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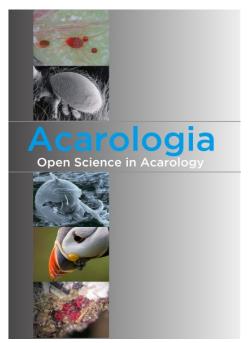
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### The detection of three new Haemaphysalis ticks (Acari: Ixodidae) in Singapore and their potential threat for public health, companion animals, and wildlife

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#### **Original research**

#### **ABSTRACT**

Efforts to safeguard human and animal health against ticks and tick-borne diseases are principally reliant on careful surveillance. Within Singapore, the importance of ticks and tick-borne diseases is increasingly recognized. However, developing foundational knowledge of the local diversity of ticks and tick-borne microbes, as well as their ecology, is ongoing. Nation-wide surveillance efforts revealed the presence of three previously unreported species of *Haemaphysalis* from Singapore, namely: *H. bispinosa*, *H. hystricis*, and *H. wellingtoni*. All three species are known to feed on humans and appear to have established populations within Singapore. We record the first infestation of humans by *H. hystricis* and *H. wellingtoni* within Singapore and present all known local host records from these three newly detected tick species. The threat posed by these species to local public health, companion animals, and wildlife is discussed.

**Keywords** ttck-borne disease; exotic tick; invasive species; Haemaphysalis wellingtoni; Haemaphysalis hystricis; Haemaphysalis bispinosa

#### Introduction

Ticks and tick-borne diseases can pose major challenges to the health of humans, animals, and ecosystems (Dantas-Torres *et al.* 2012). Human and animal health can be affected when non-native ticks invade new ecosystems, or when the ecology of native ticks is altered by anthropogenic changes (Léger *et al.* 2013; Medlock *et al.* 2013). The recent invasion of *Haemaphysalis longicornis* in North America is just one example of the serious impacts that the introductions of exotic tick species can have in new regions (Rainey *et al.* 2018). In an era of global interconnectedness, biosurveillance has never been more crucial to preventing the introduction and establishment of non-native ticks. Biosurveillance requires participation from local clinicians, veterinarians, and national ministries, as well as the general public. It is also critically dependent on both a detailed understanding of the local tick fauna and extensive monitoring efforts to detect changes in community composition or the emergence of novel species.

Singapore is a tropical nation with an abundance of both natural and artificial green spaces, as well as a high human population density (Henderson 2013). This has resulted in overlaps in land use between humans and wildlife, and occasionally direct conflict (Yeo and Neo 2010; Yong *et al.* 2010). However, this overlap can also have less obvious consequences such as an

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increased potential for zoonotic spillover. A range of zoonotic parasites and pathogens have been detected in wildlife in Singapore including parasitic worms (Mendenhall *et al.* 2018), ticks (Kwak *et al.* 2021a), bacteria (Neves *et al.* 2018) and arboviruses (Yap *et al.* 2019). Local interest in ticks and tick-borne diseases has only emerged recently and has built upon a small number of historical studies (e.g., Theis and Franti 1971; Paperna 2006; Leong 2010). In recent years, more intensive efforts have been made to survey the diversity of ticks in Singapore (Kwak 2018), along with their host associations (Kwak *et al.* 2018; 2019; 2021a), ecologies and zoonotic potential (Kwak 2021a; 2021b).

Within Singapore, three members of the genus *Haemaphysalis* have previously been recorded, namely *H. doenitzi*, *H. nadchatrami*, and *H. semermis* (Hoogstraal *et al.* 1966; Hoogstraal and Wassef 1973; Kwak 2018). However, nation-wide biosurveillance efforts in Singapore revealed the presence of *Haemaphysalis* specimens which did not match the descriptions of the three known species. Three new species were found, doubling the *Haemaphysalis* fauna known from Singapore and represents three species known to bite humans. We describe the biosurveillance work below and discuss its importance in terms of public health.

