The probable role of cannibalism in spreading *T. papuae* infection in a crocodile farm in Papua New Guinea

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Abstract

Between 2003 and 2007, 83 (50%) of 167 crocodiles (*Crocodylus porosus*) purchased as juveniles by a crocodile farm 3 or 4 years earlier from Kikori, Gulf Province, were found to be infected with *Trichinella papuae*. Between 2005 and 2007 infection was detected in a number of crocodiles at the farm obtained from six localities other than Kikori, as well as in a few animals born on the farm. Up to 2004, all juveniles at the farm, whether wild- or farm-born, were penned together; the practice was then stopped to prevent possible infection through cannibalism. The last infected animal from Kikori was seen in 2007, 4 years after the purchase of crocodiles from there ceased. The last non-Kikori infected crocodile was seen, also, in 2007. None of the 1,972 crocodiles (comprising wild- and farm-born animals) tested from 2008 to 2013, using the digestion method, was infected with *T. papuae*. This indicates that infection of non-Kikori crocodiles was the result of cannibalism within the farm during the years up to 2004 when juvenile crocodiles were kept together, and that the farm is now free of the infection.

Keywords: *Trichinella papuae*, *Crocodylus porosus*, cannibalism, crocodile farm management

1. Introduction

*Trichinella* was known only from warm-blooded animals until a new non-encapsulated species, *T. zimbabwensis* was reported in crocodiles (*Crocodylus niloticus*) in Zimbabwe in 1995 (Pozio et al. 2002).

Another non-encapsulated species, *T. papuae*, first discovered in pigs in Papua New Guinea (PNG) (Pozio...
et al. 1999), was later (2003) found infecting saltwater crocodiles (*Crocodylus porosus*) from one locality in PNG, Kikori, (Pozio et al. 2004). It has been implicated in cases of trichinellosis in humans in Thailand (Khumjui et al., 2008; Kusotsuk et al., 2010), and there is serological evidence of human infection in PNG (Owen et al., 2005).

*Trichinella* cycles in nature through host scavenging or cannibalism. Prior to the discovery of infection in crocodiles, recycled raw crocodile meat was known to be used as crocodile feed, a habit that could potentially perpetuate infection in crocodile farms (Mukaratirwa & Foggin, 1999). In PNG, saltwater crocodiles were naturally infected with *T. papuae* as a result of feeding young captured crocodiles with infected wild pig meat while they were penned before sale to a crocodile farm (Pozio et al., 2005).

PNG has one large commercial crocodile farm that holds about 30,000 *C. porosus* obtained through its own breeding programme and by purchasing eggs and young crocodiles from rural villagers under a Sustainable Management Scheme. The young, wild-born crocodiles measure about 45cm when bought and require 3 to 4 years at the farm to attain a size suitable for sale of skins. As the farm exports meat for human consumption as well as skins, quarantine authorities require testing of meat for *Trichinella*. This began in late 2002.

The crocodile farm bought wild-born animals from about 31 localities in lowland PNG between 2002 and 2012, although purchases were predominantly from Ambunti, Angoram and Wewak (East Sepik Province), Popondetta (Oro Province), Kimbe (West New Britain Province) and Kikori (Gulf Province) (Fig. 1). The farm ceased buying young crocodiles from Kikori in 2003, as soon as animals from there were found to be infected with *T. papuae*.

In 2005 *T. papuae* infection was detected in farm-born as well as crocodiles from locations other than Kikori held at the farm. The discovery prompted an investigation to determine the source and mode of transmission.
2. Materials and methods

Quarantine protocols required testing of meat from 10% of animals at each quarterly cull. Samples (2.5g each) of masseter, intercostal, biceps and tail muscle were pooled (10g) from individual crocodiles and tested separately, using the standard HCl/pepsin digestion method (Gamble et al., 2000). The work was carried out by trained personnel at the National Veterinary Laboratory, National Agriculture Quarantine and Inspection Authority (NAQIA), PNG.

