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# Comparison between Quantitative Buffy Coat (QBC) and Giemsa-stained Thin Film (GTF) technique for blood protozoan infections in wild rats

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**Abstract**. The quantitative buffy coat (QBC) technique and conventional Giemsa thin blood smear was compared to determine the sensitivity and specificity of the technique in detecting blood parasitic infection of the rodent populations from four urban cities in Peninsular Malaysia. A total of 432 blood samples from four rat species *(Rattus norvegicus*, *Rattus rattus diardii*, *Rattus exulans* and *Rattus argentiventer)* were screened using both techniques and successfully detected two blood protozoan species (*Trypanosoma lewisi* and *Plasmodium* sp.*)* with *Trypanosoma lewisi* predominantly infecting the population. Results showed that Giemsa-stained thin film (GTF) was the better detection method on blood parasitemia (46.7%) compared to Quantitative Buffy Coat method (38.9%) with overall detection technique sensitivity and specificity at 83.2% and 74.8% respectively. The sensitivity in detection of *Trypanosoma lewisi* was 84.4% with value slightly lower for *Plasmodium* sp. infections at 76.6%. Statistical analysis proved that GTF technique was significantly more sensitive in the detection of blood protozoan infections in the rodent population compared to QBC ($p<0.05$).

## INTRODUCTION

Many studies worldwide have adopted quantitative buffer coat (QBC) technique mainly for the diagnosis of malaria in human patients including Ethiopia (Spielman *et al.*, 1988), Philippines (Rickman *et al.*, 1989), Thailand (Pornsilapatip *et al.*, 1990), Venezuela (Bosch *et al.*, 1996), Nigeria (Adeoye & Nga, 2007) and Malaysia (Mak *et al.*, 1992; Alias *et al.*, 1996).

Quantitative buffy coat (QBC) technique was intended mainly for the diagnosis of *Plasmodium* spp. only (Ferreira *et al.*, 2006), meanwhile, Amato Neto *et al.* (1996) successfully evaluated the practical use of QBC for the parasitological diagnosis of *Trypanosoma cruzi* with regards to its sensitivity and practicality. Other similar studies used this technique to detect *Trypanosoma* spp. as well as for the diagnosis of trypanosomiasis (Bailey & Smith, 1992; Truc *et al.*, 1992, 1994; Ana Marli *et al.*, 2002). Zainal Abidin & Noor Azmi (1999) and Zainal Abidin & Yit Ming (1994) were the only studies which successfully adopted this method to detect *Trypanosoma lewisi* infection in blood of wild rats in Malaysia.

Quantitative Buffy Coat (QBC) technique was developed in 1983, using a capillary tube precoated with acridine orange for the rapid quantification of leucocytes in peripheral blood by staining all nucleic acid containing cells and the associated fluorescence is observable under blue-violet light through a microscope. This technique was then successfully applied for the diagnosis of malaria (Sodeman, 1970; Spielman *et al.*, 1988; Levine *et al.*, 1989) as the acridine orange staining permits differential coloration of green (nucleic) and red (cytoplasm) in stained parasites.

Spielman *et al.* (1988) found that the QBC method was at least eight times more sensitive compared to Giemsa stained thick blood smears. This technique successfully detected 10% more infected samples when compared to thick blood smears (Spielman *et al.*, 1988). In Thailand, Namsiripongpun *et al.* (1991) found the QBC had a sensitivity of 99.1% and a specificity of 96.8% in a laboratory setting. Rickman *et al.* (1989) noted sensitivity of detection in patients as few as 3 parasite/µl indicating a sensitivity of 96% (when all stages of malaria were considered) and specificity of 93% for analysis of hospital group patient. In contrast with their field study, the QBC technique had a sensitivity of 70% and a specificity of 98.4% (Rickman *et al.*, 1989). Similar result found from a field study by Mak *et al.* (1992) with 55.93% sensitivity and 94.92% specificity when the QBC tube method was compared with thick blood smears.

In contrast, diagnosis using Giemsa thick and thin blood smear technique is time consuming and requires experience for adequate preparation and interpretation. However, this technique is affordable, yet sensitive and specific in determining parasite rates, parasite densities and diversity. Another advantage is that specimens can be stored, re-examined and kept for future references unlike QBC.

Therefore the main objective of this study was to compare the sensitivity of two methods (Quantitative Buffer Coat and Giemsa Thin Film) in the detection of blood protozoan infection in the wild rat population of Peninsula Malaysia.