#### **Material and methods**

Beginning in 2018, Singapore's first nationwide tick surveillance program was initiated; it involved a network of veterinarians, clinicians, and field biologists who sourced ticks from humans, companion animals, wildlife, and the environment. Under this program, ticks were collected opportunistically from human and animal hosts by careful removal using tweezers. Opportunistic flagging was also undertaken to collect ticks from the environment (e.g., from vegetation and leaf litter). All collected ticks were fixed in ethanol (70-100%) for later identification.

Between 2018 and 2021, a total of 2,163 ticks were collected across Singapore (Figure 1) from 303 individual hosts representing 40 different species (Table 1), as well as from local vegetation. All ticks were examined using a LEICA M80 light microscope. Specimens were retained within the Singapore National Tick Reference Collection (SNTRC) presently held within the Medical Faculty (Yong Loo Lin School of Medicine) at the National University of Singapore and curated by one of the authors (M. L. Kwak). Among the specimens collected, three tick taxa were identified which did not belong to known species from Singapore. These taxa were identified to species level based on keys and descriptions from Yamaguti *et al.* (1971), Tanskul and Inlao (1989), Trapido *et al.* (1964), Hoogstraal *et al.* (1965), and Geevarghese and Mishra (2011).

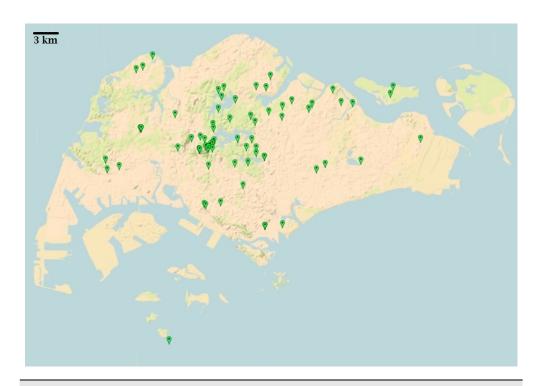
#### **Results**

The three unknown taxa were confirmed as members of the of the genus *Haemaphysalis* based on a combination of: 1) lack of eyes, 2) base of palpal article 2 flared laterally, 3) festoons. All three taxa were then confirmed as members of the subgenus *Kaiseriana* based on a combination of: 1) presence of a spur on the posteroventral edge of palpal article III, 2) presence of a spur on the posterodorsal edge of palpal article III. Females, males, nymphs, and larvae were collected for the three species (except for *H. hystricis* for which the male stage was not collected), each specimen was individually identified.

The females of the first unknown *Haemaphysalis* taxon had the following combination of characters: 1) 4/4 dentition, 2) scutum longer than wide, 3) internal, retroverted spur extending from posterodorsal edge of palpal article III, 4) internal, retroverted spur extending from posteroventral edge of palpal article III, 5) short, blunt cornuae. The males of this taxon had: 1) medium sized blunt cornuae, 2) small, blunt, posterointernally directed spur on dorsal surface of palpal article III, 3) lateral grooves on conscutum, 4) blunt, distinctive spurs of similar size on all coxae, 5) spurs on trochanters (though smaller on trochanter III and IV),

Table 1 Host species from which ticks were collected in Singapore.