The proportions of wild-born and farm-born crocodiles, and the proportions of male and female wild-born crocodiles that were infected with *Trichinella* and the 95% binomial confidence intervals were determined. The statistical significance of the difference between the prevalence of infection in male and female crocodiles was determined using the Chi-square test with a 95% confidence limit. The probability that the farm-born crocodiles present on the farm were free from infection was determined using the total number of farm-born individuals tested (1,618) and all individuals tested (1,972) from 2008-2013 as the sample sizes. Analysis was performed using Freecalc (Cameron et al., 1998) with assumptions of 70% and 100% for the sensitivity and specificity of the artificial digestion method employed (Gamble et al., 2000), respectively, with a 95% confidence limit. The design prevalence for proof of freedom was set at 0.3%.

3. Results

The results from testing crocodiles for infection with *Trichinella* are presented in Table 1. A total of 3,268 crocodiles were tested between the last few months of 2002 and the first seven months of 2013. They consisted of 1,065 wild-born (518 males and 547 females) and 2,203 farm-born animals (1,267 males and 931 females, plus 5 with no indication of sex).

The first crocodile found to be infected with *T. papuae* (5.0 larvae per g) was in the second farm cull of 2003; it arrived at the farm from Kikori in 2000. Between 2003 and 2007, 167 crocodiles originating from
Kikori were tested on reaching a size suitable for slaughter, 83 (50%) being positive for *Trichinella*. More male than female crocodiles (98:69) were tested, with 54.1% of males and 43.5% of females being positive.

In 2005 a few crocodiles (numbers in brackets) originating from Ambunti (4), Angoram (1), Murik Lakes (1), Wewak (4), Popondetta (1) and Kimbe (1) tested positive, as well as some (5) born on the farm. A further few were positive in 2006 and 2007; Ambunti (1), Timbunke (1), Popondetta (2) and farm-born (2) (Fig. 1). No crocodile tested positive for *Trichinella after 2007*.

There was a significantly greater proportion of wild-born male crocodiles infected with *Trichinella* compared to wild-born female crocodiles 9.5% (95% CIs: 7.3-12) and 5.2% (95% CIs: 3.7-7.1) respectively ($p<0.05$).

The probability of observing no infected crocodiles in a sample of 1,618 farm-born crocodiles and 1,972 farm- and wild-born crocodiles (total slaughtered from 2008 onwards), from a population with a prevalence of *Trichinella* infection of 0.3%, was 0.033 and 0.00 respectively.

4. Discussion and conclusion

The discovery of infection in a few crocodiles born on the farm, as well as in crocodiles from localities other than Kikori, presented a potential problem for the farm because it suggested that infection was occurring within the farm. All crocodiles at the farm, both purchased and farm-born, are fed exclusively on fresh poultry offal from the neighbouring chicken processing plant. Infection was not acquired from this food because *T. papuae* is not infective to chickens (La Rosa et al., 2001). Feeding of animals with recycled raw crocodile meat has never been practiced on the farm.

As animals take 3 to 4 years at the farm to attain a suitable commercial size, and the farm stopped purchasing crocodiles from Kikori in 2003, the last crocodile on the farm from Kikori was killed in 2007. It was also the year when the last crocodile from any source was found to be positive for *T. papuae*. This
means that the last non-Kikori crocodile tested positive four years after the last animal was purchased from Kikori. It also means that the first non-Kikori animals to test positive in 2005 would have been infected in 2001 or 2002, before testing had started.

It was the practice at the farm up to sometime in 2004 for all juvenile crocodiles, wherever they came from, to be placed together in the same pens and for them to remain ‘mixed’ for two or more years. Although individually tagged, neither their numbers nor health status were monitored regularly. Young crocodiles, therefore, continued to be ‘mixed’ during 2003, when purchase of Kikori crocodiles ceased, and for part of 2004. It is significant that no crocodile, although in ‘mixed’ company as a juvenile during 2004, was found infected with *Trichinella* when killed 3 or 4 years later.

With the propensity of even young crocodiles for aggression, it is common knowledge that weak or sick individuals can be attacked and eaten; Huchzermeyer (2003) states “cannibalism is a normal occurrence in crocodiles”. The observation that a higher proportion of male crocodiles was infected with *Trichinella* compared to females is not unexpected, if juvenile males have the same tendency as adults to be more aggressive compared to females.

