SIGNIFICANT BLOOD PROTOZOAN INFECTIONS, THEIR HOST RANGE AND TREND OF INFECTIONS IN DOMESTIC ANIMALS OF MALAYSIA DIAGNOSED BY THE DEPARTMENT OF VETERINARY SERVICES AND VETERINARY RESEARCH INSTITUTE (VRI) FROM 1931 TO 2010 – A HISTORICAL PREVIEW

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ABSTRACT. Blood protozoan diseases are significant to animal breeding as they result in economic loss due to mortality, reduction in production efficiency and increment in drugs usage. This historical retrospective study was carried out to investigate the most significant blood protozoan diseases, their trends as well as the host range diagnosed by Veterinary Research Institute (VRI) from 1931 to 2010 among Malaysian domestic animals. Conventional techniques such as stained thin blood film, whole blood buffy coat examination and direct wet smears were utilised for diagnosis from 1931 to 2010. Five most common significant blood protozoan diseases from the domestic animals were identified namely, theileriosis, leucocytozoonosis, plasmodiasis, babesiosis and trypanosomiasis respectively. The setting up of a systematic laboratory service in VRI has enabled more cases of blood protozoan infections to be diagnosed and this has inadvertently led to better understanding and control of the diseases thereby improving the livelihood and reducing economic loss of local farmers.

Keywords: Blood protozoa, domestic animals, Veterinary Research Institute Malaysia, historical retrospective study

INTRODUCTION

Veterinary Research Institute (VRI), Malaysia is the premier research and diagnostic facility of the Department of Veterinary Services (DVS), which was built with the aim to provide disease diagnoses of domestic animals in Malaysia. The facility started its operations in 1948 at Tanjong Rambutan Psychiatric Hospital and at Falim with a small laboratory. Prior to this, from 1931 to 1948, the Federal Veterinary Services, being the custodian of animal diseases in Malaysia, carried out diagnostic work in the facilities at Kuala Lumpur by veterinarians. Subsequently in 1953, it was upgraded to the current facilities at Jalan Sultan Azlan Shah, Ipoh. Globally, among

the diseases in animals, parasitic diseases remain as one of the major contributors to production loss. The livestock production loss due to parasitic diseases (i.e. nematodiasis) has been estimated to exceed USD$ 12million in Australia, India and Indonesia (Sani *et al.*, 2004). This current paper presents data compiled from the annual reports of the Veterinary Services of Malaysia from 1931 to 1948, together with the annual reports of the VRI from 1948 to 2010, with the aim to highlight the common as well as the five most significant blood protozoan infections in domestic animals in Malaysia. These valuable data are compiled and compared in order to evaluate the status, trends and seriousness of veterinary parasitic diseases from population to population over these years and to re-evaluate the strategies of the available parasite control program which serve to avoid further economic loss. Cases or samples were submitted by the attending veterinarians or farmers as part of the routine disease investigation procedures. Over the eight decades, it has been observed that some common diseases still persist even with modern farm management and control methods. This paper aims to provide a historical perspective that will enlighten present day veterinarians and farmers on the role of these diseases in hindering the productivity of livestock industry. An improved understanding and awareness of these diseases will benefit the current and future industry in the pursuit for higher animal husbandry standards.

MATERIALS AND METHODS

Since 1931 to 2010, a total of approximately 50,000 blood samples collected in EDTA or heparin tubes were submitted to the laboratory for the diagnosis of blood protozoan infections. This approximation is gleaned from annual reports through this period; in some cases total samples received were not mentioned.

Thin Blood Smear

A thin blood smear was prepared by placing a drop of blood at one end of a clean grease-free glass slide. By holding the spreader at an angle of approximately 45 degrees, the blood drop was pushed along the slide, drawing the blood behind it until it has all been smeared. The blood film (also known as blood smear) was then allowed to dry for 1-2 minutes. A longer time of up to 5 minutes was required if the blood film was too thick. A few drops of methyl alcohol was then applied onto the smear for three minutes to fix the smear onto the slide. The slide was then washed off and stained with 8% Giemsa stain (diluted 1/20 in buffered distilled water pH 7.2) for 45 minutes. The excess stain was removed and the slide was washed with running tap water. The slide was then allowed to dry prior to microscopic examination at 1000x magnification (Manual of Veterinary investigation Laboratory Techniques, 1978).

Whole Blood Buffy Coat Examination

As for the identification of trypanosomes, an additional test such as the whole blood buffy coat examination was done. The blood in the EDTA tube was mixed well by gently inverting the tube approximately 10 times. Blood was then drawn 2/3 of the way up a 75mm x 1.00 mm microhaematocrit capillary tube. Blood at the tip was wiped off and carefully plugged at the end with plasticine. The capillary tube was then placed in a microhaematocrit centrifuge machine with the closed ends outwards, and spun at 12,000 rpm for four minutes, depending on the model used. The capillary tube was then removed from the centrifuge machine and the buffy coat was examined under the light microscope at 100x magnification for *Trypanosoma* sp. (Manual of Veterinary investigation Laboratory Techniques, 1978). Upon microscopic examination, the common features of the blood protozoa were noted as described in Table 1.

