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Re ea ch a e ·

Ra d a d e i e detect f *Babesia bovis* a d *Babesia bigemina* b ed ated the · a a ficat c b ed th a ate a fl d t c

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ABSTRACT

Babesia . a e a c e a ·t a a a te f the ed b d ce f a a a d a e t a t ted b t c . B e babe a ca ed b *Babesia bovis* a d *Babesia bigemina* cc · ·d de, h ch a g eat th eat t a a hea th. M c · c e a at a g d ta da d f ·the d ag f babe . H e e ; t e t t t . Th t d a c d cted t e tab h a e, effic e t a d fa t LAMP LFP eth d ed f ·ea · d ag f a a babe . LAMP a de e ed th a et f f · · e · ta get g a d a f g d t ct eg f c t ch · e b ge e f *Babesia* . de · the · a c d t . Afte · a d , a ch · at g a h c ate a fl d t c (LFD) a ed t detect LAMP · d ct that e e abe ed th FITC at the 5′ e d, a d g ge e ect · h e . The LAMP LFD eth d a e · ec fic, e d g t e e t th DNA te ate f *Theileria sergenti*, *Thenileria ovis*, *Theileria equi* a d *Toxoplasma gondii*. The LAMP LFP a h gh e t e a d c d detect 0.85 fg *B. bigemina* DNA a d 0.14 fg *B. bovis* DNA, 100 f d h ghe ·tha a c e t a PCR a a . Th eth d c d be ada ted f · c a d acc ate d ag f b e babe the fie d ca e the h e b d c d be d ect ed, e ec a f · de t f g ca ··e ·a a th e · a a tae a.

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1. Introduction

Babe a a a ted ea e that ead th · gh t c ect ·. It e a a a t f the ·d e t g g eat ec c e t the e t c d t · (H e ; 2000; H fe d et a ., 2008). *Babesia bovis* a d *Babesia bigemina*, e be · f A c e a, a e the a ath ge f b e babe t · ca a d bt · ca eg (F g e ·a et a ., 1998). C ca g f b e babe c de fe e ; a e a, he g b ·a, ata a (B c et a ., 2004) a d e e death e e e fected a a f h ch *Babesia* . a be f d e ·th ·c te b d ect c · c c e a at (B e et a ., 1995). O the the ·ha d, e a a e ta t t a fect bec e ca ··e ·

eth d e e th e t t ; Yet the · e e th t ceab e fa e t e. St , e the · eth d e e h t cated e e t, ch a ea t e · a t tat e PCR (B g et a ., 2007). The ef e, t e tab h a e, effic e t, a d a d act ca *Babesia* . detect eth d that ca be ed d ag , e ec a ea · d ag f a a babe , ha ta t act ca g fica ce. L ed ated the · a a ficat (LAMP) a e ted b N t et a . (2000) a a e c e c ac d a ficat eth d de ·c ta t te e at e. It ca a f ta get DNA f · a fe c e t 10[9] c e a h t e · d f t e, a d ha bee de ed detect f a · ath ge ch a *Mycobacterium avium* (E a a et a ., 2003), *Cryptosporidium* (Ba he t et a ., 2008), *Giardia lamblia* (P te · a d Ka a , 2009), *Toxoplasma gondii* (Ga a L de a et a ., 2013) a d *Leishmania* (S · a at et a ., 2015). LAMP ha a bee a ed f ·the detect f *Babesia gibsoni* (I ada et a ., 2004; Ma da et a ., 2015), e e · a (A ha a et a ., 2007) a d *Babesia orientalis* (He et a ., 2009).

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Table 1
LAMP ' e ' (F3, B3, FIP, BIP) a d ' be ed th t d .

P ' e '	T e	Le gth	Se e ce (5′ 3′)
F3	F ' a d te ' ' e	20 e '	GTTGATTCTTTCGAATGTGT
B3	Bac a d te ' ' e '	20 e '	AATTATAACTGTTGCTCCCC
FIP	F ' a d e ' ' e (F1c + TTTT + F2)	46 e '	CAAAAGAAACACATTGAGC*TTTT* CTGAGGTTAATATGGGTTGGC
BIP	Bac a d e ' ' e (B1c+ TTTT + B2)	53 e '	GGTCCTGGTATTCAGGAATGGGGC*TTTT* CCGGAAGAACATAACCTAAGAAAG
FITC HP	H b ' d at ' be	18 e '	FITC TTCATGATGTTACATATG

