

Supplementary Material for

Kamau et. al., 2024. Comparison of test performance of a conventional PCR and two field-friendly tests to detect Coxiella burnetii DNA in ticks using a Bayesian latent class analysis

Appendix S1: ADDITIONAL DATA AND SUMMARIZED RESULTS

Table S1: A summary of tick DNA samples opportunistically collected from 17 wildlife species and cattle in wildlife conservancies in Kenya, between October 2011 and April 2019 and tested for *Coxiella burnetii* by conventional PCR, on the Biomeme *C. burnetii* platform, and a *C. burnetii* PCR HRM technique (n=179 ticks from 179 hosts).

Source host species (n individuals)	Tick genus (n)	n ticks tested* (subset with Biomeme, if different)	cPCR pos n (% pos in host species)	<u><i>C. burnetii</i> results</u>	
				Biomeme pos n (% pos in host species)	PCR-HRM pos n (% pos in host species)
Black rhino (3)	<i>Rhipicephalus</i> (3)	3	0	0	0
Cattle (44)	<i>Rhipicephalus</i> (4); Unk (40)	44 (38)	1 (2.6%)	3 (6.8%)	4 (9.1%)
Buffalo (36)	<i>Rhipicephalus</i> (21); Unk (15)	36 (35)	2 (5.7%)	8 (2.2%)	8 (2.2%)
Cheetah (1)	Unk (1)	1	0	0	0
Eland (9)	Unk (9)	9	0	0	1 (11.1%)
Elephant (17)	<i>Rhipicephalus</i> (7); Unk (10)	17	1 (5.9%)	0	2 (11.8%)
Grevy zebra (14)	<i>Rhipicephalus</i> (7); Unk (7)	14 (13)	2 (15.4%)	1 (7.1%)	4 (28.6%)
Giraffe (12)	<i>Rhipicephalus</i> (1); Unk (11)	12	0	3 (25%)	2 (16.7%)
Hartebeest (2)	<i>Rhipicephalus</i> (2)	2	1 (50%)	2 (100%)	2 (100%)
Hyena (4)	<i>Rhipicephalus</i> (3); <i>Amblyomma</i> (1)	4	0	0	1 (25%)
Impala (1)	<i>Rhipicephalus</i> (1)	1	0	0	0
Kudu (1)	Unk (1)	1	0	0	0
Leopard (1)	<i>Rhipicephalus</i> (1)	1	0	1 (100%)	1 (100%)

Lion (13)	<i>Rhipicephalus</i> (6); <i>Amblyomma</i> (1); Unk (6)	13 (12)	1 (8.3%)	1 (7.7%)	3 (23.1%)
Plains zebra (7)	<i>Rhipicephalus</i> (2); <i>Amblyomma</i> (1); Unk (4)	7	1 (14.3%)	1 (14.3%)	3 (42.9%)
White rhino (4)	<i>Rhipicephalus</i> (1); Unk (3)	4	0	0	0
Wild dog (6)	<i>Rhipicephalus</i> (4); Unk (2)	6	0	0	1 (16.7%)
Unknown/Not recorded (4)	<i>Rhipicephalus</i> (1); Unk (3)	4 (3)	0	1 (25%)	0
Totals	<i>Rhipicephalus</i> (64; 35.8% of 179); <i>Amblyomma</i> (3; 1.7% of 179); Unk (112; 62.6% of 179)	179 (169)	9 (5% of 179)	21 (12.4% of 169)	32 (17.9% of 179)

cPCR=conventional PCR; pos=positive; Unk= Unknown spp.

*One tick from each individual animal was tested for *C. burnetii*.

[illegible]

Figure S1: A figure showing the alignments of *Coxiella burnetii* and *Coxiella*-like endosymbionts

nucleotide sequences available in the GenBank database and the 3' mismatches in the *C burnetii*_HRM-R primer.