The result and data were recorded and tabulated according to the type of blood protozoan infections and the five most

Table 1. Common features of the protozoa examined from a thin blood smear stained with Giemsa (Soulsby, 1985) and wet mount thick blood film stained with the new methylene blue (Manual of Veterinary Investigation Laboratory Techniques, 1978).

Blood protozoan	Description
Babesia bovis	Small piroplasm, 2.4um x 1.5um in diameter. Vacuolated signet ring forms.
Babesia bigemina	Large piroplasm, 4-5um in length x 2um wide, pear-shaped, lie in pairs forming an acute angle in RBC.
Babesia canis	A large piroplasm, pyriform in shape, 4u-5u in length, pointed at one end and round at the other. Frequently there is a vacuole in the cytoplasm. The pyriform forms may lie at an angle to one another, but pleomorphism of shape may be seen, organisms varying from amoeboid to ring forms.
Trypanosoma evansi	In the majority of infection, this parasite is monomorphic in character but polymorphism occurs sporadically. The typical form is indistinguishable from the slender form of *T. brucei*, being 15u-34u in length. The kinetoplast is subterminal, the undulating membrane is well developed and there is a substantial free flagellum.
Anaplasma marginale	Small spherical bodies, red to dark red in colour when stained with Romanowsky stains, found inside the red blood cell of the infected animals. They are 0.2u – 0.5u in diameter, with no cytoplasm, but a faint halo may appear around them.
Anaplasma centrale	Morphologically similar to possibly a variant of *A. marginale*. As its name suggests, it is centrally placed in the erythrocyte.
Theileria orientalis (previously called *T. mutans*)	Morphologically, this parasite is indistinguishable from other species of *Theileria*. The forms in the erythrocytes are round, oval, pyriform or anaplasma-like and measure 1u-2u in diameter.

Table 2: Total number of positive blood protozoa infection cases diagnosed by VRI from 1931-2010

Blood Protozoa diseases	Total positive cases
Theileriosis	3030
Leucocytozoonosis	413
Plasmodiasis	282
Babesiosis	263
Trypanosomiasis	242
Anaplasmosis	78
Ehrlichiosis	18
Hepatozoonosis	11
Haemogregarina	1
Total	**4338**

Table 3: The various host ranges of each blood protozoan diseases diagnosed by VRI from 1931-2010

Blood protozoan disease	Host species	Number of positive samples
Theileriosis	Cattle & Buffaloes	2871
	Sheep & Goat	122
	Deer	15
	Selembu	18
	Horse	4
	Sub-total	**3030**
Leucocytozoonosis	Poultry	413
	Sub-total	**413**
Plasmodiasis	Poultry	282
	Sub-total	**282**
Babesiosis	Cattle & Buffaloes	185
	Dog	78
	Sub-total	**263**
Trypanosomiasis	Cattle & Buffaloes	95
	Dog	78
	Horse	43
	Deer	21
	Pig	5
	Sub-total	**242**
Anaplasmosis	Cattle & Buffaloes	77
	Sheep	1
	Sub-total	**78**
Ehrlichiosis	Dog	18
	Sub-total	**18**
Hepatozoonosis	Dog	11
	Sub-total	**11**
Haemogregarina	Snake	1
	Sub-total	**1**
	Total	**4338**

