Slaughterhouse survey of *Trichinella* infections in pigs of Tanzania

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SUMMARY

Trichinellosis is ahuman zoonotic disease caused by larval and adult stages of parasitic nematodes belonging to the genus *Trichinella*. The parasite has a wide range of host species, mostly mammals. Humans acquire the infection by eating raw or inadequately cooked meat infected by larvae present in the muscle cells. Worldwide, the most common sources of human infections are pig, wild boar and other game meat. In Tanzania, a human outbreak with several deaths occurred for the consumption of warthog meat in 1977. *Trichinella nelsoni* has been documented in carnivores and warthogs of Serengeti. A slaughterhouse survey was conducted in five regions of Tanzania to determine the prevalence of the nematode in domestic pigs slaughtered for human consumption in the framework of an OIE Twinning project. At least five grams of diaphragm muscle was taken from each sampled carcass. A total of 1,078 adult pigs were randomly sampled in Arusha (163), Dar es Salaam (291), Dodoma (236), Iringa (297), and Kilimanjaro (91). Magnetic stirrer method was used to digest the muscles for 30 minutes at 44 - 46^{0}C, and the digested liquid was left to sediment for another 30 minutes. The sediment was examined under a dissecting microscope at magnification of x15. No *Trichinella* larva was detected. This result suggests that the prevalence of *Trichinella* infection in domestic pigs of Tanzania if any is lower than1%. From these findings it can be concluded that the pork entering the food chain at the time of this study was safe from *Trichinella* infections.

Keywords: Magnetic Stirrer Technique, meatborneinfection, *Trichinella* larvae, Zoonosis

INTRODUCTION

Trichinellosis is a human zoonotic disease caused by larval and adult stages of parasitic nematodes belonging to the genus *Trichinella*. The parasite has a wide range of host species in the following order of importance carnivores followed by omnivores among which we have to include horses (Gottstein *et al.*, 2009). Infected animals do not show any clinical signs (hence the condition termed as *Trichinella* infections), on the opposite, when humans acquire the infection, clinical symptoms are observed though not pathognomonic, the symptoms can be severe and the disease is known as trichinellosis. Death is rare. The symptoms of trichinellosis in human being include fever, muscle pain, severe headache, chills, excessive sweating, facial oedema mainly periorbital, transient dizziness, nausea, and with or without diarrhoea (Dupouy-Camet and Bruschi, 2007). Humans acquire the infection by eating raw or inadequately cooked meat infected by *Trichinella* larvae present in the muscle cells. Worldwide, the most common sources of human infections are domestic and wild swine, carnivorous game meat, and horse meat. In Tanzania, a human outbreak with several deaths occurred in Serengeti in 1977 after consumption of warthog meat.

In animals, *Trichinella* infection is diagnosed by using direct methods to detect the presence of larvae in the muscles, on the contrary, in human diagnosis is conducted by detecting ant-*Trichinella* antibody (I-gG) in serum or plasma by serology (Gomez-Morales *et al.*, 2012). The most common serologicalmethod in human is Enzyme Linked Immunosorbent Assay (ELISA), which is frequently complemented with Western Blotting (Dupouy-Camet and Bruschi, 2007). Pozio *et al.* (1997) investigated 56 tissue samples of nine carnivore species in the Serengeti ecosystem, which demonstrated the presence of *Trichinella nelsoni* in five species of wild carnivores namely bat-eared fox (*Otocyon megalotis*), cheetah (*Acinonyx jubatus*), leopard (*Panthera pardus*), lion (*Panthera leo*), and spotted hyena (*Crocuta crocuta*). The prevalence of infection was highest in spotted hyenas, 3 out of 13 equivalent to (23%) compared to 3 out of 25 (12%) in lions. However, the numbers of carcasses of other species were too small to provide meaningful calculation of prevalence. In the recent study, *T. nelsoni* has been reported to infect leopard in Kruger National Park (KNP) and neighbouring game reserves collectively known as the greater KNP area in South Africa (La Grange *et al.*, 2014). A review made by Mukaratirwa *et al*. (2013) shows that in the sub-Saharan region four *Trichinella* species have been isolated namely *T. britovi* (in Guinea), *T.*

nelsoni (Kenya, Tanzania, and South Africa), *T. zimbabwensis* (Ethiopia, Mozambique; South Africa and Zimbabwe), and one genotype (*Trichinella* T8) in Namibia and South Africa. The review further shows that *Trichinella* infections in the region are common in carnivores (mammals and reptiles) and to a lesser extent in omnivores. These findings demonstrate that wild carnivores are the reservoirs of *Trichinella* spp. and that they may well form an important source of infections to domestic pigs in the livestock-wildlife interfaces.

