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Uses of Modified Buffy Coat Method As a Tool for Diagnosis of Bovine Hemoparasites

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MODIFIED buffy coat method (BCM) using microhematocrit tubes and acridine orange (AO) staining permitsquick(~10 min/sample) and accurate diagnosis of commonbovine hemoparasites compared to the light microscopy(LM) of Giemsa stained smears.*Babesia*, *Theileria,Anaplasma*, *Ehrlichia* and *Trypanosoma* parasites were detected. It was easy to see *Babesia* merozoites, trypomastigotes and epimastigotes of *Trypanosoma* parasite. Similarly, fluorescent microscopy (FLM), offers a method for detecting low parasitemiathat are undetectable by light microscopy. Under LM, the agreement between the twodiagnostic techniquesusing Cohen's Kappa index was perfect for *Trypanosoma* (1.000), substantial for Anaplasma(0.615), moderate for *Theileria*, and *Ehrlichia* (0.459, 0.433 respectively). BCM was able to detect all positive cases (100%) of *Babesia* infections using LM and FLM. Modified buffy coat method usingdark field microscopy (DFM) is recommended as a fast and dependable tool to detect *Babesia*and *Trypanosoma*parasites. BCMusing AO stain can be set up directlywitha low cost using anordinary LM.BCM is a valuable tool for diagnosinghemoparasitesmixed infection. Buffy coatmethodcan be used as an alternative technique for the dry stained smear for quickly screening of bovine blood samples.

Keywords: Hemoparasites, Buffy Coat, Acridine Orange, Bovine.

Introduction

Hemoparasites infections in ruminants are primarily caused by the protozoans such as *Babesia* spp., *Theileria* spp., *Anaplasma*spp., and *Trypanosoma* spp. These protozoans are transmitted by arthropod vectors such as ticks and flies. [1-5].Bovine hemoparasites are relatively importantand endemic infections,as such infectionsusually cause economic losses such asbody weight loss, reduced milk production, decreased reproductive efficacy,abortion and mortality [6]. Rapid diagnostic examinations have become an essential tool in the diseases control and management programs in the world [7]. Microscopic examination (ME) of Giemsa stained blood smear isthe gold standard tool for the diagnosis of these hemoparasites, mostlyas mixed infections dominate in cattle [8]. None of the present molecular techniques can detect all mixed infections together, but microscopic examination offers chances for this to be made in Giemsa stained blood smears.However, the process of fixing, drying and staining of the blood smears in addition to their examination are ratherlaboriousandrequire at least 60 minutes from specimen collection to the report of the result [9]. The diagnosis can be a difficulty, especially when parasitaemia are low or blood smear preparation is less than optimal [10]. To enhance and easediagnosis of hemoparasites, several methods are presented for the quick diagnosis of hemoparasites. The buffy coat method (BCM) was developed in malariologyresearches which depend on centrifugation of blood,for separation of blood cells and parasites in different layers [11].

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The quantitative buffy coat (QBC) is one of the routine techniques used worldwidefor detection and quantitation of malaria parasites in human [12, 13]. BCM is a direct and fast fluorescent method used for the identification of parasitemia depends on micro-centrifugation, fluorescence, and density of infected RBCs. This method involves centrifuging the layer of pressurized erythrocytes stained with acridine orange and then examining them under an ultraviolet light source. The whole process takes place in a thick Becton Dickson hematocrit [7]. Fluorescent microscopy is sensitive particularly the quantitative buffy coat (QBC)examination. This method can rapidly screen many samples but needs special equipment and training [14]. Motile parasitic such as *Trypanosoma* spp. and microfilaria can be screened by QBC[11,15].It iswidely used for *Trypanosoma* and microfilariae detections in humans [10] and dogs [15,16]. Also, a similar method was used to identify *Babesia* spp. [14] and *Plasmodium* spp. [18, 19] in humans. However, few studies are present on BCM application fordetecting parasites in birds [15, 17,20].

The quantitative buffy coat method imposes cost limitationsin spite of its high performance [21]. As for modern techniques, such as the focusing technique for detecting hemoparasites, it requires special equipment and cost, in addition to the need of advanced microscopes such as fluorescent microscopy, as the technique for detection and quantitative enumeration of parasites in the buffy coat QBC. The objectives of this study are: (i) To modifying the BCM for a more practical procedure in cattle blood sampling throughout fieldwork; (ii) To evaluate the performance of modified buffy coat method (BCM) as a rapid test for diagnosis of hemoparasites in cow's blood usingthe ordinarylight microscope, fluorescent and dark-field microscopy in a comparison to the Giemsa stained blood smear as a gold standard.

Materials and Methods

Ethical approval

The present study was approved by the College of Veterinary Medicine Committee.