I 2008, the ate a fl d t ' (LFD) a fi ' t ed c b at th LAMP t e tab h the LFD LAMP tech g t detect h ' Ta a d ' e ' (K at ath cha et a ., 2008). The tech e ba ed the ' c e f LAMP a ficat , 2 '3 a ' f ec fic ' e ' de g ed t detect 6 '8 eg e t f ta ' get ge e . The t ea e ' ' e ' a e abe ed b b t a d ed t a f ' d ct b B t DNA e a e de 'a c ta t te e at e (60 65 °C) f ' 1 h. Afte ' a d the fl ece th c a ate (FITC) abe ed DNA ' be th the a ge f the e ' ' e 'a ficat h b ' d e th b t ated LAMP ' d ct , a d the h b ' d at ' d ct a e detected b LFD. The tech e e e e e e t ch a a ate 'bath 'a heate ' t a ta eact te e at e a LAMP, a d g eat ed ce c t a d h te t e, et th a e t t a t , f th ghe ' tha , PCR. LAMP LFP ha bee ed the detect f h te t d ' e ' (Ja ' e a et a ., 2009), ' g e a f ca ' ' (P tha b et a ., 2009), fect ee a d d e ec ' ' (D g et a ., 2010), Ye head ' (Kh th g et a ., 2013), h a Af 'ca t ' a a (HAT) (N ' , 2011), *Plasmodium falciparum* a d *Plasmodium vivax* (Y g ett a et a ., 2014) a d c t ' H a g gb g (R ga et a ., 2014).

The a f c ' e t t d a t de e a e, effic e t, a d act ca LAMP LFP eth d f ' the detect f *Babesia* . fect . F ' ec fic ' e ' a d a FITC abe ed DNA ' be e e de g ed ba ed C t ch ' e b ge e f *B. bovis* a d *B. bigemina*, ta g t c de at f C t b ge e f *Babesia canis* a d *Babesia gibsoni* a d the eth d ca be ed t detect babe the fie d th b ' ad a cat ' ect .

2. Materials and methods

2.1. Genomic DNA preparation

T ta DNA f *B. bovis* a d *B. bigemina* a d ' ded b La h Vete ' a ' Re ea ch I t t te, Ch e e Acade f Ag ' c t a Sc e ce , La h , Ga P ' ce.

2.2. LAMP primer design

P ' e ' ec fic f ' the ec e ec fic C t ch ' e b (c tb) e e ce f *B. bovis* (Ge Ba GQ214235.1) a d *B. bigemina* (Ge Ba GQ214234.1) e e de g ed, ta e the c e ' ed eg t c de at . A e LAMP ' e 'de g g ft a e (htt :// ' e e e : /e/) a ed. The g c e t de e e ce f the LAMP ' e ' a e h Tab e 1. A ' e ' c d g the b t abe ed FIP ' e ' e e the ed b Sha gha Sa g B g ca E g ee ' g Tech g & Se ' ce C ., Ch a.

2.3. Establishment and optimization of LAMP reaction system

The LAMP te a t ed ba ed the te ate f *B. bovis*. A t a e f 25 µ LAMP c ded: 4 M MgSO4, 1 M dNTP, 0.8 M beta e (S g a, USA), 1 µM te 'a ' e ', 0.2 µM te ' ' e ', 8 U f B t DNA e a e (NEB, USA), 1 × The ' P eact b ffe ' (NEB, USA) a d 1 µ DNA te ate. The ' e ' at a d dNTP c ce t at e e t ed a e de c 'bed (De g et a ., 2015). Effect f ' e 'c ce t a t at LAMP eact a ea ed b ett g the ' e ' c ce t at at g ad e t f te ' ' e ' t e ' ' e '. The g ad e t ' e 'c ce t at at a et t 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, a d 1:9. At a at f 1:6, the LAMP ' d ct e e the t ab da t. Set g ad e t t t d the effect f dNTP c ce t at LAMP a d dNTP c ce t at e e 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 a d 1.8 M. At 1.0 M, the te t f LAMP a c a the t ' ge t. The ef e, the t a LAMP t e c ta ed 1× The ' P eact b ffe ; 8 U f B t DNA e a e, 1.0 M dNTP , 0.8 M beta e, 4 M MgSO4, 0.2 µM each te ' ' e ' (F3 a d B3), 1.2 µM each e ' ' e ' (FIP a d BIP) a d 1 µL f ta get DNA.

LAMP a a e f ' ed f 'd ffe e t a ficat t e a g g f ' 30 t 90 a d d ffe e t te e at e a g g f ' 60 °C t 65 °C t dete ' e the t c d t f 'a ficat . Acc d g t ha ' e a d b 'ght e f the LAMP ' d ct a ed de 'UV t a at ' 2% aga ' e ge ta ed th eth d b ' de, the te e at e at 64 °C a d the eact t e f 60 e e ch e f ' the b e e t LAMP a a .

