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Prevalence of Trichinellosis in Pork Meat at Slaughterhouse in Kupang City, East Nusa Tenggara Province

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Abstract: Trichinellosis is zoonotic diseases caused by infestation of nematode worms *Trichinella* spp and occurs worldwide. The prevalence of Trichinellosis in Indonesia is rare, even in the area of East Nusa Tenggara, Kupang, it is not particularly transparently reported. This study was aimed to observe the occurance of *Trichinella* spp in pork meat at slaughterhouse in Kupang City, East Nusa Tenggara Province. The research was conducted using cross sectional study involving 330 pork meat (muscles) samples which were slaughtered at the slaughterhouse in Kupang City. The meat samples were subjected to laboratory examination of *Trichinella* larvae using pooled sample digestion method (33 pooled samples of 330 muscle samples) whereas the positive results were then individually subjected to the compression method. The result showed that there were 5 pooled muscle samples were positive and by the compression method it was found 3 of 330 muscle samples (0.9%) containing larvae of *Trichinella spiralis*. The occurance of *Trichinella* in pigs slaughtered in Kupang could be a threat of human health.

Key words: Trichinellosis · Pooled Sample Digestion · Compression Method

INTRODUCTION

Trichinellosis is zoonotic disease that is caused by the nematode *Trichinella* spp. whose life cycle can take place in many different carnivorous and omnivorous animal species, including domestic pigs [1]. Throughout much of the world, *Trichinella* spp. have been found to be the causative agents of human trichinellosis, a disease that not only is a public health hazard by affecting human patients but also represents an economic problem in porcine animal production and food safety [2]. The occurrence of trichinellosis in humans is strictly related to cultural food practices, including the consumption of raw or undercooked meat of different animal origin. Therefore, most of the epidemiological data and surveys on *Trichinella* spp. in domestic or wild animals are related to outbreaks in humans [3].

Reports of cases of trichinellosis in the Southeast Asia region particularly Indonesia is very few. In Indonesia, trichinellosis has been documented ini 19.5 % of young people from Bali by serology [4] and in domestic swine from Tapanuli, the northern region of the island of Sumatra, where local customs of cooking or roasting meat greatly hinder the transmission to humans [5]. The etiology agent of trichinellosis in domestic pigs from Bali and Sumatra has never actually been identified, although it had been reported to be *Trichinella spiralis* [6]. In Malaysia, an outbreak of trichinellosis occurred in Singapura among 84 students and teachers who had visited a neighboring Malaysia Island in 1998 [7]. In Laos, a human outbreak of trichinellosis involving 51 people who had consumed pork was documented ini 1975. Trichinellosis is also present in domestic pigs from Myanmar [8], but the prevalence of infection in this animal species in unknown. Recent reports indicate

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that *Trichinella* occurs in South-East Asia and is a source of concern for food safety, human health and *Trichinella*-free pig production [9].

All species and genotypes can infect humans causing disease 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 populations of pigs in East Nusa Tenggara is 1724316 pigs, with a population in the city of Kupang has 25205 pigs [10]. Trichinellosis incidence is likely to occur and if found the incidence of trichinellosis will have an enormous impact on the sustainability of pig farming and become a public health threat in Kupang City.

The life cycle of *Trichinella* spp. relatively simple compared with a other parasites, but some patterns of transmission and prevalence rate has not been completely known. Epidemiological studies is very important and need to be done to develop a monitoring strategy in preventing transmission to humans. Research related to the identification and prevalence of *Trichinella* in pigs until now has not been reported in Indonesia, particularly for the city of Kupang, East Nusa Tenggara Province.

This research aimed to (1) identification of prevalence *Trichinella* spp. in pigs in Kupang City, (2) identify type of *Trichinella* nematodes in pig farms in Kupang City and use it as the first step to map in the East Nusa Tenggara region and as the reference for epidemiology study trichinellosis in the other region of East Nusa Tenggara Province.

MATERIALS AND METHODS

Sample Collection: The meat (masseter muscle) samples were obtained from Oeba slaughterhouse in Kupang City. A total of 330 muscle samples were collected during the period from July 2013 to April 2014. The muscle samples were examined in the Animal Health Laboratory of POLITANI Kupang for detection of *Trichinella* spp. larvae.

Design and Sample of Research: This study was a *cross-sectional* study. The muscle samples were taken by using simple random sampling. The size of samples was determined by using *software* of Win Episcope 2.0, with level of confidence of 95%, expected prevalence (estimated) of 30% and level of error of 5%, therefore, the total of muscle samples taken were 330 samples. The examination of muscle samples against larvae *Trichinella* spp. was conducted with the pooled sample digestion method and if the result is positive, individual samples were then identified by compression method.