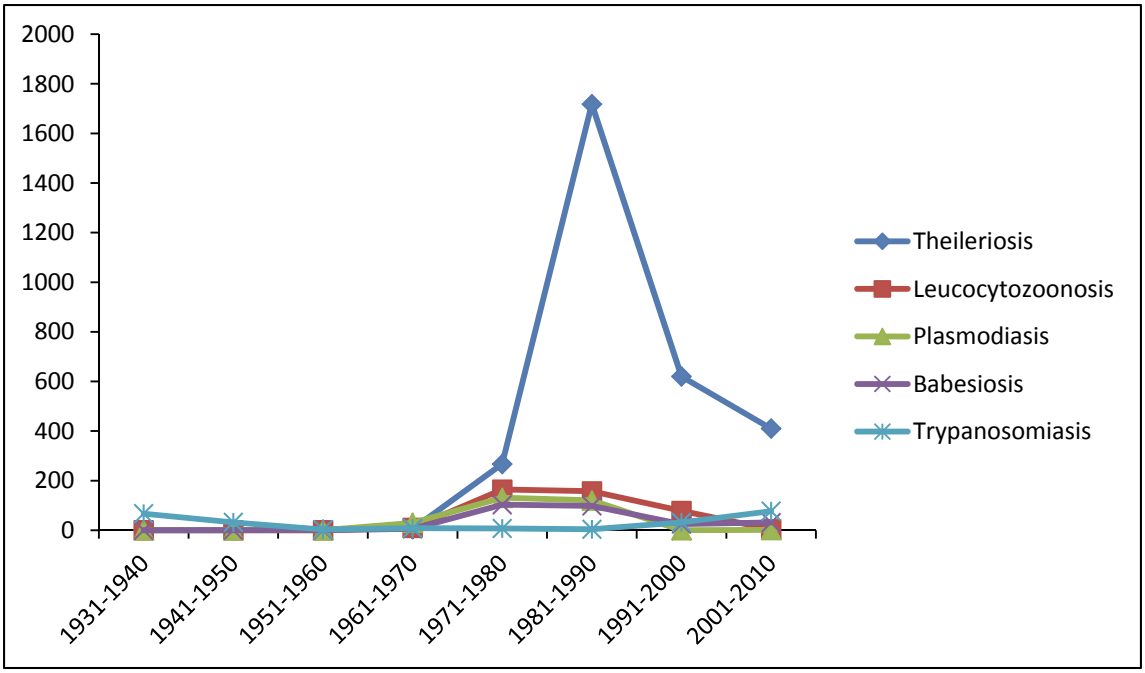

Figure 1: The five most significant blood protozoan diseases diagnosed by VRI from 1931-2010

significant blood protozoan infections with the highest number of reported cases were identified (Table 2). The data of the five most significant protozoan infections was tabulated according to a ten-year interval from 1931 to 2010 and their trends were identified (Figure 1). The host species for the significant protozoan infections were also identified and tabulated (Table 3).

RESULTS

A total 4338 received blood samples were diagnosed positive to blood parasitic infections as recorded by the Annual Reports of the Veterinary Services of Malaysia and Annual Reports of the Veterinary Research Institute, Malaysia (Table 2). Theileriosis was the commonest blood parasitic disease in domestic animals, followed by leucocytozoonosis, plasmodiasis, babesiosis, trypanosomiasis, anaplasmosis, ehrlichiosis, hepatozoonosis and haemogregarine infection. Among the blood parasites, *Trypanosoma* sp. harboured in a wide range of hosts including cattle and buffaloes, dogs, horses, deer and pigs (Table 3). With regards to *Theileria* sp., it was mostly observed in ruminants (cattle, buffaloes, sheep, goats, deer and selembu) and occasionally in horses. Apart from *Babesia* sp. (hosts: cattle, buffaloes, dogs) and Anaplasma sp. (hosts: cattle, buffaloes, sheep) which were reported to infect only three type of hosts, the rest of the blood parasites were

found more specific to a single host (i..e, *Leucocytozoon* sp. in poultry; *Plasmodium* sp. in poultry; *Hepatozoon* sp. in dogs; and *Hemogregarina* sp. in snake) (Table 3). In this paper, the focus was given to the top five commonest blood parasites reported in DVS and VRI from 1931 to 2010.

Theileriosis

Theileriosis has been considered to cause minor impact on the livestock industry, since there are no significant consequences to animal health, therefore there is a lack of clinical information focusing on this parasite. However, in the past eight decades in Malaysia, a total of 3,030 received samples were diagnosed positive for *Theileria* (Table 2). The sudden increase of Theileriosis has been observed in 1970s. The collected data demonstrated that *Theileria* positive samples were gradually elevated from as low as 3 cases in 1950's to the peak of the disease, whereby 1,718 cases were noted in 1980's (Figure 2). The increasing number of positive samples during these periods might be attributed to the importation of livestock from foreign countries, such as Australia (Rajamanickam, 1970).

Among the reported theileriosis cases through the 80 years (i.e., 1931-2010), 94.8% belonged to cattle and buffaloes, followed by 4.0% sheep and goats, 0.6% selembu, 0.5% deer and 0.1% horse (Table 3). In Malaysia, *Theileria orientalis* was the commonly reported species among domestic animals, as identified by VRI. In 1988, an epidemiological survey of bovine theileriosis was carried out and it was discovered that parasitemia ranged from less than 0.1% to 2% in 322 cattle collected from seven states in Malaysia (Kamio *et al*., 1990). In addition, 83.3% (20 of 24) of collected blood samples from the deer farm during the trypanosomiasis outbreak were positive to *T. orientalis* and the co-existence with *Trypanosome evansi* was also observed (Nurulaini *et al.,* 2007). The suspected vectors for theileriosis, such as *Haemaphysalis bispinosa* and *Boophilus microplus* were also observed in these studies (Kamio *et al.,* 1990; Nurulaini et al., 2007).

Theileriosis infection rate among the livestock (i.e. cattle) in Malaysia is high, similarly in imported temperate breeds of cattle. It has been reported that theileriosis in imported temperate breed of cattle was associated with the reduction of milk production (27.2%). However, theileriosis has the least impact compared to other diseases such as anaplasmosis, babesiosis and mixed infection and the recovery of cattle from theileriosis was reported to be high (i.e. up to 90%) (Rajamanickam, 1970).

Leucocytozoonosis

After theileriosis, the next widely reported blood parasitic disease in VRI was leucocytozoonosis and it was detected in 413 poultry blood samples. Leucocytozoonosis was firstly reported in VRI during 1961-1970 (Table 2). Similar

to theileriosis, the peak of the reported cases of leucocytozoonoosis occurred in 1971-1980 and 1981-1990. Subsequently, a slight decline of this disease was observed in 1991 until 2010 (Figure 1).