Sample collection

A total of 60 cows attended the Veterinary Teaching Hospital, University of Mosul, Iraq, with a history of fever enlargement of lymph node, and pale mucous membrane. Two milliliters of the blood from each cow were collected from the jugular vein into an EDTA container and thoroughly mixed. Blood smears were prepared and the rest of the blood samples were also tested by modified BCM.

Laboratory processing of blood samples

Thick/thin smears were made on the same slide and labeled, stained with Giemsa technique according to Gajendra et al.(22),and examined under 1000X objective of a light microscope.A thick smear was used for the rapid screening while a thin smear was used for the identification of the parasite.

Modified Buffy Coat Method

A 0.1 ml of Acridine Orange (AO 0.01%, Sigma-Aldrich) was added to 0.9 ml of blood in a glass blood container pre-coated with potassium oxalate anticoagulant in place of the conventional Becton Dickson thick capillary tube used in QBC technique and mix gently. For obtaining enough amounts of buffy coat sample fill two microhematocrit capillary tubes (5 µl size) pre-coated with EDTA anticoagulant with the mixture of blood and AO stain. Seal one end of each micro-tube with clay to prevent blood leakage. After rotating it by hand for 10 seconds, the tubes were placed in a microhematocrit centrifuge, the samples were centrifuged at 12,000 rpm for two minutes instead of 6000 rpm for 5 minutes with QBC. The microcapillary tube was cut at the desired junction between the white blood cell layer, platelets and the red blood cell layer, and by lightly pressing the sharp edge of the slide on the microhematocrit tube, the buffy coat layer with a little plasma transferred to the other slide, followed by a gentle mixing throughmoving the slide, then placing the slide cover (size 18 x 18 mm).To allow blood cells to settle on the slide, the wet preparation was left for a minute, then examined within 10 minutes using anordinary light microscope, fluorescent microscope (BX51 Olympus U-RFL-T-Japan), and dark field microscope (DK-Gundlach,Denmark), the results were marked with the presence (+) or absence (-) of parasites at the magnification power of 400 X (objective x eyepiece), but photography was done at 1000X (under oil).

Statistical Analyses

Data obtained from the study were input and analyzed with Statistical Package for Social Sciences software of computer using SPSS software version 21.0.Cohen's kappa value (κ) was calculated to determine the compatibility

between the modified buffy coat technique and Giemsa stained blood smear as a gold standard under the light microscope The Kappa value κ values were interpreted according to as: poor, κ < 0; light, κ = 0–0.20; fair, κ = 0.21–0.40; moderate, κ = 0.41-0.60; substantial, κ = 0.61–0.80; and perfect, κ = 0.81-1.00 [23].

Results

Blood samples ofa total of60 cows were tested, 18 cases were infected with*Babesia*, 17 *Theleria*, 11 *Anaplasma*, 10 *Ehrlichia*, and 17 cases with *Trypanosoma* after pooling the results of Giemsa stained smears and BCM tests (Table1).In buffy coatwet preparations, Babesia species hadthe most positive percentage, followed by *Trypanosoma* parasites.All cases (100%) of *Babesia*and *Trypanosoma* infections were detected under light microscope, fluorescent and dark-field microscopy compared to the ME using standard Giemsa stained blood smear which was able to detect only 61.11% of thecases.Regarding to *Theileria*, *Anaplasma*, and *Ehrlichia* parasites, BCM had a low positive rates. In ME of Giemsa stained smear, *Babesia* and *Trypanosoma* species had also the higher positive rates, followed by *Theileria*, *Ehrlichia,*and *Anaplasma* parasites (Table 1).The compatibility between the two diagnostic tools under LM was perfect for *Trypanosoma* (κ =1.000), substantial for *Anaplasma* (0.615), moderate for *Theileria* and *Ehrlichia* (0.459, 0.433 respectively). The BCM technique using FLM showed moderate agreement for the detection of *Anaplasma* (κ = .476), *Ehrlichia* (κ = .460) parasites, and fair agreement (κ =0.209) in detecting *Theileria* and *Trypanosoma* parasitesin a comparison with the blood smears stained with Giemsa. Fluorescent and dark-field microscopy using BCM was able to diagnose all cases (100%) of *Trypanosoma* parasite infections compared with 64.70% using the Giemsa stained blood smear. Interestingly, the BCM technique of live blood samples using dark field microscopy was able to detect all 18 (100%) cases of *Babesia* infection and 17 (100%) trypanosomes. Compared with dry-stained blood smear scanning microscopy, DFM microscopy failed to detect cases of *Theileria*, *Anaplasma,* and *Ehrlichia* (Table 1).