Pooled Sample Digestion: The International Commision on Trichinellosis (ITC) recommended muscle sample of 5 g per pig for endemic area [11]. The examination of meat samples was done based on the protocol for artificial digestion technique (magnetic stirrer method) according to the EU Regulation no. 2075/2005 [12, 13]. A total of 10 muscles samples from 10 slaughtered pigs (each 5 g) were pooled. There were 33 pooled samples from 330 muscle samples. Each pooled sample was digested using 2 l of artificial digestive fluid consisting of 1% pepsin (1:10.000 US National Formulary) and 1% HCl. The digest was stirred for 30 min at a temperature of 44-46 °C in a 3 l beaker glass using a hot plate magnetic stirrer. During this process, the *Trichinae* are released from the muscle. The digestion fluid was then poured through a sieve (mesh size of 180 mm), which keeps back any undigested tissues, but allows the passage of *Trichinella* larvae, into a 2 l separatory funnel. Larvae were allowed to settle for 30 min and then a 40 ml of sample was quickly released into a 50 ml tube. After a further 10 min of sedimentation to clarify the suspension, 30 ml of supernatant was withdrawn. The remaining 10 ml of sediment was poured into a gridded petri dish. The 50 ml tube was rinsed with 10 ml of tap-water which was added to the petri dish. Subsequently, the sample in the petri dish was examined by stereomicroscope at various magnifications (low range, mid range and high range).

Compression Method: The muscle sample was cut as thin as possible into many pieces (each piece was as long as mucle fibers). The muscle pieces were compressed between two glass plates until they became translucent [13]. The muscle was examined individually to find out the morphology shape of *Trichinella* larvae whether it belonged to either *encapsulated* or *non-encapsulated* using stereomicroscope with various magnification (*low range*, *mid range* and *high range*).

Data Analysis: The data of laboratory was analyzed descriptively to determine the prevalence of trichinellosis at the slaughterhouse in Kupang City.

RESULTS

Pooled Sample Digestion with Magnetic Stirrer Method: *Trichinella* spp. larvae were found in 5 *pooled samples* of pig muscle (sample code of E, I, K, L and T). Microscopic description of *Trichinella* spp. larvae found was shown in Figure 1.

Compression Method: The results of examination using compression method of 50 individual samples (from 5 pooled samples were positive in the pooled sample digestion test) using stereomicroscope upon the presence of *encapsulated* and *non-encapsulated Trichinella* larvae showed 3 of 330 muscle samples containing the larvae of *T. Spiralis* (Table 1). The prevalence of *T. spiralis* in pig muscles was 0.9%. The description of morphological *Trichinella spiralis* larvae in pig muscles in Figure 2.

DISCUSSION

Since 1970, various methods for artificial digestion have been introduced which allow the examination of a pool of muscle samples from up to 100 carcasses. Although the digestion method requires more technical equipment, it meets the requirements for efficiency, reliability and cost effectiveness, consequently it has become the method of choice for routine slaughter inspection in most industrialised countries [14]. The main principle of artificial digestion methods is that muscle larvae are released after digestion of the muscle tissue by means of artificial digestion fluid composed of pepsin and hydrochloric acid. Such digestion methods are used for individual or pooled muscle samples, followed by selective screening, filtration,or sedimentation procedures and a final microscopic examination for the presence of

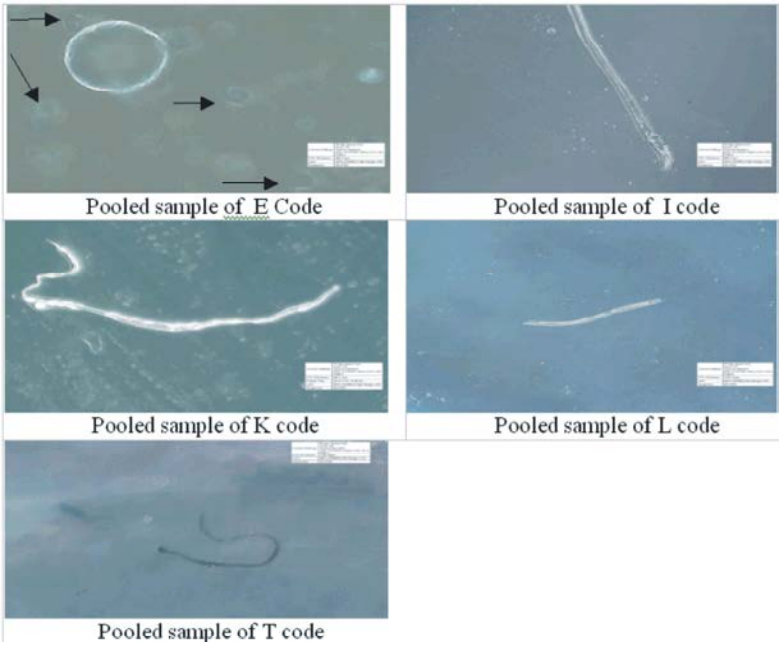

Fig 1:*Trichinella* larvae in samples of pig muscle from slaughterhouse in Kupang City