Host species	Common name	Number of hosts sampled	Captive/Wild
Boiga dendrophila	Gold-ringed cat snake	1	Wild
Callosciurus notatus	Plantain squirrel	4	Wild
Canis familiaris	Domestic dog	41	Wild
Crocodylus porosus	Saltwater crocodile	1	Wild
Cuora amboinensis	Amboina box turtle	2	Wild
Eonycteris spelaea	Cave nectar bat	14	Wild
Felis catus	Domestic cat	1	Wild
Galeopterus variegatus	Sunda colugo	1	Wild
Gallus gallus	Jungle fowl	3	Wild
Heosemys spinosa	Spiny hill turtle	2	Wild
Homo sapiens	Human	30	Wild
Lanius cristatus	Brown shrike	1	Wild
Laticauda colubrina	Yellow-lipped sea krait	3	Wild
Malayopython reticulatus	Reticulated python	53	Wild
Manis javanica	Sunda pangolin	47	Wild
Mus castaneus	Southeast Asian house mouse	1	Wild
Ophiophagus hannah	King cobra	14	Wild
Penthetor lucasi	Dusky fruit bat	6	Wild
Pitta moluccensis	Blue-winged pitta	1	Wild
Rattus annandalei	Annandale's rat	24	Wild
Rattus tanezumi	Asian house Rat	7	Wild
Rattus tiomanicus	Malaysian wood rat	1	Wild
Suncus murinus	Asian house shrew	1	Wild
Sus scrofa	Wild boar	17	Wild
Tragulus napu	Greater mouse-deer	1	Wild
Tupaia glis	Tree shrew	2	Wild
Varanus nebulosus	Clouded monitor	1	Wild
Varanus salvator	Asian water monitor	2	Wild
Acrocodia indica	Malayan tapir	3	Captive
Ailuropoda melanoleuca	Giant panda	6	Captive
Ailurus fulgens	Red panda	1	Captive
Alligator sinensis	Chinese alligator	1	Captive
Arctictis binturong	Binturong	2	Captive
Axis porcinus	Indian hog deer	1	Captive
Dendrolagus goodfellowi	Goodfellow's tree kangaroo	2	Captive
Macropus giganteus	Eastern grey kangaroo	1	Captive
Neofelis nebulosa	Asian cloud leopard	1	Captive
Nycticebus coucang	Sunda slow loris	1	Captive
Paleosuchus palpebrosus	Cuvier's dwarf caiman	2	Captive
Suricata suricatta	Meerkat	1	Captive



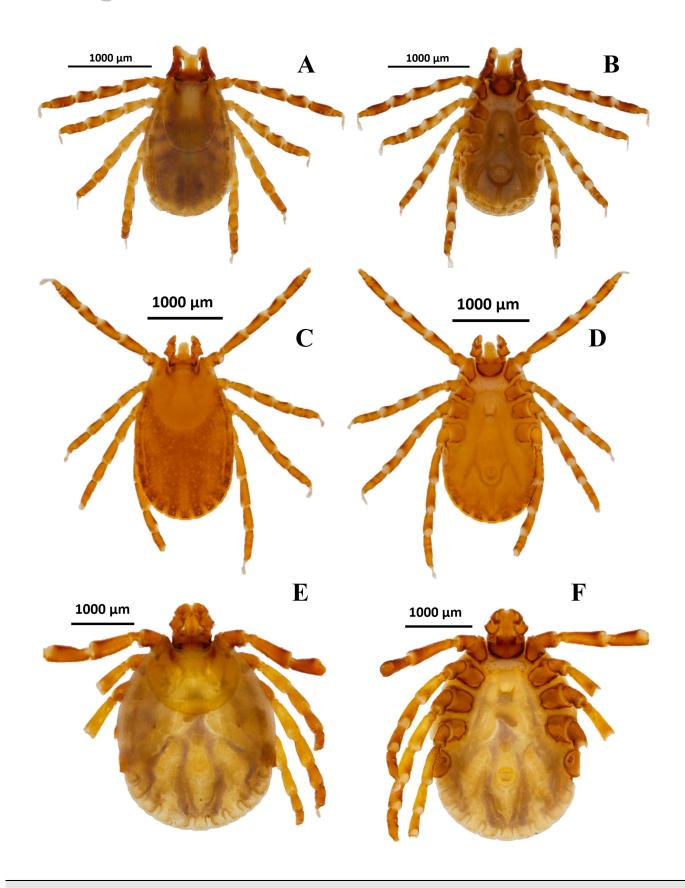
**Figure 1** Locations across Singapore from which ticks (including *Haemaphysalis*) were collected between 2018-2021 as part of nation-wide tick surveillance efforts.