During BCM examination, it was possible for the first time to observe*Babesia*spp. merozoites moving in the buffy coat wet preparations of cow blood. *Babesia* merozoites were particularly easily identified even using LM (Fig. 1: A, B). *Babesia*piroplasmwas seen with orange coloration inside red blood cells, while the yellowish merozoites were seen outside RBCs. *Trypanosoma* parasites were also easy to detectwith bright yellow colorunder the FLM (Fig. 2: C, D).All parasites were also seen inside and outside red blood cells under a fluorescent microscope in a bright golden color such as *Theileria*, *Anaplasma*, and *Ehrlichia* infections were also detected using BCM but the motile parasites such as *Babesia* merozoites and *Trypanosoma* (Trypomastigotes and Epimastigotes) are quickly seen during the rapid screening of the modified buffy coat BCM technique. Especially because of its concentration under the buffy coat layer as a result of centrifugation of the blood sample, and its movement, as well as its coloration with AO stain (Fig. 3:E, F).

TABLE 1. Comparison of Kappa value: (κ) and the compatibility between modified buffy coat method and Giemsa-stained blood smear in its ability to detect hemoparasites of cow's blood.

Blood parasites	Total No. cases	Standard Giemsa (GS)	Modified buffy coat (BCM) LM		Modified buffy coat (BCM) FLM		Modified buffy coat (BCM) DFM	
		+ (%)	+ (%)	(κ)	+ (%)	(κ)	+ (%)	(κ)
Babesia	18	11(61.11)	18 (100.0)	.000	18 (100.0)	.000	(100.0) 18	.000
Theileria	17	10(58.8)	6 (35.29)	.459	3 (17.64)	.209	0	-.080
Anaplasma	11	6 (54.54)	4 (36.36)	.615	3 (27.27)	.476	0	-.00 2
Ehrlichia	10	7 (70.00)	5 (50.00)	.443	7 (70.00)	.460	0	-.429
Trypanosoma	17	11(64.70)	11(64.70)	1.000	17 (100.0)	.209	17 (100.0)	. 209

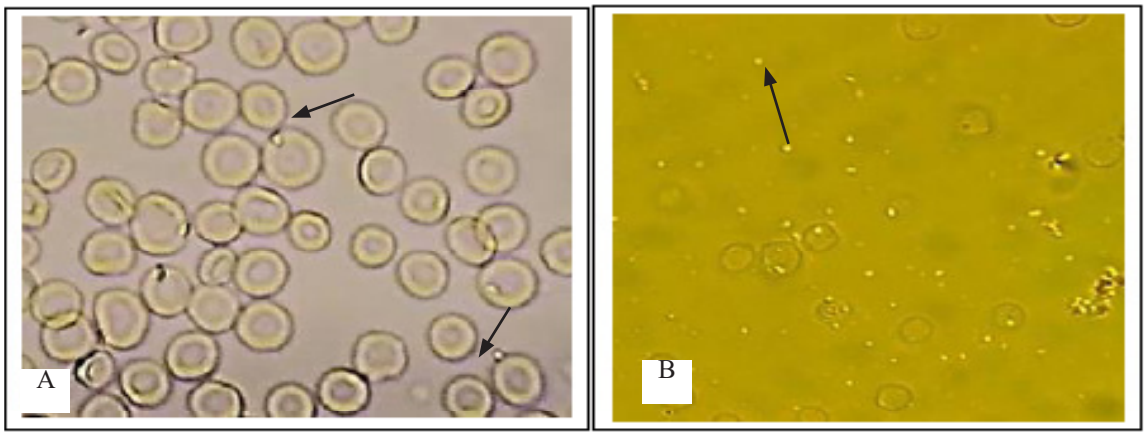

Fig.1. *Babesia* parasites inside and outside of RBC in buffy coat layer wet preparation(A) under light microscope, (B) *Babesia* merozoites outside RBC under fluorescent microscope. Oil immersion (1000X).