## MATERIALS AND METHODS

This section of study was a continuation of an earlier epidemiology study of blood parasitic infections in the urban rat population in Peninsula Malaysia (Alias *et al.*, 2014). This study was conducted primarily to compare two methods in detection of blood protozoan infection i.e.; Quantitative Buffer Coat (QBC) and Giemsa Thin Film (GTF).

**Study sites**

Fieldwork was carried out in four major cities with each location representing different unique geographical location in Peninsula Malaysia namely; Kuala Lumpur (3°82513N 101°412363E) representing the west, Pahang (3°492003N 103°202003E) representing the east, Penang (5°252003N 100°192003E) representing the north and Malacca (2°122N 102°152E) representing the south of Peninsular Malaysia. All sites were characterized by a tropical climate and high humidity throughout the year with temperatures ranging between 30°C and 36°C. Rainfall occurred periodically throughout the year with some months experiencing more rainfall than others. For this purpose, season was divided into wet and dry seasons for each year with dry months falling between January-March and June-September and wet months between April-May and October-December.

Trapping was conducted between November 2006 and November 2011 with the assistance of the municipality from each city as part of the vector control programme. All the rats were trapped alive using custom made steel wire traps measuring 29 x 22 x 50 cm using dried fish, sweet potatoes, fruits and coconut as baits. Caught rats were killed humanely by placing them into a cloth bag containing cotton wool soaked with chloroform. The study approach was approved by University of Malaya ethical committee reference number ISB/31/1/2013/SNMZ(R). Morphometric measurements of head-body, tail, ear, hind foot, weight and physical appearances were recorded. Host age (adult, sub-adult and juvenile) sex (male and female) and species for all rats were determined based on descriptions by Harrison & Quah (1962), Medway (1983) and Payne & Francis (1998).

**Giemsa-stained Thin Film (GTF)**

Blood was collected via heart puncture using a needle and syringe. A drop of blood was used to make a thin blood smear. The thin smear was fixed on a microscope slide by immersing in pure methanol for 1 minute.

Thereafter the slide was immersed to a solution of 1 part Giemsa stock to 20-30 parts of buffered water (pH 7.0 – 7.2), 20-30% Giemsa stain solution for 20-30 minutes, then flushed with water and left to air dry. The slide was mounted permanently with Depex or Canada balsam and examined under light microscopy with 400x magnification and 1000x magnification under oil immersion. Each slide was examined for gametocytes and stages of the schizogonic cycle with a total of 3 slides prepared for each rat.

**Quantitative Buffy Coat Technique (QBC)**

The QBC glass capillary tube (Becton Dickinson) is 75 mm in length and 1.677 mm in diameter (Wardlaw & Levine, 1983). The tubes are internally coated with EDTA and heparin at the fill end and with acridine orange stain and potassium oxalate at the other end. A total of 55-65 µl of blood was transferred to the QBC tube via capillary action and slowly tilted and rotated for about 10 seconds between fingers to dissolve the contained residues in the blood before the tube was fitted with a cap and labeled. Expansion of the centrifugally separated cell layers was achieved with a 20 mm plastic float. After the plastic float was inserted into the tube, approximately 40 µm of space exist between the exterior and the interior float. The plastic float has a specific gravity (1.055) that is midway between that plasma (1.028) and red blood cells (1.090). Blood filled QBC tubes were centrifuged in a QBC micro-haematocrit centrifuge (Parafuge, Becton Dickinson Inc., Franklin Lakes, New Jersey, USA) at 12,000 g for 5 minutes. The float expanded the buffy coat and the constituents of the blood then separated into bands according to density. The QBC tubes were placed in the notched Lucide block or an acrylic holder and the area surrounding the float just beneath the buffy coat was examined under ultraviolet light-source using a Paralens adapter which provided the UV light through a fiber cable connected to a light compound microscope. Individual cells within the layers can be easily seen via microscopy; with the parasites present stained green (DNA) and orange (RNA) under blue-violet light. Nucleic acid and glycosaminoglycans bounded to acridine orange will fluoresce when exposed to light with a wavelength of 490 nm. The entire circumference of the tube was examined systematically while moving away from the buffy coat through the erythrocyte layer. The tube was examined at four quadrants by turning the tube and between 1-4 minutes was spent on each tube. No attempt was made to quantify the parasitaemia in the QBC tubes. Parasites when present were identified through their morphological characteristics.