large, pointed posterointernally directed spur on ventral surface of palpal article III, 6) 4/4 dentition throughout most of hypostome. The nymphs were characterized by: 1) 2/2 dentition, 2) a distinctively larger spur on coxa II than on coxa I, 3) cornuae blunt and small, 4) coxal spurs pointed, 5) lateral salience on palpal article II shallow with dorsal surface of article II of similar width to article III, 6) obvious spurs on trochanters I and II, 7) posterointernally directed spur on dorsal surface of palpal article III. Finaly, the larvae had the following combination of characters: 1) lateral edge of palpal article II distinctly rounded, 2) cornuae absent, 3) distinctive, broad, blunt spurs present on all coxae, 4) scutum width less than 1.5 times that of length, 5) posterointernally directed spur on ventral surface of palpal article III. These character combinations distinguished each life stage as *H. wellingtoni* (Figure 2 a, b). In total, 94 *H. wellingtoni* were collected from 10 individual hosts during the period from 2018 – 2021 (Table 2). During this time period of the tick surveillance program, we recorded one case of human infestation by a *H. wellingtoni* larva.

Females of the second newly detected *Haemaphysalis* taxon had the following combination of characters: 1) 4/4 dentition, 2) palps with slight basolateral salience, 3) posterodorsal spur of palpal segment III overlapping anterior quarter or more of segment II, 4) spurs of coxa IV short, broadly ridgelike, 5) Posterodorsal spur of palpal segment III overlapping anterior

**Table 2** Numbers of each life stage of *H. bispinosa*, *H. hystricis*, and *H. wellingtoni* collected in Singapore (both on-host and off-host) between 2018 – 2021.

	Total	Females	Males	Nymphs	Larvae
H. bispinosa	251	61	86	83	21
H. hystricis	29	8	0	2	19
H. wellingtoni	94	15	66	5	8



**Figure 2** Adult female *Haemaphysalis* ticks from the three species newly recorded in Singapore. A) *Haemaphysalis wellingtoni* dorsal view, B) *Haemaphysalis wellingtoni* ventral view, C) *Haemaphysalis bispinosa* dorsal view, D) *Haemaphysalis bispinosa* ventral view, E) *Haemaphysalis hystricis* dorsal view, F) *Haemaphysalis hystricis* ventral view

third of segment II. Males were characterized by: 1) 4/4 dentition, 2) lateral grooves on conscutum, 3) large posterointernally directed spur on dorsal surface of palpal article III, 4) large posterointernally directed spur on ventral surface of palpal article III, 5) blunt salience on lateral edge of palpal article II, 6) large, blunt cornuae, 7) spurs on all coxae, 8) broad blunt spur on coxa IV, 9) spurs on all trochanters. Nymphs had the following combination of characters: 1) 2/2 dentition, 2) moderately flared lateral salience on palpal article II, 3) spurs on all coxae, 4) small, blunt, wide spur on coxa IV, 5) small, blunt cornuae, 6) small, blunt posterointernally directed spur on ventral surface of palpal article III. Finally, larvae of this taxon had: 1) 2/2 dentition, 2) weakly flared lateral salience on palpal article II, 3) spurs present on all coxae, 4) coxae II and III with a shallow, wide, blunt, spur, 5) scutum almost twice as wide as long, 6) extremely small, subtle cornuae present, 7) posterointernally directed spur on ventral surface of palpal article III. These character combinations distinguished each life stage as *H. bispinosa* (Figure 2 c, d). In total, 251 *H. bispinosa* were collected from 22 individual hosts during the period from 2018 – 2021 (Table 2).

Females of the third newly detected *Haemaphysalis* taxon in Singapore had the following combination of characters: 1) 4/4 dentition, 2) palps with slight basolateral salience, 3) posterodorsal spur of palpal segment III overlapping anterior quarter or more of segment II, 4) spurs of coxa IV distinct and not ridge-like with a narrowly rounded apex, 5) posterodorsal spur of palpal segment III overlapping anterior quarter of segment II. Nymphs were characterized by: 1) 2/2 dentition, 2) widely flared lateral salience on palpal article II, 3) lateral edge of lateral salience on palpal article II rounded, 4) small, bluntly rounded cornuae, 5) short, blunt, broad spurs on all coxae, 6) spur on coxae IV distinctly smaller than those on other coxae, 7) small, blunt posterointernally directed spur on ventral surface of palpal article III, 8) spur on coxa I short. Finally, larvae of this taxon had: 1) 2/2 dentition, 2) widely flared lateral salience on palpal article II, 3) spurs present on all coxae, 4) coxae III with extremely small, blunt spur, 5) scutum approximately 1.5 times wide as long. These character combinations were distinctive of each life stage of H. hystricis (Figure 2 e, f). In total, 24 H. hystricis were collected from 9 individual hosts during the period from 2018 – 2021 (Table 2). Notably, no males were collected. Over the 2018 – 2021 time period, we recorded two cases of human infestation, on both occasions by a female *H. hystricis*.