The information available suggests strongly that infection within the farm was spread through cannibalism through eating the flesh of juveniles that died or by killing and eating live animals as a result of the now discontinued practice of housing farm-born and village-acquired crocodiles together. The small numbers of infected wild- and farm-born non-Kikori, crocodiles, and the scattered original locations of the infected wild-born animals, would appear to support this explanation. Furthermore, the period of infection of these non-Kikori animals can be linked to the time when young Kikori crocodiles were brought into the farm and the subsequent lack of infection in later years following cessation of Kikori purchases. Cannibalism probably took place soon after arrival at the farm, as newly purchased
animals sometimes fail to adapt to the new environment, becoming weak and vulnerable to attack by stronger individuals.

Purchase of young crocodiles from Kikori had been taking place for many years prior to the discovery of infection in 2003. But finding no infection amongst non-Kikori crocodiles during 2003 and 2004, suggests that, although some Kikori crocodiles bought in 1999 or 2000 were infected – as shown by positive results in those killed in 2003 and 2004 - there is no evidence that cannibalism of infected animals occurred on the farm in those years. There is no means of ascertaining if infection was present in earlier years.

The failure to detect any infected animals in the farm- and wild-born crocodiles tested from 2008 onwards is sufficient evidence to prove that the farm population is free from *Trichinella* infection at a design prevalence of 0.3%.

This observation has been confirmed statistically using Freecalc, which calculates the exact probability of detecting infected animals in a population given the sensitivity and specificity of the tests used, the population size and design prevalence (lowest level detectable). The design prevalence in this case was set at a level below which transmission would not be sustained. This can be attributed to crocodiles no longer being purchased from Kikori since 2003 and the cessation since 2004 of the practice of placing all young animals together, irrespective of their place of origin.

**Conflict of interest statement**

Two of the authors, E.L. and W.S., are employed by the crocodile farm.

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Fig. 1. Map of Papua New Guinea indicating coastal provinces and locations mentioned in the text, and including the number of crocodiles that tested positive for Trichinella papuae, together with the number of crocodiles tested from each locality or source, during the period 2002 to 2013.

Note: ★ - indicates location of crocodile farm.
Table 1. The proportion (%) of wild-born and farm-born crocodiles from Mainland Holdings Crocodile Farm, PNG, that were infected with *Trichinella papuae* from 2002-2013 (95% confidence intervals in parentheses).

| Year | Wild-born animals | | Farm-born animals | |
|------|------------------|--|--|------------------|--|
|      | No. test | No. pos | % pos (95% CIs) | No. Test | No. pos | % pos (95% CIs) |
| 2003 | 69       | 16      | 23.2 (13.9, 34.9) | 30*      | 0       | 0 |
| 2004 | 116      | 37      | 31.2 (23.6, 41.2) | 31       | 0       | 0 |
| 2005 | 222      | 39      | 17.6 (12.8, 23.2) | 109      | 5       | 4.6 (1.5, 10.4) |
| 2006 | 196      | 6       | 3.1 (1.1, 6.5)    | 149      | 1       | 0.7 (0, 2.0)    |
| 2007 | 108      | 1       | 0.9 (0, 5.1)      | 266      | 1       | 0.4 (0, 2.1)    |
| 2008 | 86       | 0       | 0                  | 364      | 0       | 0 |
| 2009 | 106      | 0       | 0                  | 397      | 0       | 0 |
| 2010 | 101      | 0       | 0                  | 303      | 0       | 0 |
| 2011 | 23       | 0       | 0                  | 202      | 0       | 0 |
| 2012 | 8        | 0       | 0                  | 262      | 0       | 0 |
| 2013 | 30       | 0       | 0                  | 90       | 0       | 0 |

**TOTAL** | 1065 | 99 | 9.3 (7.6, 11.2) | 2203 | 7 | 0.3 (0.1, 0.7) |

*9 of these were tested in late 2002.*