**Data analyses**

Data collected was analyzed using SPSS version 16.0. The results are presented as prevalence. The total data comprised of three intrinsic factors (host species, sex and age) and two variables (prevalence of infection using QBC and GTF method). In all cases significance was set at $p<0.05$. Non parametric test was performed as the data collected is nominal and non- normally distributed to determine the sensitivity between the two techniques (QBC and GTF) in the detection of blood protozoa. The analyses began with the changing of host species label into nominal data (1= *Rattus norvegicus*; 2= *Rattus rattus diardii*; 3= *Rattus exulans*; 4= *Rattus argentiventer*). Similarly, numerical values were given to the two techniques in the detection of blood protozoa with 3 levels (0= negative for all parasites, 1= positive for one parasite, 2= positive for both parasites). In all cases significance was set at $p< 0.05$.

Following this, parasitic infections were analyzed using Binary Logistic Regression to determine the effect of factors (host species, age and sex) on the prevalence of blood parasites harboured by the rodents. The dataset was grouped accordingly to two variables i.e; the detection technique and the parasite recovered. The groupings; QBCTry/ GTFTry and QBCPlasmo/ GTFPlasmo was put as the dichotomous criterion variables and the host species, age and sex were set as dichotomous predictors variables. We then coded the categorical variables (0=

absent of blood parasites and 1= present of blood parasites) and species (1= *Rattus norvegicus*; 2= *Rattus rattus diardii*; 3= *Rattus exulans*; 4= *Rattus argentiventer*), sex (1=M; 2= F) and age (1=Adult, 2= Sub-adult; 3= Juvenile). The model involving all main effects and interaction combination stepwise, and the significance of its contribution was noted. The dependent variable was analyzed in turn but each was replaced in the model before evaluating of the remainder. The model was then re-run, including those combinations which had shown significance (maximum likelihood), to locate at which significant variation resided within the factors. The probability was predicted based on the log linear analysis of contingency tables, the result were given in the text as $\chi 2$ value associated with probabilities in full logistic regression model.

For calculation of technique sensitivity and specificity, the thin blood smear count was used as standard. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and concordance were calculated as follows:

$$\text{Sensitivity} = \frac{\text{Number of specimens positive by both QBC \& thin smears}}{\text{Total number of positive thin smears}} \times 100$$

$$\text{Specificity} = \frac{\text{Number of specimens negative by both QBC \& thin smears}}{\text{Total number of negative thin smears}} \times 100$$

$$\text{PPV} = \frac{\text{Number of positives by both QBC \& GTF}}{\text{Total number of positives by QBC}}$$

$$\text{NPV} = \frac{\text{Number of negatives by both QBC \& GTF}}{\text{Total number of negatives by QBC}}$$

$$\text{Concordance} = \frac{\text{Number of positives and negatives by both QBC \& GTF}}{\text{Total no. of samples examined}}$$

## RESULTS

A total of 432 wild rats from four rat species *(R. norvegicus, R. rattus diardii, R. exulans* and *R. argentiventer)* were captured from the surrounding areas of urban wet markets of Kuala Lumpur, Malacca, Penang and Pahang during dry and wet seasons (Table 1). *Rattus norvegicus* (60.6%) was the dominant species followed by *R.rattus diardii* (36.8%), *R. exulans* (1.9%) and *R. argentiventer* (0.7%). More females were captured (51.6%) compared to males (48.4%) with higher number of adults (75.2%) followed by juveniles (13.7%) and sub-adults (11.1%). The rat population was dominated by two main species namely; *R. norvegicus* and *R. diardii*. The numbers of *R. exulans* and *R. argentiventer* trapped did not exceed more than 3% of the total captured and therefore not considered further in the statistical analyses.

Table 2 summarized the overall blood protozoan prevalence of infection in the rodent population. Results showed that Giemsa-stain thin film (GTF) technique recorded a total of 226 (52.3%) infected blood samples while 202 (46.8%) samples were detected by Quantitative Buffy Coat (QBC). Statistical analysis showed high significant association ($p<0.05$) between the techniques used for blood protozoan detection with GTF the better detection technique ($\chi 2= 13.8$, df= 1, standard residual value= 67.0), compared to QBC ($\chi 2= 12.8$, df= 1, standard residual value= 46.0).