All life stages of all taxa were also matched using DNA barcodes generated as part of this work (methods and data not shown). This data will soon to be available in a published DNA barcode library for the ticks of Singapore using the Cytochrome c oxidase subunit I (COI) locus.

An index was compiled of host species in Singapore from which *H. bispinosa*, *H. hystricis*, and *H. wellingtoni* were collected (Table 3). A key to distinguish among the six *Haemaphysalis* species found in Singapore is included in this paper for females (Figure 3), males (Figure 4), and nymphs (Figure 5). As the larvae of *H. semermis* and *H. nadchatrami* have not yet been described, no key is presented for larval *Haemaphysalis* of Singapore.

#### **Discussion**

#### **Exotic invaders or overlooked natives?**

Identifying a tick fauna and their ecology are key factors to consider when developing management policy for ticks and tick-borne diseases. This information, in conjunction with host interaction data, can help determine whether local eradication efforts could or should be undertaken; especially given that during the early stages of invasion, there is sometimes a chance of eradicating exotic ticks to minimize their impact on native ecosystems. However, the status of particular tick species as native or exotic can be challenging to determine as it can be difficult to differentiate between natural range expansions and anthropogenic introductions of ticks. It can also be complicated by cases of local tick extirpations (both historically or prehistorically) followed by recolonization of their former range. *Dermacentor auratus* 