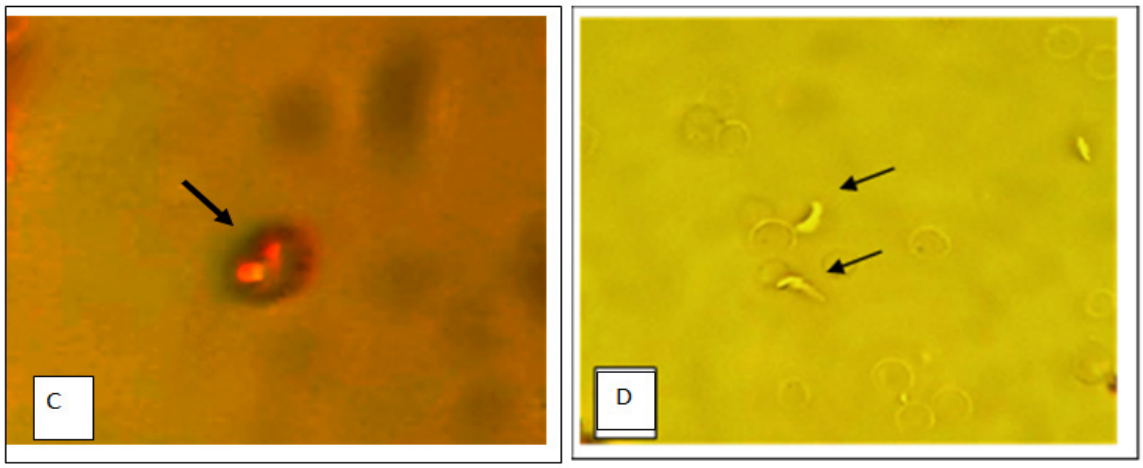

Fig. 2. The buffy coat layer wet preparation in which (C) *Babesia*merozoites appear inside the red blood cells in a reddish-orange color of the cytoplasm with the brilliant yellow nucleus. (D) *Trypanosoma* outside erythrocytes. Fluorescent microscope magnification power 1000 X.

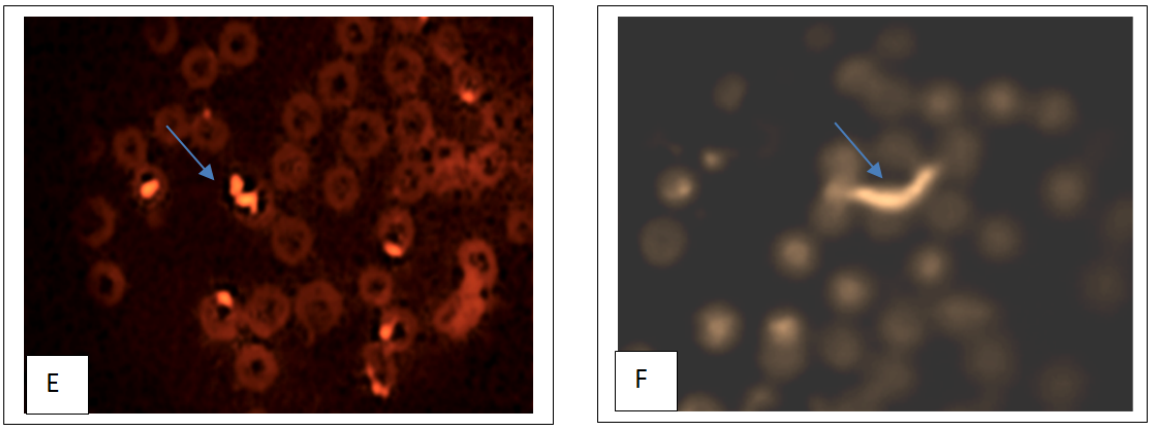

Fig. 3. (E) The buffy coat layer wet preparations show the brightness of *Babesia* parasites inside red blood cells. (F) *Trypanosoma* outside erythrocytes. Under dark field microscope magnification power 1000 X.

Based on the microscopic examination of the concentrated live blood samples using dark-field microscopy, detailed observations were made about the processes of invasion or egress from red blood cells mediated by *Babesia* parasites. Many unexpected results related to the dynamics of their ability to deal with the red blood cells of cows under laboratory conditions, and despite the speed with which these processes occur and the small size of the parasites, infective merozoites were moving fast and making surprising abnormalities in the erythrocytes membrane during the active invasion. large quantities of merozoites in a short period of time.

The merozoites of *Babesia bovis* enter red blood cells and invasion into the membrane of red blood cells through their gliding movement, as the merozoites recognize red blood cells through their apical end, which is the guiding part of *Babesia* parasite during the process of invasion into the red blood cell, and cause an initial decline in the membrane of the host cell at the entry location, a tight connection is initiated between the plasma membrane of the red blood cell and the free merozoite membrane, with clear entry from the front pointed end, which contains the apical complex. *Babesia* merozoites appeared with different instantaneous morphology moving forcefully in all directions to invade suitable RBCs. Their movement was in different styles, such as straight, curvilinear, zigzag, front and back…etc.

Different intraerytrocytic dynamic stages were observed such as single pyriforms, combined pyriforms, two paired pyriforms, tetrads, and multiple parasite stages resulting from exit of a large number of merozoites within relatively short period (Fig. 4 G, H).