2.4. LAMP effectiveness and repeatability test

A *B. bovis* t e c t ' a e a d t egat e c t ' a e f ee f

2.6. PCR assay

A 25 μ PCR t e c ta ed 10 M T HC (H 8.3), 50 M KC , 1.5 M MgC 2, 0.2 M dNTP , 0.2 μM each f F3 a d B3 e , 5 U t f Ta DNA e a e (Ta a a, Ch a), a d 1 μL te ate DNA. A ficat c d t c ded a t a c ba t at 94 °C f 3 , f ed b 35 c c e f 94 °C f 30 , 52 °C f 20 a d 72 °C f 30 . A fi a e te te a c d cted at 72 °C f 5 . The d ct e e the a a ed b 2% aga e. The 264 b f ag e t e e e ected f the e ect e ge e ta get f *B. bovis* a d *B. bigemina*.

2.7. Sensitivity by PCR, LAMP by gel electrophoresis (LAMP-AGE) and LFD

DNA c ce t at e e dete ed g a Na d ec t h t ete (Mae t Ge I c., USA). The e t t f the LAMP a a a te ted th *B. bovis* DNA a d *B. bigemina* DNA a e , h ch e e e a d ted at c ce t at f a ate 14 g/μL t 0.014 fg/μL a d a ate 85 g/μL t 0.085 fg/μL. O e c te f DNA a ed a the te ate f b t abe g LAMP acc d g t the t ted c d t . The a e a t f te ate a a ed f PCR. DNA f *Babesia* . f ee c a ed a egat e c t . The d ct e e e a ed b 2% aga e ge e ect h e ta ed th eth d b de a d b LFD a de c bed ab e.

2.8. PCR, LAMP by gel electrophoresis and LFD specificity test

The ec fic t e f the LAMP a d PCR a a e e dete ed th DNA te ate f *B. bovis*, *B. bigemina*, *Theileria sergenti*, *Theileria ovis*, *Theileria equi* a d *T. gondii* a ed ab. DNA f *Babesia* . f ee c a et a egat e c t . The a fied d ct e e a a ed b 2% aga e ge e ect h e a d LFD.

3. Results

3.1. LAMP effectiveness and repeatability test

Th ee te t e t e e c ete c te t. The e e ta e t h ed that the *B. bovis* c t a e a ea ed t e a d t c t a e f ee f *Babesia* . egat e. The e e ta e t e e acc da ce th e t g ta get . The ab e d cate th LAMP e eatab e a d acc ate.

3.2. Analytical sensitivity of the PCR, LAMP by gel electrophoresis and LFD

T e a ate the e t t f LAMP, c a ed th PCR, DNA a e f 10 f d e a d t f *B. bovis and B. bigemina* e e b ected t LAMP a d PCR (F g . 1 a d 2). U g a e e d t f DNA e t acted f *B. bovis* a d *B. bigemina*, LAMP AGE ga e t e e t t d t f 1 × 10^{-5} (*B. bovis* 0.14 fg a d *B. bigemina* 0.85 fg) (F g . 1 a a d 2 a), h e t e PCR a cat e e b e ed t d t f 1 × 10^{-3} (*B. bovis* 14 fg a d *B. bigemina* 85 fg) (F g . 1 b a d 2 b). Th , LAMP AGE a 100 t e e e t e tha PCR. S a t LAMP AGE,

