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CANINE HOOKWORM PRESENCE IN TONGATAPU, TONGA

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ABSTRACT

Dogs are common in Tonga, where they exist in a close relationship with humans as pets and guard dogs and often are free-roaming due to limited property fencing and the need to scavenge for food. Little is known about the health status of these dogs and the type and level of pathogens carried by them. In this study, blood and fecal samples were analyzed to identify the presence of endoparasites and determine packed cell volume (PCV) values for dogs in Tonga. The current data deficit in this area made it necessary to develop a methodology and collect preliminary data to assist with future research in Tonga. Fecal analysis revealed hookworm in 73% of dogs, primarily those 4–12 mo old. Hematologic evaluation revealed low to borderline low PCV in 87% of dogs. The zoonotic nature of some of the pathogens identified poses a public health risk, with a particular risk for children.

With a population of 105,000 people, the Kingdom of Tonga has a dog:human ratio of 1:4 (G. D. Aguilar, unpubl. data) similar to that in other developing countries such as Nepal, Indonesia, and the Philippines (Jackman and Rowan, 2007; Traub et al., 2015). The dogs of Tonga are well known to the local population as free-roaming animals due to limited property boundaries and the need to scavenge to meet their nutritional requirements (Spennemann, 1990). Although some studies have been conducted on the prevalence of intestinal worms, such as hookworm, in similar socioeconomic environments (Chu et al., 2014; Keutchazoue et al., 2020), limited data have been collected on the health and parasite status of dogs in Tonga, including parasites that pose a zoonotic risk. The most prominent parasites found in humans in previous studies in similar socioeconomic settings (Chu et al., 2014; Keutchazoue et al., 2020) were *Ascaris* spp., hookworm, and *Trichuris* spp.

Keutchazoue et al. (2020) focused on parasites transmitted to humans via soil in Cameroon, Africa and considered methods of transmission related to personal hygiene, water quality, and agriculture practices. However, dogs as possible parasite vectors do not appear to have been evaluated in that study; thus, a clearer understanding of present endoparasites of dogs in Tonga may establish a link between human and zoonotic canine parasites, which may lead to methods for improving human health in the Kingdom of Tonga.

Through an annual week-long desexing clinic, data on packed cell volume (PCV) values and endoparasites burdens were collected. The desexing clinics are run by qualified New Zealand veterinarians and veterinary nurses with help from veterinary nursing students and are supported by the charity group South Pacific Animal Welfare. Although this study is likely the first systematic analysis of the health of dogs in Tonga, some bias may have been introduced because samples were collected from only those dogs presented to the desexing clinic. A wider sample population should be investigated for future studies.

One hundred two dogs were evaluated for endoparasites, blood chemistry, and PCV values. Because of time restraints, not all patient fecal samples could be processed, and only 78 fecal samples were evaluated.

During anesthesia for the desexing surgery, a blood sample of up to 5 ml was collected via intravenous catheter, and a fecal sample was collected per rectum. Blood samples were placed in labeled vacutainer tubes (free of lithium-heparin and additives) and stored in a standard refrigerator to allow clotting and separation of blood samples. Fecal samples were stored and refrigerated in additive-free tubes. All samples were tested within 24 hr of collection. Sex, age (where known), and weight of each dog also were recorded. Ethics approval for this study was obtained from the AgResearch Animal Ethics Committee, Ruakura, New Zealand (application number 14846). All experiments were conducted per AgResearch Animal Ethics Committee guidelines.

PCV was assessed by spinning a sample of blood in a standard hematocrit placed in a centrifuge at a relative centrifugal force of 12,298 for 6 min. Following separation by centrifuge, PCV results were read with a micro-hematocrit reader (Hawkesley, London, Great Britain) and recorded as a percentage.

A fecal flotation test was carried out by placing at least 1 g of fecal matter into a Fecalyzer® tube (Vetoquinol, Fort Worth, Texas), using approximately 20 ml of saturated sugar solution with a specific gravity (SG) of 1.27, which is ideal because most parasite eggs have an SG of 1.05–1.23 (David and Lindquist, 1982). The solution was made fresh daily to avoid crystallization issues associated with sugar solutions. The modified Sheather's