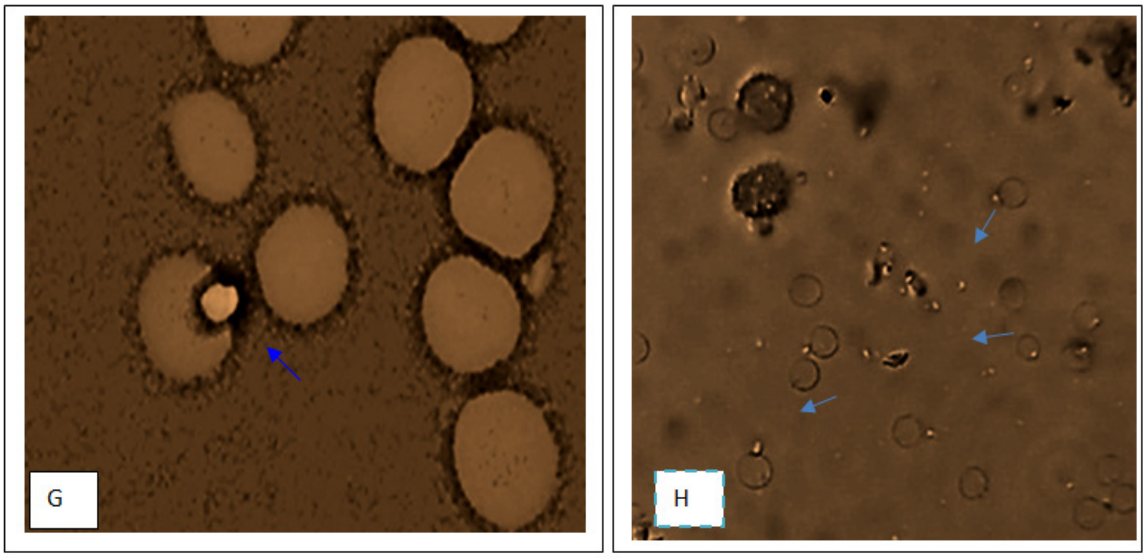

Fig. 4. (G) BabesiaMerozoite egress into the red blood cells from the front end with the decrease of the red blood cell membrane. (H) Free merozoites, with different morphologies moving forcefully in different directions Dark Field microscopy, 1000x.

Discussion

For a reliable diagnostic method, the higher the positivity percentage of known cases the more useful method. The opposite is commonly the case for negativity percentage which is predictable to be attentive towards zero for good diagnostic techniques. In the study, the positivity rate for *Babesia* cases was 100% by modified buffy coat method while Giemsa staining method recorded it as (61.11%). Whereas the negativity rates were 0% and 38.89% respectively.One of the most interesting notes is that the agreement between the modified buffy coat and Giemsa stained smear using LM was considered as perfect for *Trypanosoma* detectionusing the Cohen's kappa value. It is commonlypreferred to decrease the time required for sample screening inthe fieldwork, substituting the time-consuming techniques with rapid ones, which undoubtedly is the case with the BCM. However, there is less

agreement between BCM and ME in detecting parasites other than *Trypanosoma* species. This work indicates that the present method is more reliable in the detection of Babesia and *Trypanosoma* infections. This technique could also be used for the detection of *Theileria*, *Anaplasma*,and *Ehrlichia* infections under LM and FLM, but it is not recommended for *Theileria*, *Anaplasma* and *Ehrlichia* species diagnostics using DFM. This conclusion is significant for clinical and field work when infected animals should be rapidly screened for the assessment of treatment and prevent the complications of the infections in an endemic area.Accurate and quick diagnosis represents the essential weapon in the fight against parasitic infections [7]. This method is a simple and rapiddiagnostictooland can be used at everylaboratorywitha simpleLM microscope and microhematocrit centrifuge equipment. The main modification of the present BCM used for diagnoses of hemoparasites is that the new protocol does not require specialized capillary tube,ordinary microhematocrit tube was used after adding acridine orange stain to blood sampleand concentration with high speed centrifugation at 12,000 rpm for two minutes instead from 6000 rpm for 5 minutes with commercial QBC. The modified BCT is based on using only the staining buffy coat wet preparationswith the fluorescent vital stain acridine orange (AO) to make parasites more visible in blood samplesand toimprovethe image contrast, as exemplified by the QBC technique. In a comparison with previous modifications of buffy coat method, the protocols usedfor the detection of malaria in human [19, 24] was applying capillary tubes containing acridineorange stain and required fluorescence microscopyfor parasite observation, several flourchrome particularly the nucleic acid binding stain acridine orange has been used for the rapid detection of Apicomplexa, Rickettsia, and *Trypanosoma* parasites in blood smear using fluorescence microscopy [25,26,27]. In the current study; a conventional laboratory microscope (LM) is suitable to permit aquickdiagnosing of the parasites. *Babesia*, *Theileria*, *Anaplasma*, *Ehrlichia*, and *Trypanosoma* parasites were detected easily in AO staining buffy coat using the light microscopic examination because the parasites are rapidly absorbed the stain and appeared with luster inside or outside red blood cells which facilitate the rapid detection of parasites in even cases with low parasitaemia in the comparison to the Giemsa method. Detection of *Trypanosoma* parasite depends on the volume of the blood sample examined. Centrifugation of blood samples with high speedcause separation of the buffy coat, where most trypanosomes concentrate [28], and their detection is mostly dependent on their motility because they are translucent and colorless [15].