Tanzania has about 1,581,396 pigs across the country, and 95% of those are kept in the smallholder backyard and some practising free-ranging system especially in the time of post harvest of crops. Most farmers have 2-8 pigs but with a range of 2 to 48 pigs per farm. Large farms in Tanzania are those keeping 100 pigs and above and there 108 farms in the country with a total of 80,316 pigs (NBS, 2012; MLFD, 2013). The free-range management system predisposes the pigs to come in contact with thrown away flesh from game meat sold in livestock-wildlife interface areas. The objective of this study therefore was to investigate the presence of *Trichinella* larvae in the pigs of Tanzania that have reached the human food chain.

MATERIALS AND METHODS

Study area and period

The surveillance study was conducted in four zones where pig production and/or pork consumption is relatively high in the country. The zones included Eastern zone (Dar es Salaam region); Central zone (Dodoma region); Northern zone (Arusha and Kilimanjaro regions); and Southern Highland Zone (Iringa region). The study based on abattoir slaughters. Arusha region included two districts namely Arusha City and Arusha District Council; Dar es Salaam region included three municipal councils namely Ilala, Kinondoni and Temeke; Dodoma region included Kongwa district; Iringa region included Iringa municipal council; and Kilimanjaro included Moshi urban district. The abattoir survey was conducted from October 2015 to September 2016. A total of 14 slaughter slabs were visited including Arusha (2), Kilimanjaro (1), Dar es Salaam (8), Dodoma (1), and Iringa (2) (Figure 1). Twelve of those were privately owned, whereas only two, all in Iringa, were public facilities.

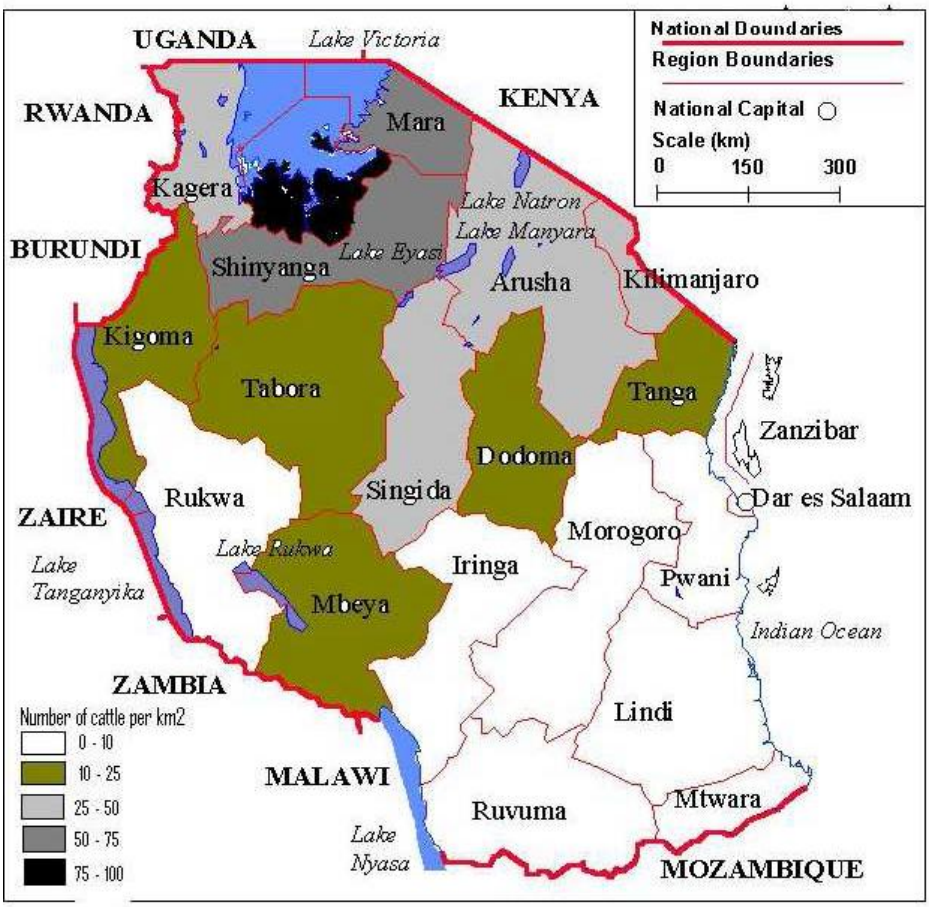

Figure 1. A map of Tanzania showing study areas

Target animals

In the sylvatic cycle, wild carnivores are the main reservoirs of *Trichinella* infections through their predation feeding, scavenging, and carrion feeding. In this regard, wild carnivores from national parks or game reserves were target of the surveillance. Samples from wildlife were expected from dead animals through road kills/accidents, diseases, or famers fighting back predators in their farms.