Among the reported *Leucocytozoon* sp. positive samples, a total of 26 samples were identified as *Leucocytozoon caulleryi* (data not shown). *L. caulleryi* infections have been reported in West Malaysia (i.e. Port Swettenham; Sungai Buloh, Selangor; Kuala Kangsar; Chemor, Perak; Pusing, Perak; Buntong, Perak; Penang; and Province Wellesley) in 1966 and 1967 (Omar, 1968). These leucocytozoonosis (*L. caulleryi*) were accompanied by other infections such as *Staphylococcus pyogenes*, *Plasmodium juxtanucleare*, Newcastle disease virus, fowl pox virus, caecal coccidiosis and ascaridiasis.

Apart from *L. caulleryi*, *Leucocytozoon sabrazesi* was observed among poultry in Malaysia (Omar, 1968) and it has been detected in Perak and Johor. Co-infections of *L. sabrazesi* and other blood parasites seemed more common as reported in a plasmodiasis outbreak in 1961 (Omar and Ismail, 1962) and four of the *P. juxtanucleare* infection cases at 1966 and 1967 (Omar, 1968).

Plasmodiasis

Plasmodiasis was the third most common disease reported in VRI with a total of 284 *Plasmodium* sp. positive samples (Table 2) comprising of 226 chicken and 8 pigeon, while 49 samples were recorded as poultry in general (data not shown). Apart from that, a human *Plasmodium* positive sample was also reported in VRI. All the recorded samples were generally reported as plasmodiasis and there were five samples successfully identified as *P. juxtanucleare* in chicken (data not shown).

Plasmodiasis was first observed in 1961-1970. Then in the next two decades (i.e. 1971-1980 and 1981-1990), plasmodiasis cases exceeded 120 but has since then dropped drastically to nil. (Figure 1). An outbreak of plasmodiasis occurred in April of 1961 at a poultry farm (majority chicken White Leghorn crosses) located in Puchong, Kuala Lumpur (Omar and Ismail, 1962). A total 110 out of 200 poullets died during the outbreak. The infected chickens demonstrated several clinical signs including paleness on the parts of combs and wattles, lethargy, stopped taking feeds and were found dead after three to four days. During the investigation, *Plasmodium gallinaceum* was identified based on microscopic examination on the thin blood smears (Omar and Ismail, 1962). In addition, several outbreaks (1964 to 1966) due to *P. gallinaceum* were also reported in Penang (7 outbreaks) and Kajang, Selangor (1 outbreak) (Omar, 1968). These outbreaks contributed to 2,500 deaths. Apart from that, *P. juxtanucleare* infection in chicken has been reported in White Leghorn breed with heavy parasitemia and co-existence with other protozoan parasites (i.e. *L. caulleryi*, *L. sabeazesi*) (Omar, 1968). However, due limited accessible studies

focusing on plasmodiasis in poultry, the trends of this particular disease remain unknown.

Babesiosis

Babesiosis was the top four reported blood parasitic diseases in the past 80 years. Similar to leucocytozoonosis and plasmodiasis, reports of positive cases started between 1961 and 1970 (Figure 1). *Babesia* has been detected in cattle, buffaloes and dogs and its vectors have been recognized as *Boophilus* sp and *Haemaphysalis* sp. The present result showed a total number of 264 cases of babesiosis diagnosed by VRI from 1931-2010 (Table 2). Of these 264 cases, 186 cases were diagnosed from cattle and buffaloes while 78 cases were from domestic dogs (Table 3).

Bovine babesiosis comprised 70.5% or 186 of the total *Babesia* positive samples diagnosed by DVS and VRI in the past 80 years. The data showed that bovine babesiosis was first reported in 1951-1960 (the number of samples was not recorded). Thereafter, the number of babesiosis sharply increased in 1971-1980 and 1981-1990 (achieved 80 samples in each decade) and dropped to 8 and 12 positive samples in the past two decades (1991-2000 and 2001-2010, respectively). Based on the current data, *Babesia bigemina* and *Babesia bovis* were the only species identified among the diagnosed samples. Both species were the most widespread species in cattle throughout Peninsular Malaysia (Chandrawathani *et al*., 1994).

Bovine babesiosis is common in the local breed of cattle due to the lack of proper tick management (Rahman *et al*., 2010). This particular disease was also observed in imported cattle from temperate countries such as Australia, in 1970's (Rajamanickam, 1970). Among the 118 imported cattle, 29 (24.6%) died due to various diseases but 12 (8.5%) died of suspected babesiosis (Rajamanickam, 1970). However, babesiosis in imported cattle has now decreased or even disappeared. This situation might be due to the supply of high quality foods, constant monitoring and deployment of de-ticking programmes as well as the use of vaccination for tick fever on imported cattle (Chandrawathani, 2000).