Rodents with single infection either with *Trypanosoma* or *Plasmodium*, both recorded prevalence > 10% therefore was considered further for analysis of factors affecting the prevalence and abundance of infection. Overall, 189 blood samples (43.8%) were infected with *Trypanosoma* with a marginally higher prevalence detected using GTF compared to QBC technique (Table 2). The minimum sufficient model confirmed significantly higher number of positive samples using GTF (n=99) than QBC (n= 90)

Table 1. Captured Rodent population distributed by species, age and sex from four urban cities in Peninsula Malaysia

| Season | Age | Sex | Species | Sampling sites | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | | | Kuala Lumpur | Malacca | Penang | Pahang | |
| Dry | Adult | Female | *Rattus norvegicus* | 2 | 23 | 5 | 19 | 49 |
| | | | *Rattus rattus diardii* | 0 | 6 | 0 | 0 | 6 |
| | | | *Rattus exulans* | 3 | 0 | 0 | 0 | 3 |
| | | | *Rattus argentiventer* | 1 | 0 | 0 | 0 | 1 |
| | | | Total | 6 | 29 | 5 | 19 | 59 |
| | | Male | *Rattus norvegicus* | 4 | 10 | 4 | 34 | 52 |
| | | | *Rattus rattus diardii* | 1 | 9 | 0 | 0 | 10 |
| | | | *Rattus exulans* | 2 | 0 | 0 | 0 | 2 |
| | | | Total | 7 | 19 | 4 | 34 | 64 |
| | Sub-adult | Female | *Rattus norvegicus* | 0 | 2 | 1 | 5 | 8 |
| | | | *Rattus rattus diardii* | 0 | 4 | 0 | 0 | 4 |
| | | | *Rattus exulans* | 1 | 0 | 0 | 0 | 1 |
| | | | *Rattus argentiventer* | 1 | 0 | 0 | 0 | 1 |
| | | | Total | 2 | 6 | 1 | 5 | 14 |
| | | Male | *Rattus norvegicus* | 1 | 0 | 1 | - | 2 |
| | | | *Rattus rattus diardii* | 0 | 2 | 0 | - | 2 |
| | | | Total | 1 | 2 | 1 | - | 4 |
| | Juvenile | Female | *Rattus norvegicus* | 3 | 1 | 2 | 5 | 11 |
| | | | *Rattus rattus diardii* | 0 | 4 | 0 | 0 | 4 |
| | | | *Rattus exulans* | 2 | 0 | 0 | 0 | 2 |
| | | | Total | 5 | 5 | 2 | 5 | 17 |
| | | Male | *Rattus norvegicus* | 1 | 0 | - | - | 1 |
| | | | *Rattus rattus diardii* | 0 | 2 | - | - | 2 |
| | | | Total | 1 | 2 | - | - | 3 |
| Wet | Adult | Female | *Rattus norvegicus* | 2 | 14 | 18 | 30 | 64 |
| | | | *Rattus rattus diardii* | 15 | 3 | 13 | 0 | 31 |
| | | | Total | 17 | 17 | 31 | 30 | 95 |
| | | Male | *Rattus norvegicus* | 4 | 20 | 19 | 22 | 65 |
| | | | *Rattus rattus diardii* | 22 | 0 | 19 | 0 | 41 |
| | | | *Rattus argentiventer* | 0 | 1 | 0 | 0 | 1 |
| | | | Total | 26 | 21 | 38 | 22 | 107 |
| | Sub-adult | Female | *Rattus norvegicus* | 0 | 5 | 0 | 2 | 7 |
| | | | *Rattus rattus diardii* | 4 | 2 | 1 | 0 | 7 |
| | | | Total | 4 | 7 | 1 | 2 | 14 |
| | | Male | *Rattus rattus diardii* | 11 | - | 5 | - | 16 |
| | | | Total | 11 | - | 5 | - | 16 |
| | Juvenile | Female | *Rattus norvegicus* | 0 | 0 | 3 | - | 3 |
| | | | *Rattus rattus diardii* | 10 | 3 | 8 | - | 21 |
| | | | Total | 10 | 3 | 11 | - | 24 |
| | | Male | *Rattus rattus diardii* | 13 | - | 2 | - | 15 |
| | | | Total | 13 | - | 2 | - | 15 |

among the rodent population ($p< 0.05$). There was an absence of interaction in the prevalence of *Trypanosoma* between host species, sex and age effect with only prevalence of adults harbouring *Trypanosoma* were significantly higher ($p< 0.05$) (Table 4).