In this study, domestic pigs preferably adult free-ranging pigs were the main focus. In this regard, the sampling was conducted in slaughter slabs where 3-20 pigs are slaughtered 1-2 times per week. Adult pigs of over nine months of age were randomly selected for sampling. Pigs originating from intensive management system were excluded from the study.

Sample collection, storage and preservation

At least five-gram of meat muscles were taken from the diaphragm of domestic pigs. At least one gram was intended to run in each pool, which was up to 100 g.

The site of cut was mainly the pillar of the diaphragm at the transition to the sinewy part though masseter muscles and the tongue are equally suitable sites. Fat and fascia were dissected from the muscles before digestion. Fresh samples from Dar es Salaam slaughter facilities were examined on the same day or stored in a refrigerator until the following day. However, samples from Arusha, Dodoma, Iringa and Kilimanjaro were preserved in 0.1% merthiolate until examination.

The technique

During sample processing, the Magnetic Stirrer Technique (MST) for the diagnosis of *Trichinella* infections in tissue was used. Samples preserved in 0.1% merthiolate were washed in tap water before a sub-sample was taken for a pool. Fresh pig tissues were chopped to obtain a 1g from each individual pig. A complete pool had 51 to 100 g, whereas pools containing 15 to 50 g were processed separately as half pool protocol. The procedure was as follows:

a) Two litres of tap water was added in a 3 L capacity beaker and preheated to temperature between 46^0C and 48^0C
b) 10.8± 0.2 ml of 37% hydrochloric acid was added into a 3 L beaker containing 2 L of tap water, preheated between 46°C to 48°C; a stirring rod was placed in a beaker, the beaker was placed on the preheated plate and the stirring started;
c) 10 ± 0.2 g of Pepsin was added to the 2 L tap water beaker (1:10,000 **NF);**
d) A pool of 100 g of pig muscles or depending to number of animals was chopped in the blender;
e) Chopped meat was transferred into a 3L beaker containing preheated water, Pepsin and hydrochloric acid;
f) The mincing insert of the blender was immersed repeatedly in the digestion fluid in the beaker and blender bowl was rinsed with small quantity of digestion fluid to remove any meat adhering on it;
g) The beaker was covered with aluminium foil;
h) The temperature of the Magnetic stirrer was adjusted so that it maintains a constant temperature of 44°C to 46°C throughout the operation. During stirring, the digestion fluid was allowed to rotate at a sufficiently high speed to create a deep whirl without splashing;
i) The digestion fluid was stirred until the meat particles disappeared (approx. 30 min). Then the stirrer was switched off and the digestion fluid poured through the standard sieve into a sedimentation funnel. Longer digestion time at times was extended but not exceeding 60 minutes;
j) Weighing the undigested tissue. The digestion process was considered satisfactory if less than 5% of the starting sample weight remained on the sieve;
k) The digestion fluid was allowed to stand in the separatory funnel for 30 min;
l) After 30 min, a 40 ml sample digestion fluid was quickly run into a Falcon tube;
m) The digestion fluid and other liquid waste were kept in tray/ sedimentation funnel until reading of the results was completed;

Sample size

The sample size was pre-determined by simple random sampling (SRS) formula given by Thrusfield (2007), which is $n=Z^2PQ/L^2$. The value of Z score is 1.96 at 95% Confidence level; P is estimated prevalence, in this case the prevalence is not known therefore in order to have a maximum sample size the value was set at 50%; Q is equal to 100%-P = 50%; L is pre-set error rate, and in this case was set at 3%; and n= the sample required. Therefore, the sample size (n) was 1,067. This number was sufficient owing to the low number of pigs slaughtered from each slaughter slab per day, the budget allocated for the sampling, the distance required to travel and get the samples in the correct manner.

n) The 40 ml fluid was allowed to stand for 10min, thereafter 30 ml of the supernatant was carefully withdrawn by suction or decanted to remove the upper layer and leave a volume of not more than 10 ml;
o) The remaining 10 ml sample of the sediment was poured into a larval counting basin or engraved Petri dish;
p) The Falcon tube was rinsed with not more than 10ml of tap water, which had to be added to the sample in the larval counting basin or Petri dish. The sample was examined by stereo microscope at a 15 to 20 times magnification;
q) All digests were examined as soon as they were ready and under no circumstances examination was postponed until the following day.

