for the successful implementation of the test.

Samples

A total of 442 serum samples were obtained from 20 pig farms in the northern states of Perak and Penang (Table 1). The samples obtained from Perak originated from 115 sows and 109 porkers at 10 farms, while all 218 samples from Penang came from sows in 10 pig farms.

TABLE 1. Seroprevalence of *Trichinella* infection in pigs in two northern states of Malaysia as determined by ELISA.

State	No. Examined	No. Positive	Percentage (%)
Penang	218	0	0 %
Perak	224	9	4.0 %
Total	442	9	2.0%

Test

A commercial ELISA kit (PrioCHECK®) using the excretory/secretory antigens of *T. spiralis* was used to detect anti-*Trichinella* antibodies in serum samples according to the manufacturer's instructions (Prionics, Wagistrasse 27a, CH-8952, Schlieren-Zurich, Switzerland). Five ELISA plates were used and consisted of Positive Control, Weak Positive Control, Negative Control and 90 serum samples to be tested each. All solutions were derived from the test kits. All samples were conducted at dilutions of 1:200.

Validation criteria

All plates were required to pass validation criteria to ensure the reliability of the test. The criteria included the mean OD₄₅₀ of the Positive Controls which must be >1.0, the mean percentage of positivity of the weak positive control which must be >35%, and the mean OD₄₅₀ of the negative controls must be <0.2. If any criteria were not met, the results were invalid and the samples have to be retested.

Interpretation of OD readings

The cut-off level of OD reading used to determine positive results was established by dividing the OD of the sample by the OD of the positive control and then multiplying by one hundred.

Results obtained above or equal the cut-off of 15% positivity are considered positive while results obtained below the cut-off of 15% positivity are considered negative.

RESULTS

Of the 442 of serum samples tested for Trichinellosis nine were positive for antibodies to *Trichinella*. All nine positive samples originated from 224 animals from Perak.

DISCUSSION AND CONCLUSION

This is the first evidence of trichinellosis in pigs in Malaysia. Although there have been no previous surveys specifically for *Trichinella* in the country, we are not aware of any direct or indirect evidence of the parasite in humans, livestock or wildlife

in Malaysia. However, recent reports indicate that *Trichinella* occurs in South-East Asia and is a source of concern for food safety, human health and *Trichinella*-free pig production (Takahashi *et al.*, 2000). The seroprevalence of *Trichinella* infection in pigs has been reported at 4% for Thailand, 19.9% for Vietnam and up to 12% for China. The overall 2% prevalence revealed in the present study appears low, but is of considerable significance, as it indicates the occurrence of the parasite in the country even after a sub-population of the country's pigs in only 2 of 13 states. It is interesting that all positive samples originated from Perak and none from Penang. The distribution of the positive cases could indicate if bordering Thailand may be important as an extension of infected pigs from that region. For this reason, a survey of pigs from the states of Kedah, Kelantan and Perlis should be seen as a priority.

Considering the positive findings in this study, it is important to increase the number of pigs tested in Perak and include samples from all parts of the country to be statistically representative of the pig populations in the country as well as in each of the states. It is also important to determine the species or genotype of *Trichinella* present in Perak. *Trichinella* larvae recovered from tissue digestion assay in a farm trace-back epidemiological investigation are needed for this purpose. Furthermore, any rodents or other wild various animals near infected premises

should be investigated for any potential reservoirs of *Trichinella*.

The low seroprevalence of porcine trichinellosis in pigs in Malaysia needs to be confirmed by other methods and the survey expanded. Because false positive reactors in pigs raised outdoors are a possibility, it is essential to recover and identify muscle larvae in a follow-up study. Whether *Trichinella* was long established or recently introduced in Malaysia would be difficult to resolve. However, the lack of trichinellosis in humans in Malaysia is probably due to the culinary practices which require pork and pork products to be well cooked.

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