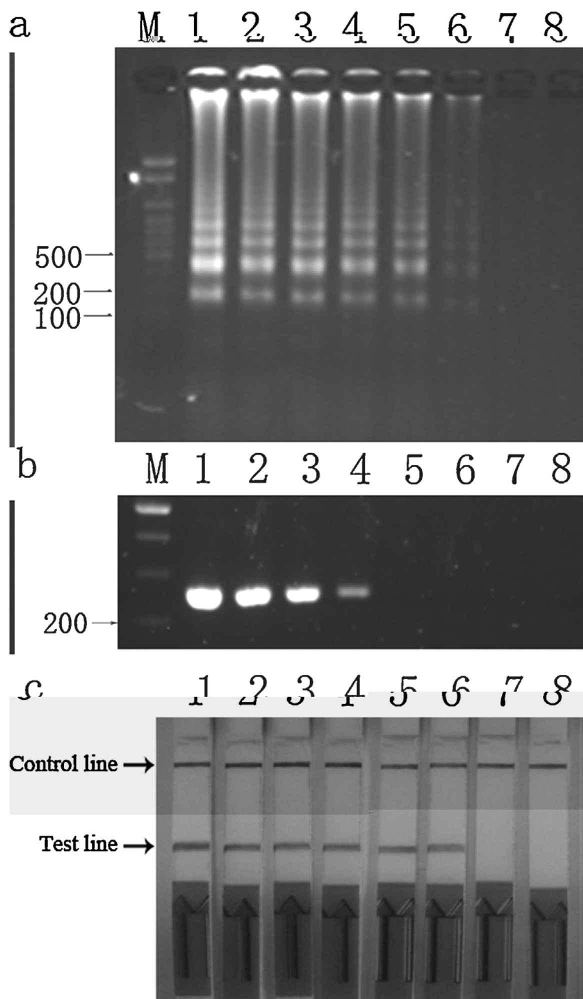

Fig. 2. C a at e e t t e b (a) LAMP AGE, (b) PCR AGE, a d (c) LAMP LFD f the detect f *Babesia bigemina* ba ed C t b a ficat .
M:100 b DNA Ma e ; La e 1 7: a t f DNA te ate e e, de ; 85 g, 8.5 g, 850 fg, 85 fg, 8.5 fg, 0.85 fg a d 0.085 fg *B. bigemina* DNA; La e 8: egat e c t . LAMP AGE: LAMP c b ed th aga e ge e ect h e ; PCR AGE: PCR c b ed th aga e ge e ect h e ; LAMP LFP: LAMP c b ed th LFP. The e t t f LAMP AGE a d LAMP LFD a 0.8 fg, h e that f PCR AGE a 85 fg.

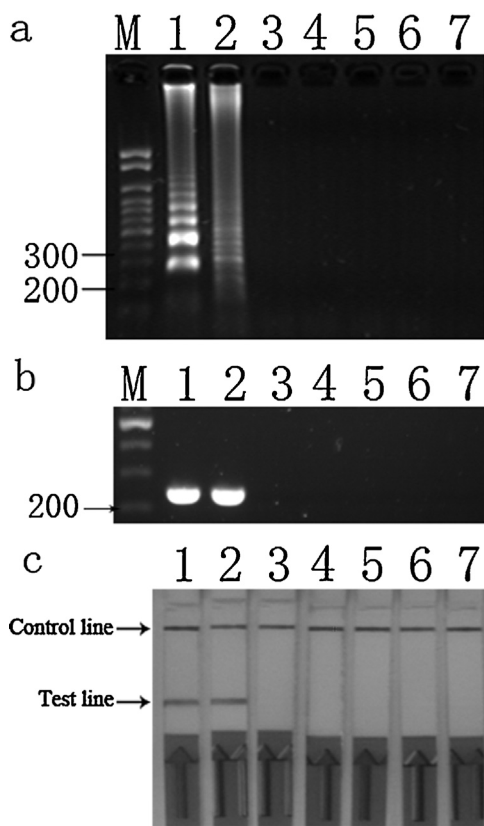

Fig. 3. S ec fic t f (a) LAMP AGE, (b) PCR AGE, a d (c) LAMP LFD ba ed C t b a ficat .
M:100 b DNA Ma e ; La e 1: *Babesia bigemina* DNA; La e 2: *Babesia bovis* DNA; La e 3: *Theileria sergenti* DNA; La e 4: *Theileria ovis* DNA; La e 5: *Theileria equi* DNA; La e 6: *Toxoplasma gondii* DNA; La e 7: egat e c t .

4. Discussion

Babe , ca ed b *Babesia* . t s a, a c a a t c d ea e. The t s a a ade ed b d ce f b e, h e (d e , e) a d the a a (Pe sh , 2006). *Babesia* . c de *B. bovis*, *B. bigemina*, *Babesia divergens*, *B. canis*, *Babesia caballi* a d *Babesia ovis*, th d ffe e t deg ee f h t ec fic t (Pe sh , 2006). The d ea e a cc t ca a d bt ca a ea , b g g h ge e t the e t c d t a d the g ba ec , c ea g ece a t detect the e t c d t a d the fie d (Pe es de Le et a ., 2010).

C e t , the g d ta da d f d ag f babe d ect c c f b d ea : Th eth d e a d fa t D ad a tage c de e t t a d de a d g h gh t a ed e e. D ffe e t ec e f a a te a be a h g that the a e d t g hab e. I add t , th eth d t tab e f detect f a a th a a te b de the e he a b d a d ca e ca e a a th e a a tae a (A e a et a ., 2001). Se g ca eth d a effect e ea f e de g ca e ea ch, b t t ca t d t g h fected a a f e ta t e afte fect , e the

dete e c fect , acc ate te ca g h t f t age h t (Wag e et a ., 1992).