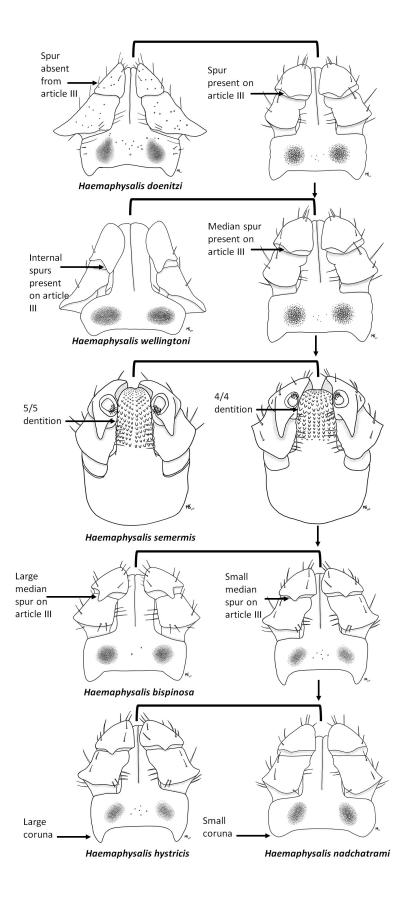


Figure 3 Visual key to female Haemaphysalis ticks of Singapore

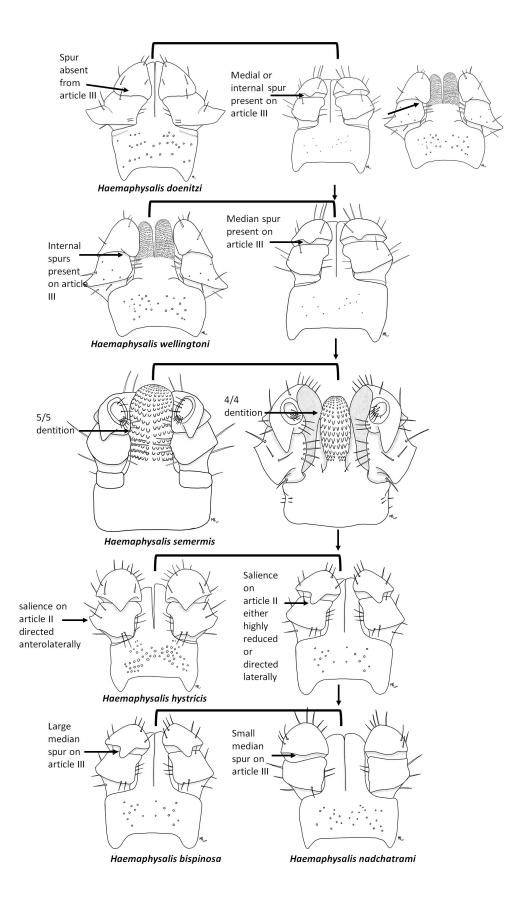


Figure 4 Visual key to male Haemaphysalis ticks of Singapore

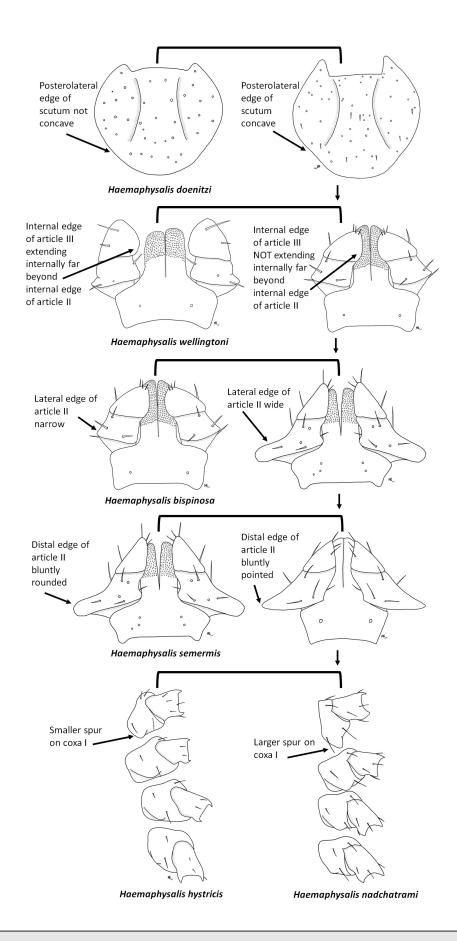


Figure 5 Visual key to nymphal Haemaphysalis ticks of Singapore

recently emerged as a major tick of public health importance in Singapore (Kwak *et al.* 2021a). Although apparent absence may initially be interpreted as evidence of *D. auratus* being exotic (Hoogstraal and Wassef 1985), biogeographic and host data suggest that the emergence of *D. auratus* in Singapore is simply an example of a native species reestablishing into a section of its former range after local extirpation (Kwak *et al.* 2021a). While *D. auratus* appears to be native, two non-native ticks have been recorded in Singapore, namely *Rhipicephalus sanguineus* and *R. microplus* (Kwak 2018). The former is a specialist parasite of dogs and the latter (now locally extinct in Singapore) is a specialist of cattle (Guglielmone *et al.* 2014; Kwak 2018).

Among the three *Haemaphysalis* species newly detected in Singapore, *H. bispinosa* is of particular interest as it has long been considered an exotic species on the Malay Peninsula, but native to India (Hoogstraal *et al.* 1969). This is largely because of its synanthropic occurrence in tropical southeast Asia on domestic dogs (*Canis lupus familiaris*), and its general absence from forested habitats and the native species of Carnivora and Artiodactyla which occur within them. The majority of specimens examined in this study were also collected from domestic dogs, though a small number also came from the native banded pig (*Sus scrofa vittatus*) (Table 1). We therefore consider *H. bispinosa* to be an exotic species that was likely introduced with domestic dogs. Although this is the first time *H. bispinosa* has been officially reported from Singapore, historical specimens held in the US national tick collection indicate that this species was present in Singapore at least as far back as 1913 (specimen reference code: USNTC 111274) and was also collected in 1963 (specimen reference code: USNTC 87282). Theis and Franti (1971) undertook an extensive study on dog ticks in Singapore. However, as their study focused on *R. sanguineus* and they did not mention *H. bispinosa*, it is unclear if this species was collected during their work.