Staining of different forms of *Babesia* and *Trypanosoma* parasites exhibits light scattering when BCM wet preparations are viewed by dark-field microscopy due to their brightly illuminated appearance.Dark-field microscopy fails to detect *Theileria*, *Anaplasma*, and *Ehrlichia*infectionsin modified BCM preparations mainly because some of these hemoparasitesare immotile and most of them are intracellular which remain in the erythrocyte layer beneath the buffy coat [29]. Using light microscopy, Babesia merozoites wereeasily visible moving inside and outside RBCs for the first time in BCM preparationsof cattle blood samples.Some aspects of the process of *B. divergence* and *B. bovis* invasion in humans have been previously observed in culture medium by optical, fluorescent and vector electron microscopy [30,31]. Egress and invasion are important processes in the *Babesia* life cycle, where the merozoites are vulnerable and can bea very good therapeutic target. Thus transition was made from the dry and silent blood smear to parasite kinematic dynamics. These improved techniques will greatly facilitate epidemiological studies, as well as studying these parasites from many aspects, such as following up on the effect of different treatments on them and their different stages inside and outside red blood cells. In addition to studying some aspects of the pathogenesis and immunology of these parasites.Some previous studies indicated that the buffy coat method is highly sensitive in detecting human malaria parasites [18, 31, 32, 33]. Whereas others showed a low sensitivity [34, 35]. Adeoye & Nga [14] suggested that it is a valuable protocol inearly human malaria therapeutic inter position owing to there duced time of diagnostics. The present work supports this conclusion, mainly because modified BCM can provide possibility to rapid diagnosis of hemoparasites, permitsstarting of treatment and/or control measures tobe taken as soon as possible and to prevent furtherspread of hemoparasites infections.

Conclusion

Modified BCM is a sensitive and recommended method which is quick and reliable in detecting *Babesia* and *Trypanosoma* parasites in blood samples of cattle using light, florescent,and dark-field microscopy during fieldwork. BCM is a helpful methodin detectinghemoparasitewith low parasitemia. Its application can be drawn-out to studies of hemoparasites in other animals through field conditions.

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Competing interests

The authors declare that they have no competing interests.

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References

1. Rhaymah, M.S.H. and AL-Badrani, B.A. Clinical and diagnostic study of trypanosomiasis of cattle in Mosul, Iraq. *Res. Opin. Anim. Vet. Sci*., **2**(1), 23-26(2012).
2. Weny, G., Okwee-Acai, J., Okech, S.G, Tumwine, G., Ndyanabo, S., Abigaba, S. and Goldberg, T.L. Prevalence and risk factors associated with hemoparasites in cattle and goats at the edge of Kibale National park, western Uganda. *J. Parasitol*., **103**(1), 69–74 (2017).
3. Abdullah, D.A, Ali, M.S, Omer, S.G, Ola-Fadunsin, S.D, Ali, F.F. and Gimba, F.I. Prevalence and climatic influence on hemoparasites of cattle and sheep in Mosul, Iraq. *J. Adv. Vet. Anim. Res.,* **6**(4),492–498 (2019).
4. Alfatlawi, J.A, Jarad, N.I. andKlaif, S.F. Clinical and molecular identification of ruling *Theleria annulata* strains in cattle calves in Al-Diwaniyah province, Iraq. *Iraqi J. Vet. Sci.*, **35**(1),111-115 (2021).
5. Dahl, O.M., Hamdoon, O.K.H. and Abdulmonem, O.N. Epidemiological Analysis for medical records of Veterinary Teaching Hospital, University of Mosul during 2017 to 2019. *Iraqi. J. Vet. Sci.,***35**(3),541-548 (2021).
6. Nayel, M., El-Dakhly, K.M., Aboulaila, M. Elsify, A., Hassan, H., Ibrahim, E., Salama, A., and Yanai, T. The use of different diagnostic tools for Babesia and Theileria parasites in cattle in Menofia, Egypt. *Parasitol. Res.*, **111**(2), 1019–1024 (2012).
7. Momčilović, S. Cantacessi, C., Arsić-Arsenijević, V., Otranto, D.and Tasić-Otašević, S. Rapid diagnosis of parasitic diseases: current scenario and future needs. *C.M.I*., **25**(3), 290-309. (2019).
8. Nourollahi- Fard, S.R., Khalili, M.and Ghalekha, N.Detection of Theileria annulata in blood samples of native cattle by PCR and smear method in Southeast of Iran.*J. Parasit. Dis.,***39**(2),249–252 (2015).
9. Sathpathi, S., Mohanty, A.K., Satpathi, P., Mishra, S.K., Behera, P.K., Patel, G. and Dondorp, A.M. Comparing Leishman and Giemsa staining for the assessment of peripheral blood smear preparations in a malaria-endemic region in India. *Malar. J.*, **13**(512), 2 – 5 (2014).
10. Jangale, N. and Waghmare, A. Acridine orange for diagnosis of malaria – Our experience. *South East Asia J. Pub. Health*., **6**(1),49-51 (2016).
11. Marcos, R., Pereira, C., Santos, M., Luzzago, K., Lauzi, S., Maia, J.P., Faustino, A. and Puente-Pay, P. Buffy coat smear or Knott's test: which to choose for canine microfilariascreeninginfieldstudies? *Vet.Clin. Pathol.*, **45**(1),201-205(2016).
12. Liartee, D.B., Mendonça, I.L., Luz, F.CO., de Abreu, E.A.S., Mello, G. W.S, Farias, T.J.C., Ferreira, A.F.B. and Millington, M. A. Cost, C.H.N. QBC® for the diagnosis of human and canine American visceral leishmaniasis: preliminary data. *Rev. Soc. Bras. Med. Trop.*, **34** (6),134-145(2001).
13. Salih, D.A., El-Hussein, A.M. andSingla, L.D. Diagnostic approaches for tick born hemoparasitic diseases in livestock. *J. Vet. Med. Anim. Health*., **7**(2),45-56 (2015).
14. Adeoye, G.O. and Nga, I.C. Comparison of Quantitative Buffy Coat technique (QBC) with Giemsa-stained Thick Film (GTF) for diagnosis of malaria. *Parasitol Int.*, **56**(4),308-312 (2007).
15. Chagas, C. R., Binkienė, R., Ilgūnas, M., Iezhova, T., andValkiūnas, G. The buffy coat method: a tool for detection of blood parasites without staining procedures. *Parasit. & Vec.*, **13**(1), 1-12 ((2020).