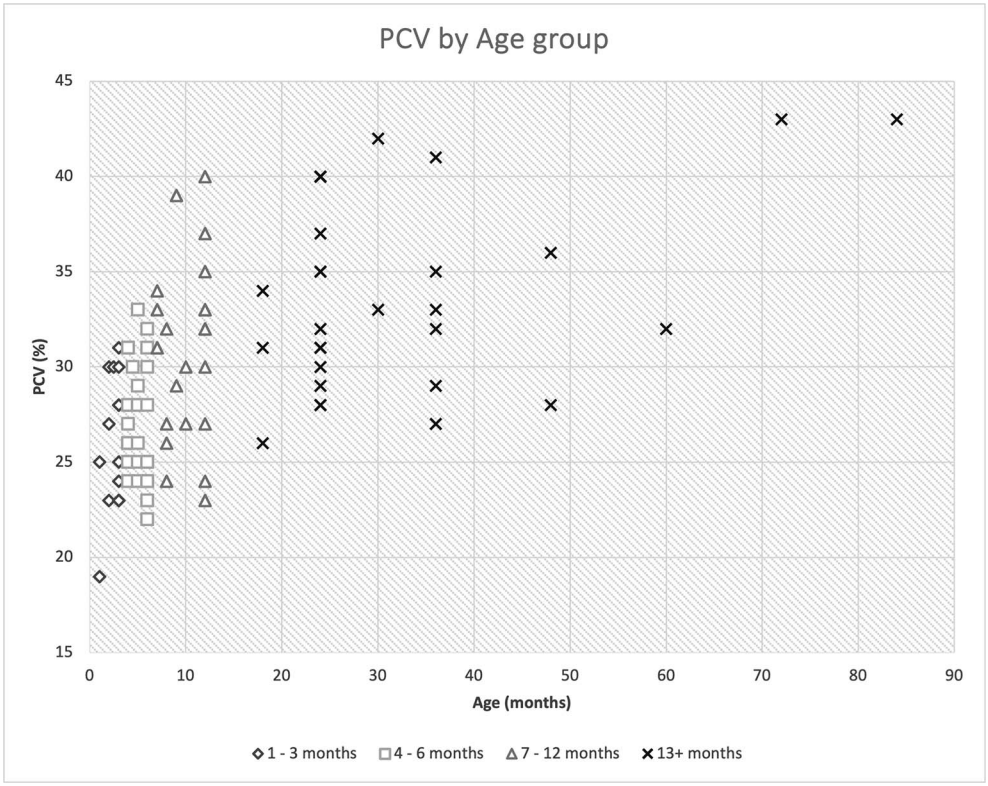

Figure 1. Packed cell volume (PCV) ranges in dogs by age. The normal range for PCV for dogs from birth to 3 mo is 32.5–40.55%, with a 4- to 6-mo average of 45.2% and a >6-mo range of 37–45% (Bird, 2011).

solution (Dryden et al., 2005) was the preferred option, but because of lack of accessibility, formalin was not added to the solution. Samples were placed in the Fecalyzer tubes, which were half-filled with the sugar solution and then agitated for approximately 20 sec. The Fecalyzer tubes were then filled with the solution until a meniscus was formed, and a cover slip was placed on top of the tube. Samples were left to sit for at least 15 but no more than 20 min to allow any eggs present to float and adhere to the cover slip. The cover slip was carefully removed and placed onto a labeled microscope slide. Slides were examined with a standard compound microscope and photographed for analysis using protocols described by Singaravel and Aleem (2016). Because the materials required to perform the McMaster method were not available, the slide was systematically scanned, and photographs were taken when endoparasite eggs were visible. *Toxocara canis* and *Trichuris vulpis* were recorded as the total number of eggs seen, and hookworm eggs were categorized into 5 burden grades (no eggs seen = 0, low = <30 eggs, moderate = 30–60 eggs, high = 60–90 eggs, very high = 90+ eggs) due to the substantially larger number of eggs present.

Eighty-six percent of dogs (88/102) had a low to borderline low PCV of <36% (Moxon et al., 2023), and each age group had an average PCV well below this level in some cases (Fig. 1). An independent *t*-test revealed a significant difference between samples values and the mean PCV (Bird, 2011) ($t = -29.315$, df = 98, n = 99, $P < 0.001$). Hookworm burden was an ordinal variable in this study, and Spearman's rank correlation test revealed a correlation of −0.226 ($P = 0.049$) between the PCV values and the burden of hookworm in these dogs.

Hookworm was the most common endoparasite identified in fecal flotation tests; hookworm eggs were identified in 73% of samples examined (57/78) (Fig. 2). The hookworm was identified as *Ancylostoma* spp. based on peer-reviewed literature (Brioudes et al., 2014; Chu et al., 2014; Traub et al., 2015; Bradbury et al., 2017; Carslake et al., 2017), but the identity could not be confirmed due to lack of access to molecular diagnostic techniques. The roundworm *To. canis* and whipworm *Tr. vulpis* were identified in a small number of samples (13/78 and 5/78, respectively), a substantially smaller number of samples than contained hookworm. Of the parasites found, *To. canis* and hookworms are considered zoonotic (Overgaauw and van Knapen, 2013; Carslake et al., 2017). All cases of *To. canis* infestation were found in dogs <9 mo old.