In addition, the present data demonstrated that the number of bovine babesiosis samples has decreased after the year 1991. However, a comprehensive serological study covering 9 states in Peninsular Malaysia reported contrasting results. The serological test on 271 bovine serum samples revealed that 74.4% were positive to *B. bovis* and co-occurrence of *B. bovis* and *B. bigemina* occurred in 72.6% of tested samples (Chandrawathani *et al*., 1994). In addition, a very low *Babesia ovata* infection was also reported (Chandrawathani *et al*., 1994). In addition, the World Organization for Animal Health Report (2008) demonstrated 29 outbreaks in 1996, 15 outbreaks with 17 deaths in 1997, 3 outbreaks with 4 deaths in 1998,

15 outbreaks with 11 deaths in 1999 but a sharp increase of bovine babesiosis cases (263 cases) in 8 outbreaks reported in 2001. Recently in 2010, another seroprevalence study was conducted on 100 serum bovine samples collected from all states in Malaysia obtained from Serological Unit, VRI, Malaysia reported a low prevalence of *B. bovis*, *B. bigemina* and 9% for the co-occurrence of both species (Rahman et al., 2010). The decline of the bovine babesiosis cases might also be due to the better husbandry management systems, veterinary care and availability of drugs for treatment (Rahman *et al.*, 2010).

It is clearly demonstrated that babesiosis in dogs was endemic in Malaysia from 1971 to 2010. The current data showed that *Babesia gibsoni* and *Babesia canis* were the two species identified from dog samples. However, the prevalence of *B. gibsoni* and *B. canis* remained low in Malaysia and it was supported by several studies in Malaysia. During the nine years study (from 1973 to 1981) in small animal clinics of Kuala Lumpur, Petaling Jaya and Klang, 17.7% of *B. gibsoni* and 1.1% of *B. canis* have been detected from a total of 4,084 urban dogs (Rajamanickam *et al.*, 1984/85). Furthermore, within the last ten years, several studies also reported a low prevalence of babesiosis among stray dogs (0 to 4%) (Lim, 2007; Yeoh, 2009) and owned dogs (1.82 to 10.0%) (Lim, 2007; Yap, 2004). However, the morphologically based diagnostic technique (i.e. microscopy screening) might contribute to misidentification of *Babesia* species, therefore, the gold-standard tool, Polymerase Chain Reaction (PCR) assay can been performed for this particular purpose. In Malaysia, only few studies have applied the molecular technique on the detection of *Babesia* species in animals, PCR amplification based on 18SrRNA gene revealed that 2.85% prevalence among stray dogs and the sequences analysis shows the amplified *B. gibsoni* have closer relationship to the other *Babesia* species, namely *Babesia vogeli* (Zulkifli, 2011). In addition, a more recent study focusing on the molecular detection of *B. gibsoni* and *Anaplasma platys* reported low *B. gibsoni* (3.3% of 30 dogs) infection among the local mongrel dogs which were captured in an animal shelter (Mokhtar *et al.,* 2013).

Trypanosomiasis

Trypanosomiasis was the fifth most common blood protozoan parasitic disease diagnosed by DVS and VRI in Malaysia. The present data demonstrated that trypanosomiasis is endemic in dogs, horse, cattle and buffaloes since 1931 (data not shown). Furthermore, this disease was also reported in pig (since 1995) and deer (since 2000). Generally, the number of trypanosomiasis positive samples has been consistent through the eight decades (Figure 1). The reports showed that there were a total number of 309 trypanosmiasis cases, involving several hundred samples, diagnosed by VRI from 1931-2010, which comprised of 242 from local cattle and

buffaloes, 78 cases from domestic dogs, 43 cases from horses, 21 cases from deer and 5 cases from pigs (Table 1 and Table 3). Although there were no identified trypanosome species recorded, previous studies reported that *Trypanosome evansi* was the dominant *Trypanosome* found among the livestock in Malaysia (Buniamin *et al.*, 2002; Cheah *et al.,* 2000; Rahman *et al.,* 2002).

Bovine trypanosomiasis is the most common typanosomiasis reported in Malaysia. OIE World Animal Health Information Database (WAHID) documented the outbreaks of bovine trypanomiasis in Malaysia, particularly in Sabah (i.e. first half of 2005, March 2007, July 2008, January 2009) and Johor (i.e. January 2009). In addition, another trypanosomiasis outbreak has been reported in early 2012 in Perak. During the outbreak, a total 102 whole blood samples (46 of deer, 20 of cattle, 20 of buffaloes, 16 of pigs) were collected from neighbouring farms (Nurulaini *et al.*, 2013). The routine diagnosis revealed that 61.8% of tested samples (73.9% of deer, 55% of cattle, 25% of buffaloes, 81.3% of pigs) were positive for trypanosome infection. The outbreak was attributed to the high population of biting flies (i.e., *Tabanus* sp. and *Stomoxys* sp.) in these farms during the wet rainy seasons, which transmitted the *Trypanosome* (Nurulaini et al., 2013).

Swine trypanosomiasis was first detected in one of the samples received in VRI in 1995. An outbreak occurred in a pig farm in Malaysia causing mortality in 11 sows and a boar as well as abortions in more than 20 sows (Arunasalam *et al.*, 1995). After the investigation on six pigs in the farm, *T. evansi* was identified as a causative agent in this outbreak. This could be due to infection of *T. evansi* transmitted by biting flies from a nearby buffalo farm which was 3 km away from the pig farm, wherein buffaloes are noted to be efficient asymptomatic carriers of trypanosomiasis (Arunasalam *et al.*, 1995).