16. Mylonakis, M.E., Papadopoulos, E., Koutinas, A.F., Paitaki, C. and Leontides, L. Comparative methodology for the detection and differentiation of circulating microfilariae of *Dirofilaria immitis* in the dog. *J. Helminthol*., **78**,137–140 (2004).

17. Atawal, A.F., Mgbeahuruike, A.C and Hammers, M. Microfilarial infections associatedwith body mass loss of village weavers Ploceus cucullatus. *Ostrich.,* **90**, 41–44 (2019).

18. Kocharekar, M.M, Sarkar, S.S and Dasgupta, D. Comparative study of modified quantitative buffy coat and two rapid tests in comparison with peripheral blood smear in malaria diagnosis in Mumbai, India. *J. Parasitol. Res.,* 194651(2014). doi: 10.1155/2014/194651.

19. Ifeorah, I.K, Brown, B.J and Sodeinde, O.O. A comparison of rapid diagnostic testing by Plasmodium lactate dehydrogenase), and quantitative buffy coat technique in malaria diagnosis in children. *Afr. J. Infect.Dis*., **11**, 31–38 (2017).

20. Bukauskaitė,D., Chagas, C.R.F., Bernotienė, R., Žiegytė, R., Ilgūnas, M.and Iezhova, T. A new methodology for sporogony research of avian haemoproteids in laboratory-reared Culicoides spp., with description of complete sporogonic development of Haemoproteus pastoris. *Parasit. Vec.*, **12**,582(2019). DOI:10.1186/s13071-019-3832-x

21. Kochareka, M., Sarkar, S., Dasgupta, D.and Aigal, U. A preliminary comparative report of quantitative buffy coat and modified quantitative buffy coat with peripheral blood smear in malaria diagnosis. *Pathog. Glob. Health*, **106** (6), 335-339 (2012).

22. Gajendra, S., Jha, B., Goel, S., Sahni, T., Sharma, R., Shariq, M., Jaiswal, S., and Sachdev, R. Leishman and Giemsa stain: a new reliable staining technique for blood/bone marrow smears. *Inter. J. Lab. Hematol*., **27** (5), 774-782 (2015).

23. Landis, J.R. and Koch, G.G. The measurement of observer agreement for categorical data. *Biometrics*., **33**,159–174 (1977).

24. Abubakari, A.A, Abdulraheemia I.A. andNassar, S.A. A trial study on a mixture of Acridine and Giemsa stains and modified Quantitative Buffy Coat for detection of malaria parasites. *Int. J. Biol. Chem. Sci*., **13**(4),2074-2081 (2019).

25. Ravindran, R., Lakshmanan, B., Sreekumar, C., John, L., Gomathinaygam, S., Mishra, A.K., Tewari, A.K. and Rao, J.R. Acridine Orange staining for quick detection of blood parasites. *J. Vet. Parasitol*., **21**(1),85-86 (2007).