Although some dogs in Tonga are kept as pets and cared for, many are considered community owned and roam freely. In developing countries, the practice of keeping dogs as pets is becoming more common (Traub et al., 2015), possibly because of increasing urbanization and wealth. Nearly all dogs in Tonga have poor nutrition due to the high cost of importing commercially prepared dog food. Thus, many dogs are fed scraps such as coconut meat and fish remains or rely on scavenging or foraging for their food (Spennemann, 1990). Regular veterinary care is also minimal because of the absence of a permanent veterinary clinic and veterinarian in the Kingdom of Tonga. The continuing collection of data is an important tool for monitoring the overall health of the dog population in Tonga and for determining the possible presence of zoonotic parasites. The purpose of this study was to establish baseline data, adapt a plan for future canine health studies in Tonga, and investigate the presence of zoonotic and/or debilitating parasites. Hookworm is a high-risk parasite for young puppies and a possible causative agent of eosinophilic enteritis in humans (Bowman et al., 2010; Xie et al., 2019).

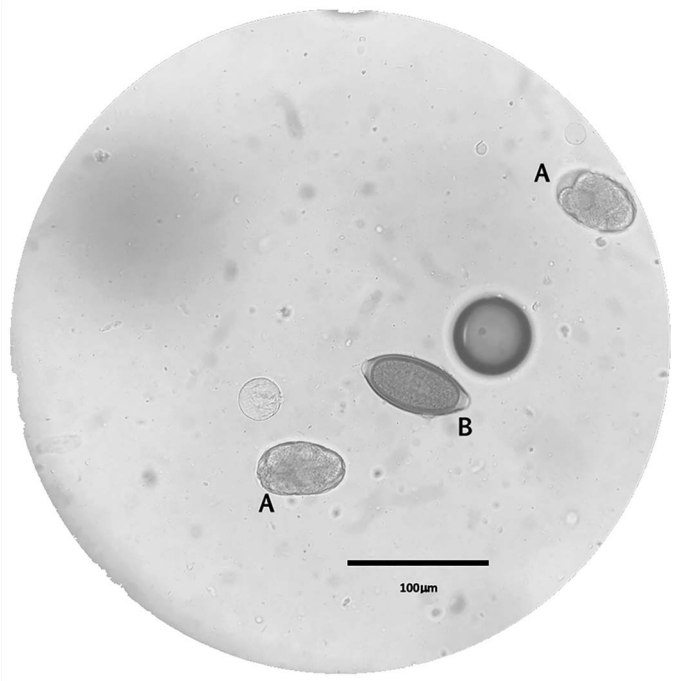

Figure 2. Fecal flotation results showing hookworm (A) and *Trichuris vulpis* (B) at ×40 objective on a standard compound microscope (photo by K. Harder). Approximate measurements of the hookworm eggs are 65 by 45 µm, which aligns with findings by Lucio-Forster et al. (2012).

Although *Tr. vulpis* was detected in this study, it is not zoonotic, and future studies will focus on hookworm because of its prevalence and high zoonotic risk.

The large number of dogs with low PCV, commonly associated with anemia (Bird, 2011), was expected. Tongan dogs typically present with pale gums, a high ectoparasite burden (primarily ticks and fleas), and poor nutrition—all common signs or causes of anemia. Twenty-two percent of dogs (23/103) presented with a PCV of <25%, a value at which many veterinarians would consider avoiding elective surgery such as desexing. Dogs commonly have a higher PCV at birth, which then decreases at 2 mo of age then rebounds to normal adult levels by 6 mo (Bird, 2011). Our results revealed a similar trend (Fig. 1); however, the youngest dogs sampled were 4 wk old, and only 14 dogs in the 1- to 3-mo age group were sampled (Table I).

The prevalence of the zoonotic roundworm *To. canis* in puppies and juvenile dogs was expected because this parasite is common in dogs of this age (Roddie et al., 2008), whereas hookworm was more prevalent in dogs 4–12 mo old (Fig. 3). More than 60% of infectious animal diseases are zoonotic (Bidaisee and Macpherson, 2014), and public health risks should be considered due to the proximity of humans and animals in living conditions in developing countries (Naden, 2020). Children under 6 yr old are at particular risk of intestinal parasite–induced physical developmental deficiencies, anemia, and malnutrition due to poorer hygiene practices (Rajoo et al., 2017). Zoonotic parasites such as *To. canis* are commonly found on the coats of younger dogs (Roddie et al., 2008), presenting a high risk for toxocariasis infection in humans.