It is important to note that trypanosomiasis outbreaks are common in Malaysia and the *Trypanosome* (i.e., *T. evansi*) infections among animals were suspected transmitted by biting flies rather than blood-sucking ticks (Nurulaini *et al.,* 2007). In 2006, 17 adult java deer in government deer farm located in Lenggong, Perak were reported dead a week after suffering from clinical signs of dullness, inappetence, anaemia, anorexia, respiratory distress and recumbency. Post-mortem on the deer carcasses and blood samples from the remaining surviving deer also showed *T.evansi* as the main parasite contributing to the high mortality in the deer farm. Apart from this outbreak, *Trypanosome* outbreaks and infections were also reported in horses (Ng and Vanselow, 1978), cattle and buffaloes (Sani *et al.,* 1989; Zary, 1999; Ahmad Hafiz *et al.,* 2002; Rahman *et al.*, 2012) in Malaysia.

Other blood parasitic diseases

Apart from the top five blood parasite diseases in Malaysia as described above,

several diseases (anaplasmosis, ehrlichiosis, hepatozoonosis, haemogragarine infection) were also reported with less than 100 cases during the eight decades. Among the diseases, *Anaplasma* infection was observed in 78 blood samples received in VRI of which 15 cattle samples were identified as *Anaplasma marginale*. With regards to ehrlichiosis and hepatozoonosis, both diseases were reported in dogs and infected by the species *Ehrlichia canis* and *Hepatozoon canis*, respectively. The lowest number of detected blood parasite, *Haemogregarina* sp. was reported in a snake in Malaysia in 2008, however, due to the serious deficiency of the data to this infection in Malaysia, the trend of this disease remains unknown.

GENERAL DISCUSSION

A number of limitations need to be acknowledged in the present study. First of all, in many cases the clinical history is unclear and the animal may have concurrent illnesses which are not reported or recorded. Besides, the results also depended on the variation of technician's diagnostic expertise over the eight decades and the availability of diagnostic technology to detect these blood protozoan parasites. Furthermore, submission of samples was also solely depended on the veterinarians or veterinary representatives from each farm and clinic. There may be instances whereby they only submitted samples extracted from the clinically sick animals, excluding the possibility of the sub-clinically infected animals.

Table 2 shows the total number of positive blood protozoa infection cases diagnosed by VRI from 1931-2010. The five most common and significant protozoan diseases seen over the eight decades in domestic animals of Malaysia were theileriosis, leucocytozoonosis, plasmodiasis, babesiosis and trypanosomiasis.

Several thousand samples have been tested during this period and Figure 2 shows the trend and seriousness of the five most significant protozoan diseases over that period of time from 1931-2010. There were a total of 3030 samples positive for theileriosis, of which 2,817 cases are from cattle and buffaloes, 122 cases are from sheep and goat, 15 cases are from deer, 18 cases are from Selembu and 5 cases were diagnosed in pig. *Theileria sp.* is the most common non-species specific protozoan parasite diagnosed. Since it is a non-pathogenic organism, its existence is usually ignored. As *Theileria* is benign, farmers seldom treat the condition unless it is accompanied by anaemia and other observable clinical symptoms.

With regards to babesiosis, *Babesia* organism has been detected in cattle, buffaloes and dogs, and the recognised vectors include *Boophilus* sp. and *Haemaphysalis* sp. A total of 263 cases of babesiosis were diagnosed by VRI from 1931-2010, which comprised of 185 cases diagnosed from cattle and buffaloes while 78 cases from domestic dogs.

From the results, both trypanosomiasis and babesiosis was observed in the early 1980's, most probably due to the ruminant importation programme which led to a large number of temperate breeds of cattle being brought into this country, thus acting as a source of infection to the susceptible animals in Malaysia. Besides this, newly established laboratories and techniques could heighten the accuracy of the diagnosis of blood protozoan diseases in samples submitted for diagnosis. The trend of both blood protozoan diseases, however, declined in 1991 to 2010, as more veterinarians were available to diagnose diseases clinically and there was a wide variety of drugs available for treatment. Besides this, more animals from Thailand were being imported from the year 2000 onwards , most of which were crossbred tropical breeds which can withstand tick burdens and more acclimatized to tropical climates. As for babesiosis in canines, small animal practitioners sent blood specimens for diagnosis for export purposes as well as clinical diagnosis as this involves a skill in identifying Babesia organisms in red blood cells , which needs expertise and familiarity available in the VRI, thus more babesiosis cases were reported compared to other host species. The trend of these blood protozoan diseases seen in dogs declined after 1990s due to the rapid establishment of the private clinics and personal laboratories, therefore fewer samples were sent to VRI and thus the number of cases reported from VRI reduced. Several temperate cattle breeds from Australia, South America and Africa as well as their crosses were used for breed improvement and production, and this posed new challenges for farmers in vector control and protozoan disease management. In the ruminant and domestic dog industry, there was much dependence on the government diagnostic laboratory instead of private diagnostic laboratory. It was noted that there were less positive samples from the poultry and swine industry as they depended on the private diagnostic laboratories which emphasises confidentiality status. Trypanosomiasis and Babesiosis was observed especially in imported cattle within a few months of arrival until they are acclimatized. From the results, it is seen that there were a total number of 242 trypanosmiasis cases diagnosed by VRI from 1931-2010, which comprise of 95 from local cattle and buffaloes, 78 cases from domestic dogs, 43 cases from horses, 21 cases from deer and 5 cases from pigs. The infection is mechanically transmitted by blood-sucking insects of the genera *Tabanus, Stomoxys, Atylotus* and *Lyperosia*. By nature, trypanosomiasis is subclinical in cattle, buffaloes and horses as well as dogs, but occasional outbreaks of acute disease might transmit to other susceptible species such as deer and pigs, leading to sudden deaths and development of its reservoir. These animals are also considered to be the main reservoirs of horse infection, when there were no strict screening procedures being enforced when importing these exotic breeds (Shalm & Reed, 1998).