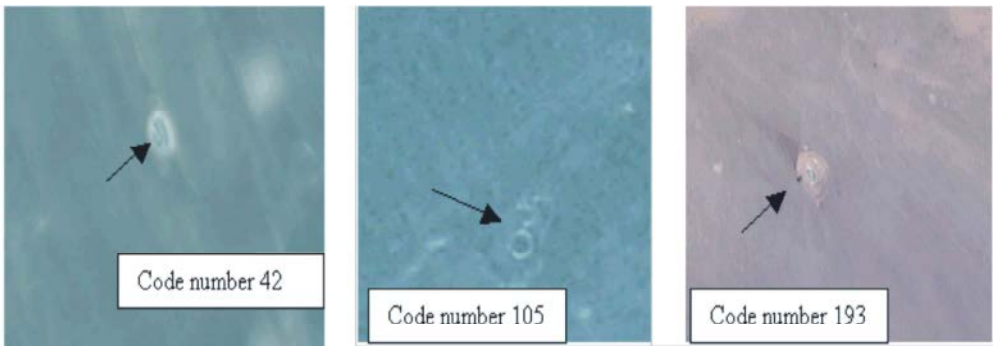

Fig 2:*Trichinella spiralis* larvae in pig muscles using compression method

Table 1: Positive pork muscle samples subjected to digestion and compression method

Code number	Date of sampling (M/D/Y)	Breed of pigs	Age/sex of pigs
42	11/16/2013	Local	Above 1.5 years/male
105	12/08/2013	Exotic	Above 3 years/female
193	01/18/2014	Triple cross	Above 3 years/female

larvae [15]. The efficacy of digestion tests may vary because digestibility depends on muscle types and animal species. For routine *Trichinella* meat inspection in pigs, the time for digestion not more than 30 min at 44°C to 46°C [16]. The magnetic stirrer method is considered the gold standard because it is a method specifically designed for pooled samples and it has been subjected to validation studies [16]. This widely used magnetic stirrer method for pooled samples can be employed in a variety of circumstances with a minimum of equipment [15].

As early as the 1860s, microscopy and later trichinoscopy or compression method, were introduced in slaughterhouses for systematic inspection of pigs for *Trichinella* larvae [13]. However, it is a laborious method for the inspection of individual carcasses and it is time-consuming. After slaughter, muscle tissue is collected from each of prescribed predilection sites (e.g. maseter or diaphragm crus in pigs).

Therefore this study design was reliable to represent the prevalence of slaughtered pigs in the Kupang City, East Nusa Tenggara Province. This study was the first study for the detection of *Trichinella* larvae in maseter muscles. Identification *Trichinella* larvae is important and necessary to develop a monitoring strategy in preventing transmission to humans. The pooled sample digestion with magnetic stirrer method followed by the compression method had be a success to detect *Trichinella* larvae from meat samples 5 g muscle per pig was used. The prevalence of *T. spiralis* in pig muscles at Oeba slaughterhouse in kupang city was 0.9%.

The occurance of *Trichinella spiralis* in pigs at Oeba slaughterhouse in Kupang City was higher comparing to its occurance in pig diaphragms collected from slaughterhouse in the northern of Thailand (0.005%) and in Chiang Mai (0.01%) [9]. This prevalence in Kupang City was supposed to be related with traditional farming practices, feeding pigs with home or restaurants garbage and lack of farmers' knowledge on trichinellosis. The management practices of farms are associated with the occurrence of *Trichinella* [17]. Pigs can only become infected with *Trichinella* by ingesting raw or undercooked meat containing infective larvae [18]. Kitchen waste, which is not heat treated properly is a risk factor for infection [19]. Kitchen waste and offal should be sterilized before feeding to pigs. *Trichinella* positive meat should not be delivered to the market unless it is well cooked. Similarly, intensive pig farming with adoption of proper biosecurity measures is advocated to prevent the transmission of *Trichinella* [20].

CONCLUSION

The prevalence of *Trichinella spiralis* in pork meat at the slaughterhouse in Kupang City was 0.9% which was mostly found in female pigs. This occurance of *Trichinella spiralis* should be considered as a threat to consumer health.

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