A total of 266 positive detections of *Plasmodium* with a significantly higher result using GTF technique as compared to QBC ($\chi2= 50.7$, df= 1, $p\leq 0.05$) (Table 2). The only significant effect on prevalence of parasite harboured was observed in *R. norvegicus* ($p\leq0.05$) where up to 17% more *R. norvegicus* harboured *Plasmodium* infection compared to other rat species (Table 4). There was also an absence of interaction for the prevalence of *Plasmodium* infection between the host species, sex and age.

Rodents with mixed infections of both *Trypanosoma* and *Plasmodium* were low and only found in 2.8% (12 samples) by QBC technique and 15 samples by GTF technique ($\chi2= 13.8$, df= 1, standard residual value= -129.0). Results also indicated that GTF was better in detection of both parasites compared to QBC ($p<0.05$)

The overall sensitivity of parasite detection was 83.2% with specificity of 74.8% (Table 3). However, detection of *Trypanosoma* infection was better (sensitivity: 84.4%; specificity: 93.3%) compared to *Plasmodium* (sensitivity: 76.6%; specificity: 84.7%).

## DISCUSSION

As in many other cities around the globe, the rat population in peninsular Malaysia was dominated by two main species living commensally with humans, namely *R. norvegicus* and *R.ratttus diardii*, with *R. norvegicus* being the dominant species. Mohd Zain *et al.* (2012) also noted this trend, however, with *R. rattus diardii* being more prominently dominant than *R. norvegicus*.

More females were captured compared to male rats with higher number of adults compared to juveniles. Other rat epidemiology studies however, showed almost equal numbers between males and females captured, highlighting the scavenging behaviour of commensal rats

Table 2. Prevalence of blood protozoan between Quantitative Buffy Coat (QBC) and Giemsa-stained Thin blood Film (GTF) method

| GTF | QBC | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | All infected no. (%) | | | *Trypanosoma lewisi* infected no. (%) | | | *Plasmodium* sp infected no. (%) | | |
| Status | Positive | Negative | Total | Positive | Negative | Total | Positive | Negative | Total |
| Positive | 168 (74.3) | 58 (25.7) | 226 (52.3) | 76 (76.8) | 23 (23.2) | 99 (22.9) | 95 (66.9) | 47 (33.1) | 142 (32.9) |
| Negative | 34 (16.5) | 172 (83.5) | 206 (47.7) | 14 (4.2) | 319 (95.8) | 333 (77.1) | 29 (10.0) | 261 (90.0) | 290 (67.1) |
| Total | 202 (46.8) | 230 (53.2) | 432 (100) | 90 (20.8) | 342 (79.2) | 432 (100) | 124 (28.7) | 308 (71.3) | 432 (100) |

Table 3. Technique sensitivity and specificity in blood protozoan detection

| Status | All infected (%) | *Trypanosoma lewisi* infected (%) | *Plasmodium* sp infected (%) |
|---|---|---|---|
| Sensitivity | 83.2 | 84.4 | 76.6 |
| Specificity | 74.8 | 93.3 | 84.7 |
| PPV | 74.3 | 76.8 | 66.9 |
| NPV | 83.5 | 95.8 | 90.0 |
| Concordance | 78.7 | 91.4 | 82.4 |

* PPV- Positive Predictive Value, NPV- Negative Predictive Value

Table 4. Logistic regression prediction values between in the detection of blood parasitimae according to host species, sex and age

| Variation | Wald | df | *p* | Exp(B) |
|---|---|---|---|---|
| *Trypanosoma* | | | | |
| Species(1) | 0.000 | 1 | 0.999 | 5.109e$^{8}$ |
| Species(2) | 0.000 | 1 | 0.999 | 7.143e$^{8}$ |
| Species(3) | 0.000 | 1 | 1.000 | 1.119 |
| Sex(1) | 0.358 | 1 | 0.550 | 1.146 |
| Age(1) | 3.857 | 1 | 0.050* | 0.516 |
| Age(2) | 0.731 | 1 | 0.392 | 0.691 |
| *Plasmodium* | | | | |
| Species(1) | 4.440 | 1 | 0.035* | 0.170 |
| Species(2) | 2.480 | 1 | 0.115 | 0.269 |
| Species(3) | 1.589 | 1 | 0.208 | 0.152 |
| Sex(1) | 0.988 | 1 | 0.320 | 0.816 |
| Age(1) | 0.642 | 1 | 0.423 | 0.775 |
| Age(2) | 0.939 | 1 | 0.333 | 1.475 |