Hookworm is one of the most detrimental endoparasites for dogs and zoonotic infestations for humans (Bowman et al., 2010; Xie et al., 2019). *Ancylostoma caninum* in particular (the suspected parasite in this study) is likely to cause anemia which can result in the death of puppies with severe burdens and is found in subtropical settings (Bowman et al., 2010; Xie et al., 2019; Beknazarova et al., 2020). Statistical analysis of canine PCV and hookworm burden revealed a minor negative correlation, supporting one of the main clinical signs of hookworm, i.e., anemia. Although this correlation was weak, future studies should include more rigorous data collection with use of appropriate methodologies such as the McMaster or FLOTEC technique (Cringoli et al., 2010; Levecke et al., 2012) and a larger sample size to improve statistical power. Data on confounding variables such as diet and whether bitches are presented to the clinic in season should be collected because these variables can influence the PCV levels of a patient. Many dog owners create home-cooked meals for their pets because of the lack of access to commercial dog food, and this variable needs to be included in future studies (Naigamwalla et al., 2012). PCV values also can vary greatly during the estrus cycle of dogs (Moxon et al., 2023); therefore, these factors need to be included as variables in future studies.

Although *A. caninum* could not be confirmed in this study because of limited resources, there is evidence of the parasite in the Asia-Pacific region, including the Northern Territory of Australia (Beknazarova et al., 2020) and China (Xie et al., 2019), both of which have strong trade links to Tonga.

Literature reviews of peer-reviewed studies (Spennemann, 1990; Brioudes et al., 2014; Chu et al., 2014; Bradbury and Traub, 2016; Bradbury et al., 2017; Carslake et al., 2017; Beknazarova et al., 2020) over the last 20 yr in the Pacific Island region for diseases found in domestic animals reveal a clear lack of data for hookworm and need for further research in this area. Hookworm infections in humans can result in debilitating illness, especially infection by *A. caninum,* highlighting the importance for human health of molecular identification of the hookworm species present in Tonga. This study was conducted to address gaps in this data-deficient area and to better understand the general health of

Table I. Distribution of dogs examined by age group, sex, average packed cell volume (PCV), and fecal samples positive for parasites. Average PCV was calculated based on all 102 blood samples collected; only 78 of these samples analyzed for parasites.

	Sex (n = 102)			PCV	Number of positive fecal samples (n = 78)		
Age	Male	Female	Total	(n = 102) (%)	Hookworm	*Toxocara canis*	*Trichuris vulpis*
0–3 mo	7	7	14	26.14	9	4	0
4–6 mo	16	14	30	26.96	20	5	2
7–12 mo	14	11	25	29.64	11	4	1
13 mo +	16	14	30	32.7	15	0	2
Unknown	2	1	3	32	2	0	0
Total	55	47	102		57	13	5

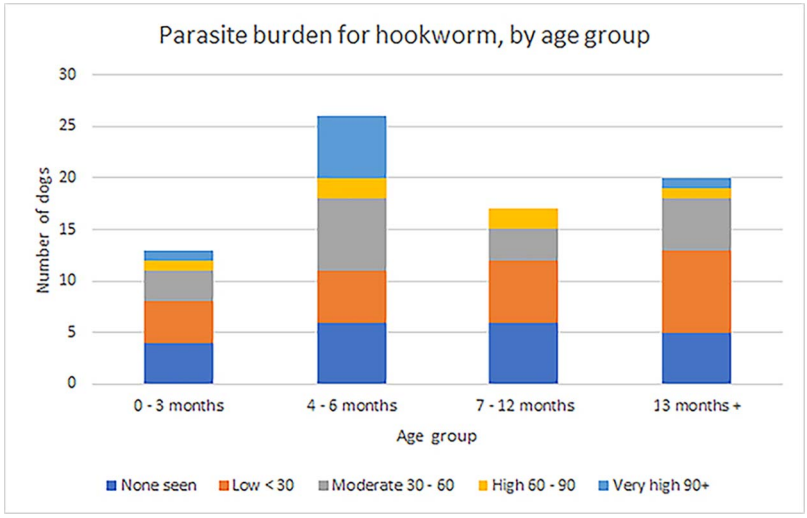

Figure 3. Hookworm burden by age group as determined by fecal flotation results. No eggs seen = 0, low burden = <30 eggs counted per slide, moderate burden = 30–60 eggs, high burden = 60–90 eggs, very high burden = >90 eggs. Color version available online.

the canine population while gathering data on zoonotic parasites that may affect the human population of the Kingdom of Tonga.

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K.H. developed the method for and conducted fecal analysis, photographed and collated data, analyzed data, and cowrote the text. K.N. developed the concept, supervised the project, collected biological samples, analyzed the results, cowrote the text, and prepared figures. S.E. conducted biochemical analyses and PCV studies and collated data. All authors reviewed the manuscript.

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