Haemaphysalis hystricis is a widely distributed species throughout East Asia and occurs from southern Japan as far south as Indonesia and west into India (Hoogstraal *et al.* 1965; Petney *et al.* 2019). Although it is commonly called the East Asian Mountain haemaphysalid because it is relatively common at high altitudes, *H. hystricis* can also sometimes occur at

Table 3 Number of H. bispinosa, H. hystricis, and H. wellingtoni ticks collected from different host species in Singapore between 2018 – 2021.

Tick species (number of ticks collected on- host/off-host)	Host species	Common name	Wild/Captive	No. ticks
H. bispinosa	Acrocodia indica	Malayan tapir	Captive	10
(250/1)	Axis porcinus	Indian hog deer	Captive	43
	Canis familiaris	Dog	Wild & Captive	195
	Rattus tanezumi	Tanezumi rat	Wild	1
	Rusa unicolor	Sambar deer	Wild	1
H. hystricis	Canis familiaris	Dog	Wild	3
(10/19)	Homo sapiens	Human	-	2
	Rattus annandalei	Annandale's rat	Wild	1
	Rattus tiomanicus	Malayan field rat	Wild	1
	Rusa unicolor	Sambar deer	Wild	1
	Sus scrofa	Wild boar	Wild	2
H. wellingtoni	Gallus gallus	Jungle fowl	Wild	87
(94/0)	Homo sapiens	Human	-	1
	Ixobrychus flavicollis	Black bittern	Wild	1
	Lanius cristatus	Brown shrike	Wild	1
	Pitta moluccensis	Blue-winged pitta	Wild	4

sea-level (Hoogstraal *et al.* 1965). As Singapore is well within the broad range of *H. hystricis*, we regard this species as a previously unreported native species. This is further supported by the local host associations; *H. hystricis* commonly infests both *C. l. familiaris* and *S. sc. vittatus* throughout its range (Hoogstraal *et al.* 1965). In addition, this species typically occurs close to the equator (Hoogstraal *et al.* 1965), and Singapore is almost exactly on the equator. It is noteworthy that this species was collected infrequently in this study, and although a viable local population of *H. hystricis* seems to be present, it's possible that it does not thrive in the lowland equatorial conditions that prevail in Singapore –the highest elevation on the island is 164m above sea-level (Bukit Timah).

Haemaphysalis wellingtoni is also widely distributed throughout East Asia, and although it occurs primarily in the Oriental Zoogeographic Region, it is also known from New Guinea (the northern Australasian region) (Petney et al. 2019). Singapore is well within its range and we therefore consider the species to be native (Petney et al. 2019). H. wellingtoni feeds predominantly on gallinaceous birds, and within Singapore, the red jungle fowl (Gallus gallus) serves as its main host, though other birds (and in rare cases mammals) are also occasionally infested (Table 1).

#### **Health risks?**

Given its small size, Singapore hosts a surprisingly rich tick fauna, of which a number of species infest humans (Kwak 2018). Ticks have become an increasingly important threat to local public health, particularly with the increased abundance of *D. auratus* since its recent recolonization of the island (Kwak *et al.* 2021a). To inform public health policy and biosurveillence, it is crucial to understand the potential zoonotic risk posed by ticks, including the three newly detected *Haemaphysalis* species.