It is obvious that the increasing trend of trypanosomiasis cases from 1931 to 1950, was most probably recognised in the laboratory diagnosis as productivity was affected in terms of morbidity and mortality, therefore samples were routinely sent in for laboratory diagnosis and thus the number increased. The trend was found to be decreasing from 1951-1990 probably due to treatment administered to the infected animals as well as preventive measures taken to prevent further infection to the animals. Treatment such as berenil and ectoparasiticides was given simultaneously as a measure of vector control in most of the farms. However, the trend was seen slightly increasing from the year 1991 to 2010 probably due to the development of resistance of *Trypanosome* sp. towards the intensive utilization of the same drug as the treatment option over the past 40 years.

Leucocytozoon sp. and *Plasmodium sp.* were the only blood protozoans detected in poultry. The majority of cases were diagnosed after 1965 with the setting up of the Parasitology division in VRI and acquisition of trained technical expertise in the form of technicians and veterinarian. After 1970s, more samples were sent in with the development of the livestock and poultry industry in Malaysia. Dramatic growth and development of the poultry industry caused the introduction of various diseases which included blood protozoan diseases. However, with the improvement of management and disease control programme, these protozoan parasitic infections dropped dramatically in the year 1990.

CONCLUSION

The studies showed that the five most significant blood protozoan diseases in domestic animals of Malaysia over the eight decades from 1931 to 2010 were theileriosis, babesiosis, leucocytozoonosis, plasmodiasis and trypanosomiasis, some of which, are still common diseases persisting until today, even with modern farm management and control methods. It is also observed that these blood protozoan diseases affect not only a single host but having a wide host range and intermediate vectors, whereby cattle is the most susceptible host to most of these protozoan infections such as theileriosis, babesiosis and trypanosomiasis, followed by poultry which was susceptible to leucocytozoonosis and plasmodiasis. This showed that treatment of infected animals does not guarantee that these protozoa would be completely eliminated and animals safe from protozoan diseases. The analysis of these data also showed that there was an increasing trend of all these five significant blood protozoan diseases from 1961-1990, with the most probable reason being the expansion of laboratory services at this point of time whereby several cases were submitted for diagnosis of these diseases. The decreasing trend from 1990 to 2010 could most probably be due to higher level of awareness among the farmers and preventive measures implemented which

had greatly controlled the transmission of these protozoan diseases among the domestic animals in Malaysia. Government sectors and institutions such as VRI play a major role in diagnosing these significant blood protozoan diseases, thus, it is essential to provide good diagnostic services with expertise and knowledge that is able to garner confidence in the public and private veterinary sectors. Obviously, clinical and laboratory expertise as well as sustainable practices can significant reduce the incidence of diseases. Besides, it is equally beneficial for the government veterinary sectors and research institutions to work closely with the human health sectors and private laboratories to establish a diplomatic and informational yet sustainable relationship in order to investigate and control these vital diseases. This will in turn help boost food production by increasing productivity at the farm level.