26. Suleiman, E.G.and Altaee, A.F. Microscopic study for prevalence of *Babesia bovis* and *Babesia bigemina* in cattle in Mosul city. *Iraqi. J. Vet. Sci*., **31**(2),57-66 (2017).

27. Chandra, B.S., Rajkumar, K., Barathidasan, R. and Vijayalakshmi, P. "Acridine Orange Staining Technique - A Quick Diagnostic for Babesia gibsoni Infection." *Intas.Polivet.,* **19**(2), 328 (2018).

28. Sahimin, N., Alias, Woh, S.N., Edah, M.A. and Zain, S.N. Comparison between Quantitative Buffy Coat (QBC) and Giemsa-stained Thin Film (GTF) technique for blood protozoan infections in wild rats. *Trop. Biomed*., **31**(3), 422–431 (2014).

29. Jamjoom, G.A. Dark-Field Microscopy for Detection of Malaria in Unstained Blood Films. *J. Clin. Microbiol*., 17 (5), 717-721(1983).

30. Sevilla, E., González, L.M., Luque, D., Gray, J. and Montero, E. Kinetics of the invasion and egress processes of Babesia divergens, observed by time-lapse video microscopy. *Sci. Rep*., **8**,14116 (2018).

31. Asada, M., Goto, Y., Yahata, K., Yokoyama, N., Kawai, S., Inoue, N., Kaneko,O. andKawazu, S. Gliding motility of Babesia bovis merozoites visualized by time-lapse video microscopy. *PLoS One*., **7**(4),1-6(2012).

32. Gurung, B., Bairy, I., Jagadishchandra, J. and Manohar, C. Evaluation of falcivax against quantitative buffy coat (QBC) for the diagnosis of malaria. *Inter. Med. Public. Health*., **2**(1),132–140 (2010).

33. Bhandari, P., Raghuveer, C.and Rajeev, A. Comparative study of peripheral blood smear, quantitative buffy coat and modified centrifuged blood smear in malaria diagnosis. *Indian. J. Pathol.Microbiol*., **51**(1),108–112 (2008).

34. Amir, A., Cheong, F.W., De-Silva, J.R. and Lau, Y.L. Diagnostic tools in childhood malaria. *Parasit. Vec*., **11**(2), 53-56 (2018).

35. Parija, S.C, Dhodapkar, R., Elangovan,S.and Chaya, D. R. A comparative study of blood smear, QBC and antigen detection for diagnosis of malaria. *Indian. J. Pathol.Microbiol.*, **52**(3),200-202(2009).

استخدام طريقة المعطف المعدلة كوسيلة للتشخيص المجهري لطفيليات دم الابقار

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تمكنت طريقة المعطف المعدلة باستخدام أنابيب ميكروهيماتوكرت وصبغة أكريدين البرتقالي من التشخيص السريع (١٠دقائق/عينة) والدقيق للطفيليات الدموية الشائعة في الأبقار مقارنة بالفحص المجهري لمسح الدم الجافة المصبوغة بكيمزا. تم تشخيص طفيليات البابيزيا ، الثايليريا، والانابلازما ، والايرليخيا ، والمثقبيات. على الكشف بسهولة عن المراحل المتحركة وغير المتحركة للطفيليات الدموية بوساطة المجهر الضوئي. بالمثل ، كما وفر المجهر التألقي طريقة للكشف عن وجود أعداد صغيرة جدًا من الطفيليات التي لا يمكن كشفها بوساطة الفحص المجهري التقليدي. وفقًا لمؤشر كوهين كابا ، فإن الاتفاق بين التقنيتين في قابليتهما لتشخيص طفيليات دم البقر تحت المجهر الضوئي كان مثاليًا لطفيلي التريبانوسوما (١,٠٠٠) ، وحقيقي في الانابلازما (٠,٦١٥) ، ومتوسط للثايليريا والايرليخيا (٠,٤٥٩، ٠,٤٣٣ على التوالي). تمكنت تقنية المعطف الابيض المعدلة من اكتشاف جميع الحالات الإيجابية (١٠٠ ٪) من عدوى البابيزيا و التريبانوسوما باستخدام المجهر الضوئي و التألقي و مجهر المجال المعتم. . يمكن اجراء تقنية المعطف المعدلة باستخدام صبغة الاكردين البرتقالي بسهولة وبتكلفة منخفضة باستخدام مجهر ضوئي عادي. اظهرت طبقة المعطف المصقولة المحورة (BCM) بانها أداة مفيدة لتشخيص العدوى المختلطة لطفيليات الدم ويمكن استخدامها كأسلوب بديل للمسحة الجافة المصبوغة بصبغة الكيمزا للفحص السريع لعينات دم البقر تحت المجهر الضوئي.