18S RNA ge e a ch e a a ta get d ag f b e babe beca e the f at e e a t the c e e tge e ca be ea bta ed f ec a data ba e. H e e ; the c be f th ge e the *Babesia* ge e . The e a e th ee c e *B. bovis* ge e (Da e, 1990), t c e *T. parva* ge e (K be et a ., 1994). The a e t at f *P. falciparum* (C ed a d E ea, 1994; Da e a d McC tcha , 1983) a d *Cryptosporidium parvum* (Le B a c et a ., 1997) e t, each th 4 5 c e . C t ch e b (C t b) ge e f *Babesia* . e t e ta ch a t ch d a DNA, c a ed t 18S RNA ge e, th h ghe c be (Sa e et a ., 1999). PCR e t t c e e ated t the be f ta get ge e. Se ect g ge e f h gh c be a ta get ge e g eat c ea e the e t t f the e t g PCR eth d . Sa e et a . (1999) ed C t b ge e a ta get ge e t detect *B. bovis* a d *B. bigemina* b PCR a fica t a d d c e ed t a 20% e e t t e tha 18S RNA ge e a a ta get. Rega d g C t b ge e a a ta get ge e, a a t tat e PCR eth d a ab e t detect 0.1 g 0.1 fg f DNA *B. bovis* a d *B. bigemina* (B g et a ., 2007). The ef e, C t b ge e a ch e the c e t t d f de e g a LAMP LFP et h d, h ch e c e e t a d a cab e tha the c e t a PCR.

The LAMP e tab hed b N t et a . (2000) e tab e f c c detect a d ha bee a ed t detect f a ath ge , beca e f h gh ec fic t , e t t a d eed f e e e e t e the a c c e : A cat f LAMP t t c ba ath ge ha bee e e ed b Ka a a d O ge th (2009). LAMP a e f ed e f detect f *B. gibsoni* b I ada et a . (2004), th the detect t 0.0005%

a a tae a a d a e a e t t f LAMP a d PCR. T c fi the LAMP eact d ct e b ta ce a be ed ch a eth d b de e ab (Ma da et a ., 2015). I addt , fa e t e e t cca a cc he the t b d t a d fl e ce ce detect eth d a e e f ed. LAMP LFD ba ed LAMP a d de e d the ec fic h b d zat f the eact d ct th DNA be . U g *B. bigemina* C t b a d *B. bovis* C t b c e ed e e ce t de g e , the LAMP LFD e tab hed ca acc ate de t f *B. bigemina* a d *B. bovis*, h e the the ge e a f t a *T. sergenti*, *T. ovis*, *T. equi* a d *T. gondii* e e t detected, gge t g that the detect eth d h gh ec fic t , acc da ce th e t d e (N , 2011; Y g et a et a ., 2014). Se t t f the LAMP LFD eth d a f the c fi ed. B t g the e c ce t at at , dNTP a d the c d t , the e t t f the LAMP LFD 100 t e h ghe tha ge PCR, th a detect th e h d f 0.85 fg *B. bigemina* DNA a d 0.14 fg *B. bovis* DNA, th a c a ab e e t t f the a t tat e PCR e tab hed bef e (B g et a ., 2007). LAMP a a e t d e h ed d ffe e t e t t . LAMP eth d f the de t ficat f Ch e e *B. bovis* a d *B. bigemina* (L et a ., 2012) a th a t detect f 0.1 g DNA, 1000 f d e e t e tha the c e t a PCR a a . M e e ; the LAMP a a c d detect 12 g DNA f *B. gibsoni*, 10 t e g eate tha e ted PCR (Ma da et a ., 2015). I c t a t, LAMP e e f d e e t e tha PCR (M e et a ., 2010). Re at e , LAMP LFP e e t t d a e e t e. LAMP LFD a fi t a ed t the detect f *B. bigemina* a d *B. bovis*, th h gh ec fic t a d e t t , h t t e c g a d e e t dge e t, h ch fit the eed f act t e a d ha a de a cat f t e.

5. Conclusion

The LAMP LFD eth d e tab hed the c e t t d ca ec fica detect *B. bigemina* a d *B. bovis* t 70 e c d g DNA e t act a d e a at t e. Th eth d, th h gh ec fic t a d e t t a d th t e e e t f fe a e e t, e tab e f fie d a cat a d et h ef t be c a ed fa t detect .

Conflict of interest

The a th ha e c fl ct f te e t.

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