REFERENCES

1. Ahmad Hafiz B, Wahab AR, Zaini CM and Chandrawathani P (2002). The incidence of *Trypanosoma evansi* in cattle and buffaloes around Ipoh. In: *Proceedings of the 12th Federation of Asian Veterinary Association Congress,* Kuala Lumpur. P 181.
2. Arunasalam V, Chandrawathani P, Sivanandan S (1995). An outbreak of *Trypanosoma evansi* infection in pigs. *J. Vet. Malaysia* **7**(2): 71-73.
3. Buniamin AH, Rahman AWA, Zaini CM and Chandrawathani P (2002). The incidence of *Trypanosoma evansi* in cattle and buffaloes around Ipoh. In: *Proceedings of the 12th Federation of Asian Veterinary Association Congress* 26-28 August 2002, Selangor.
4. Chandrawathani P (2000). Status of babesiosis in Malaysia. In: *Proceedings of the Third Obihiro International Symposium on Protozoan Diseases*. August 31- September 1, 2000. National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.
5. Chandrawathani P, Tsuji N, Kawazu S, Ishikawa M, Fujisaki K (1994). Seroepidemiology studies of bovine babesiosis caused by *Babesia ovata*, *B. bigemina* and *B. bovis* in Peninsular Malaysia. *Journal of Veterinary Medical Science* **56**(5): 929-932.
6. Cheah TS, Chandrasekaran S, Ramlan M and Chandrawathani P (2000). Humoral Response to Haemorrhagic Septicaemia Alum Precipitated Vaccine in Cattle experimentally infected with *Trypanosoma evansi*. In: *Proceedings of the 12th Veterinary Association Malaysia Scientific Congress*, 14 Sept. 2000, Kuantan, Malaysia.
7. Soulsby EJL, Helminths, Arthropods & Protozoa of Domestic Animals (6th Edition)
8. Kamio T, Rajamanickam C, Kawazu SI, Fujisaki K (1990). Epidemiology and pathogenicity of bovine theileriosis in Malaysia. JARQ. *Japan Agricultural Research Quarterly* **24**(3): 231-234.
9. Lim CW (2007) *Prevalence of blood parasites in canine pet and stray populations: prevalence and effect on hematological parameters*. DVM Thesis. Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang. Unpublished data.
10. Mokhtar AS, Lim SF, Tay ST (2013). Molecular detection of *Anaplasma platys* and *Babesia gibsoni* in dogs in Malaysia. *Tropical Biomedicine* **30**(2): 345-348.
11. Ng BKY and Vanselow B (1978). Outbreak of surra in horses and the pathogenesis of the anaemia. *Kajian Veterinar* **10**(2): 88-98.
12. Nurulaini R, Jamnah O, Adnan M, Zaini CM, Khadijah S, Rafiah A, Chandrawathani P (2007). Mortality of domesticated java deer attributed to Surra. *Tropical Biomedicine* **24**(2): 67-70.
13. Nurulaini R, Premaalatha B, Zaini CM, Adnan M, Chandrawathani P, Fazly Ann ZA, Enie Aryanti A, Ramlan M (2013). Trypanosomiasis outbreak in deer, cattle, buffaloes and pigs in Perak. *Malaysian Journal of Veterinary Research* **4**(1): 55-58.
14. Omar AR (1968). Haemoprotozoan infections of poultry in Malaysia. *Kajian Veterinaire* **1**(3): 109-124.
15. Omar AR, Ismail Y (1962). *Plasmodium gallinaceum* infection among chickens in Malaya. *J. Malay. Vet. Med. Assoc.* **3**: 75-80.
16. Rahman AWA, Yahaya ZS, Cheah TS and Chandrawathani P (2002). An assessment of the prevalence of *Trypanosoma evansi* in cattle from some farms in Perak, Peninsular Malaysia, using the QBC technique. *Jurnal Biosains* **13**: 35- 37.

17. Rahman WA, Fong S, Chandrawathani P, Nurulaini R, Zaini CM, Premalaatha B (2012). Comparative seroprevalences of bovine trypanosomiasis and anaplasmosis in five states of Malaysia. *Tropical Biomedicine* **29**(1): 65-70.
18. Rahman WA, Lye YP, Chandrawathani P (2010). The seroprevalence of bovine babesiosis in Malaysia. *Tropical Biomedicine* **27**(2): 301-307.
19. Rajamamickam C (1970). Blood protozoan diseases of imported temperate breeds of cattle in West Malaysia. *Kajian Veterinaire, Malaysia-Singapore* **2**(3): 145-152.
20. Rajamanickam C, Wiesenhutter E, Zin FMD, Hamid J (1984/85). The incidence of canine hematozoa in Peninsular Malaysia. *Veterinary Parasitology* **17**: 151-157.
21. Sani RA, Chandrawathani P and Rosli M (1989). Gastrointestinal parasites and *Trypanosoma evansi* in buffaloes. In: *Proceedings of the final research co-ordination meeting on the use of nuclear techniques to improve domestic buffalo production*, Australia. pp. 201-212.
22. Schalm Charles J Price, FAO and Josephine E Reed (1998). Veterinary Haematology by & Practical Parasitology – General Laboratory Techniques and Parasitic Protozoa (3rd edition)
23. Yap BK (2004) *Survey of blood protozoon and Ehrlichia spp. in dogs in Klang, Selangor.* DVM Thesis. Faculty of Veterinary Medicine and Animal Science, Universiti Putra Malaysia, Serdang. Unpublished data.
24. Yeoh WH (2009) *Blood parasites in dogs in Klang Valley: Prevalence and diagnosis.* DVM Thesis. Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang. Unpublished data.
25. Zary Shariman Y (1999). *Kadar prevalens Trypanosoma evansi di ladang ternakan lembu di sekitar kawasan Ipoh*. BSc thesis, Universiti Sains Malaysia.
26. Zulkifli AR (2011) *Molecular study of Babesia in canine blood and comparison between conventional and molecular diagnostic methods.* DVM Thesis. Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang. Unpublished data.