Species (1= *Rattus norvegicus*; 2= *Rattus rattus diardii*; 3= *Rattus exulans*;), sex (1=Male) and age (1=Adult, 2= Sub-adult)
* Significant $p<0.05$

were independent from one another (Paramaswaran *et al.*, 2005, 2009a, 2009b; Syed Arnez & Mohd Zain, 2006; Mohd Zain., 2008; Mohd Zain *et al.*, 2012; Alias *et* al., 2014). The lower success rate of trapping juveniles observed in the present study could be attributed to inherently limited activity and home range. It is also suggested that the greater number of adult rats generates imbalanced social competition, therefore restricting the overall movement of the juveniles. Other findings with similar observations for host age were also reported (Paramaswaran *et al.*, 2005; Syed Arnez & Mohd Zain, 2006; Mohd Zain., 2008; Mohd Zain *et al.*, 2012; Alias *et* al., 2014).

The Quantitative Buffy Coat (QBC) technique has been well documented in many countries including Ethiopia (Spielman *et al.*, 1988), Philippines (Rickman *et al.*, 1989), Thailand (Pornsilapatip *et al.*, 1990), Venezuela (Bosch *et al.*, 1996), Nigeria (Adeoye & Nga, 2007) and Malaysia (Mak *et al.*, 1992; Alias *et al.*, 1996) mainly for diagnosis of malaria in humans. In Malaysia, this technique was successfully used to screen *T. lewisi* in wild rats from Kuala Lumpur (Zainal Abidin & Noor Azmi, 1999; Zainal-Abidin & Yit-Ming 1994). This study reports for the first time an epidemiology study of blood protozoan in wild rat population in Peninsula Malaysia using the quantitative buffy coat (QBC) technique as well as to compare the effectiveness of this method with the conventional GTF technique.

The sensitivity and specificity of QBC and GTF in the detection of blood protozoan infections in wild rats were 83.2% and 74.8% respectively. Sensitivity and specificity was higher for *Trypanosoma* infection (84.4% and 93.3%) compared to *Plasmodium* (76.6% and 84.7%). The sensitivity and specificity recorded in the present study was low compared to results from previous studies which ranged from 75.5% to 99.6% and 82% to 98.4% respectively (Rickman *et al.*, 1989; Pornsilapatip *et al.*, 1990; Wongsrichanalai *et al.*, 1991; Baird *et al.*, 1992; Cabezos & Bada 1993; Gay *et al.*, 1994; Benito *et al.*, 1994). Alias *et al.* (1996) noted lower sensitivity in the field setting due to delayed specimen processing (up to 8-10 hours after

collection), as well as exposure to high ambient temperatures and humidity. Rickman *et al*. (1989) and Mak *et al.* (1992) noted that the sensitivity values were observed high only in a laboratory setting while it was low in the field.

Low sensitivity was also detected in Nigeria (Adeoye & Nga, 2007) at 55.9% and specificity was 88.8%. Their study showed low sensitivity under routine laboratory conditions and concluded that QBC was clearly unreliable at low parasitaemia level.

The advantages of the QBC technique are endless from time saving and easier technique to learn compared to the GTF technique. This method performs rapid identification of malaria infection especially in busy blood bank and outpatient clinics (Mak *et al*., 1992) as well as microfilaria infection, it has the ability to screen two parasitic diseases with a single sample of blood, particularly in areas where these diseases are endemic (Pius *et al.*, 1993). Although considerable time and experience are required for adequate preparation and interpretation of the blood smears, QBC is a less labour-intensive alternative to GTF. The principal advantages of the original QBC method were sensitivity, rapidity of staining, and ease to interpret in an endemic area.

However, the main disadvantage of this method was the cost as this method requires a fluorescent microscope (with 0.3 mm or greater working distance of objective lenses) and the QBC microcentrifuge, as well as limitation in estimating parasite density. It is also not suitable for field studies as it requires storage for further examination for quality control checking at a regional or central laboratory. Specimens also need careful handling during preparation and examination and prone to leakage and breakage of the capillary tubes during centrifugation if the centrifuge lid is not properly closed (Mak *et al*., 1992; Petersen & Marbiah, 1994; Alias *et al*., 1996).

To conclude, this study showed that GTF is the more reliable method in the detection of blood parasitic infections compared to QBC. QBC technique had limitations in the detection of blood protozoa than the conventional GTF technique. GTF was still the best in the overall detection of parasites and the cheapest to run. QBC technique may be very useful for quickly picking positive infection, however it still requires GTF in order to confirm the species of parasites and for quantification of parasitaemia.

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