Haemaphysalis bispinosa occasionally infests humans through its range, and infestations have been recorded in India (Sharma 1993), Sri Lanka (Ariyarathne et al. 2016), and Thailand (Tanskul and Inlao 1989). Although the authors have seen no cases of this species infesting humans in Singapore, local cases of human infestation may emerge in the future. This is particularly likely given the propensity of this species to infest local dogs, which are a highly synanthropic species in Singapore and are now commonly seen in local parks, gardens, and nature reserves. Pathogenic bacterial genera have been detected in H. bispinosa in the region, including Rickettsia (Malaisri et al. 2015) and Bartonella (Kho et al. 2015). The detection of B. bovis in H. bispinosa, in neighboring Malaysia (Koh et al. 2015), is of particular interest given that this pathogen infects ungulates and could impact wildlife health in Singapore, particularly sambar deer (Rusa unicolor) and mouse deer (Tragulus spp.) (Chua et al. 2009; Chua and Lim 2011). Hemaphysalis bispinosa has also been found in association with Babesia infected dogs, though the role of H. bispinosa as a vector remains unclear (Augustine et al. 2017). Nonetheless, the close association this tick has with dogs in Singapore indicates that it may serve as a vector of some tick-borne pathogens in local canines. Research should now focus on studying the pathobiome of *H. bispinosa* in Singapore to better understand which microbes are present in the Singaporean population. Special attention should also be given to this tick as bites from its sister species, H. longicornis, have been suggested to cause mammalian red-meat allergy in other parts of Asia, as well as numerous other diseases of humans (Chinuki et al. 2016).

Haemaphysalis hystricis also occasionally feeds on humans and records exist from China, Japan, Taiwan, Vietnam, Laos, Thailand, and Myanmar (Guglielmone and Robbins 2018). Despite its apparent rarity within Singapore, we recorded two separate local human infestations by H. hystricis. This tick species feeds on a wide range of host species including members of numerous mammal families, and occasionally also birds (Guglielmone et al. 2014). While the two records of human tick bites by H. hystricis in Singapore mentioned in the present study represent the first recorded cases in Singapore, further infestations will likely occur in coming years. Indeed, many of the rodent and large mammal host species used by H. hystricis have

expanding populations within Singapore (e.g., wild pigs, samba deer, wild dogs) bringing them into increasing contact with humans and urban greenspaces. A number of important microbial genera have been detected in *H. hystricis* including an undescribed *Borrelia* species from the relapsing fever clade (Khoo *et al.* 2017), as well as *Coxiella*-like bacteria (Arthan *et al.* 2015), *Ehrlichia* and *Rickettsia* (Mahara 1997; Parola *et al.* 2003). The pathogenic protozoan *Babesia gibsoni* has also been detected in *H. hystricis* and is known be transmitted transovarially between generations (Jongejan *et al.* 2018). The wide range of pathogenic genera recorded from *H. hystricis* suggest that it may be a vector of importance in Singapore and therefore requires further study to determine its potential threat to local public health.

Although *H. wellingtoni* has been recorded feeding on humans, this appears to occur more rarely than with *H. hystricis* and *H. bispinosa* (Guglielmone and Robbins 2018). Just one case of human infestation by *H. wellingtoni* has been recorded in Thailand (Parola *et al.* 2003) and may be due to the species' preference for avian hosts (Guglielmone *et al.* 2014). The record of human infestation by *H. wellingtoni* presented in this study represents the first from Singapore and the second globally. Though fewer pathogenic microbes have been detected within *H. wellingtoni*, it has been shown to be a competent vector of Kyasanur forest disease virus, which is frequently deadly to humans and other primates (Bhat and Naik 1978). Therefore, the microbiome (including the virome) of this tick species should be carefully studied in Singapore to ascertain public health risk. This is particularly important given the rapid population expansion of red jungle fowl (*Gallus gallus*) in Singapore, which serve as the primary host of this tick (Wu *et al.* 2020).

Surveillance of ticks and tick-borne diseases is crucial for safeguarding public health in Singapore. The recent detection of *H. bispinosa*, *H. hystricis*, and *H. wellingtoni* in Singapore highlights the need for more extensive research into these parasites and potential disease vectors, both within the island nation and across Asia more broadly. Expanded research into Singapore's tick fauna should not only focus on their microbiomes, but also their ecologies, as understanding their life histories and habitat needs is key to predicting and mitigating future disease spillover.

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