Note: Minimum weight was 15 g whereas the maximum was 115 g for a pool. Pools between 15 and 50 g the digestion fluid and the ingredients were reduced to 1L of tap water, 5.4 ml of 37% hydrochloric acid, and 5g of Pepsin. On the other hand, pools with weight between 51 and 115 g were treated as 100 g pool.

RESULTS

From the above study, a total of 1,078 samples were collected and examined from the pre-determined four zones in Tanzania. Examination of digested samples revealed that all samples were negative. Unfortunately, no sample was obtained from the wildlife sector. The distribution of samples across the study areas is shown in Table 1.

Table 1. Sampled diaphragm muscles from domestic pig in Tanzania from 2015 to 2016.

District/ Slaughter facility*	Central Zone/Dodoma	Eastern Zone/ D'Salaam	Northern Zone/Arusha and Kilimanjaro		Southern Highland/ Iringa	Total
			Arusha	Kilimanja		
Arusha City						
Sanawari*			130			130
Arusha Council						
Sakina*			33			33
Ilala						
Karakata*		4				4
Pondi*		30				30
Sitakishari*		2				2
Ukonga*		4				4
Iringa Municipal						
Ipogolo*					103	103
Kihesa*					194	194
Kinondoni						
Kimara*		143				143
Kwa Urasa*		7				7
Ubungo*		53				53
Kongwa						
Kibaigwa*	236					236
Moshi Urban						
Kiborloni*				91		91
Temeke						
Temeke*		48				48
TOTAL	236	291	163	91	297	1,078

Note: * is the name of the slaughter facility

DISCUSSION

Trichinella infection is an infection of wide range of animals ranging from mammals, reptiles to birds. *Trichinella* infections have been observed in a wide range of animals across the world. In this regard, it is imperative to note that no area can be considered a free zone for this zoonotic helminth.

From the results of this study, it was noted that all pig carcasses examined were free from *Trichinella* infections, however this could not rule out the presence of *Trichinella* infections in some areas, which were not included in the study. In a previous study (Pozio *et al.,* 1997), *T. nelsoni* was detected in hyenas, lions, leopard, cheetah, and bat-eared fox out of 56 wild carnivores from the Serengeti ecosystem. It may well be that failure to obtain muscles from wildlife carnivores in this study may have contributed to failure to isolate the helminth parasite. In this case, more study is recommended to establish the threat in wild animals. Furthermore, in 1977 in the Serengeti area, a number of people were infected with *Trichinella* after eating warthog meat.

In conclusion, we can say that even if the prevalence of *Trichinella* infections could be lower than 1% in domestic pigs of the investigated areas of Tanzania, the Tanzanian population could be at risk if raw pork is consumed and if the pig population will increase as expected.

REFERENCES

Dupouy-Camet J, Bruschi F. Management and diagnosis of human trichinellosis. In: FAO/WHO/OIE guidelines for the surveillance, management, prevention, and control of trichinellosis,edDupouy-Camet J, Murrell KD. FAO/WHO/OIE, Paris, pp 37-68, 2007.

Gomez-Morales MA, Ludovisi A, Amati M, Blaga R, Zivojinovic M, Ribicich M, Pozio E. A distinctive Western blot pattern to recognise Trichinella infections in humans and pigs. *Intern J of Parasitol* 42: 1017-1023,2012.

Gottstein B, Pozio E, andNockler K. Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clin Microbiol Rev* 22: 127-145, 2009.

La Grange LJ, Reininghaus B, and Mukaratirwa S. First report of a mixed infection of *Trichinella nelsoni* and *Trichinella* T8 in a leopard (*Pantherapardus*) from the greater Kruger National Park, South Africa, 2014.

MLFD (Ministry of Livestock and Fisheries Development. Basic data for Livestock and Fisheries Sectors Tanzania Mainland, Annual report, 2013

Mukaratirwa S, La Grange LJ, Pfukenyi D. *Trichinella* infections in animals and humans in sub-Saharan Africa: A Review. *Acta Trop* 125: 82-89, 2013.

National Bureau of Statistics (NBS). National Sample Census: Livestock Data, 2012.

Pozio E, De Meneghi D, Roelke-Praker ME, and La Rosa G. *Trichinellanelsoni* in carnivores from the Serengeti ecosystem, Tanzania. *JParasitol* 83 (6): 1195-1198(1997).

Thrusfield M. Veterinary Epidemiology.Wiley-Blackwell, 2007. ISBN: 978-1-4051-5627-1