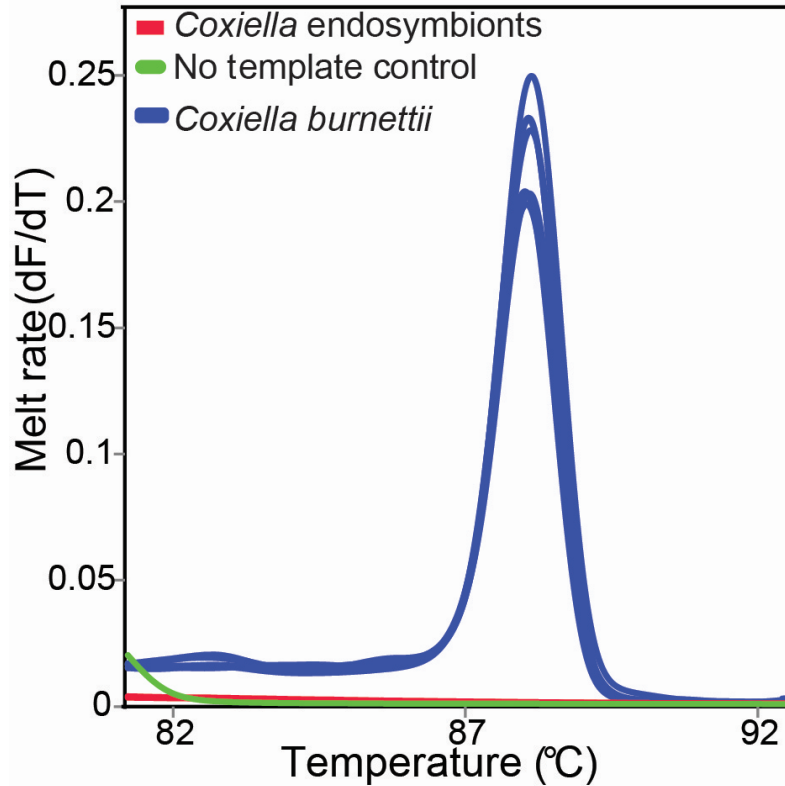


Figure S2: Figure showing the melt profiles of samples that had previously been confirmed to be positive for *Coxiella burnetii* and *Coxiella endosymbionts* as well as a no template control used to test the specificity of the *C_burnetii*_HRM-F and *C_burnetii*_HRM-R primers.

Appendix S2: STARD-BLCM CHECKLIST

Table S1. Checklist of items included for reporting the Bayesian Latent Class Model to aid in transparency, replicability, and integrity of the analysis. Checklist is from the Standards for the Reporting of Diagnostic Accuracy studies that use Bayesian Latent Class Models (STARD-BLCM), reported by Kostoulas et. al., 2017.

Section and topic	Item	STARD BCLM	Section (section no.)
Title/ Keywords	1	Identification as a study of diagnostic accuracy, using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC) and Bayesian latent class models	Title; adjusted to best represent the purposes of the study
Abstract	2	Structured summary of study design, methods, results, and conclusions	Abstract
Introduction	3	Scientific and clinical background, including the intended use and clinical role of the tests under evaluation	Introduction (1)
	4	Study objectives and hypotheses, such as estimation of diagnostic accuracy of the tests for a defined purpose through BLCM	Introduction (1)
Study design	5	Whether data collection was planned before the tests were performed (prospective study) or after (retrospective study)	Methods (2.1)
Participants	6	Eligibility criteria and description of the source population	Methods (2.1)
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Not applicable
	8	Where and when potentially eligible participants were identified (setting, location, and dates)	Methods (2.1)
	9	Whether participants formed a consecutive, random or convenience series	Methods (2.1)
Test methods	10	Description of the tests under evaluation, in sufficient detail to allow replication, and/or cite references	Methods (2.2)
	11	Rationale for choosing the tests under evaluation in relation to their purpose	Methods (2.2)
	12	Rationale for test positivity cut-offs or result categories of the tests under evaluation, distinguishing pre-specified from exploratory	Methods (2.2)
	13	Whether clinical information was available to the performers or readers of the tests under evaluation	Not Applicable

Analysis	14a	BLCM model for estimating measures of diagnostic accuracy	Methods (2.3.1); Results (3; Table 3)
	14b	Definition and rationale of prior information and sensitivity analysis	Methods (2.3.1)
	15	How indeterminate results of the tests under evaluation were handled	Not applicable; no indeterminate results in this study
	16	How missing data of the tests under evaluation were handled	Methods (2.3.1)
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Not applicable; not done in the present study
	18	Intended sample size and how it was determined	Not applicable; sample size was determined opportunistically
	19	Flow of participants, using a diagram	Not applicable
	20	Baseline demographic and clinical characteristics of participants	Partially applicable as stated in Methods (2.3); tick hosts are reported in Appendix S1, Table S1
	21	Question not applicable per STARD BLCM standards	Not applicable
	22	Time interval and any clinical interventions between the tests under evaluation	Not applicable
Test results	23	Cross tabulation of the tests' results (or for continuous tests results their distribution by infection stage)	Table 2
	24	Estimates of diagnostic accuracy under alternative prior specification and their precision (such as 95% credible/probability intervals)	Not applicable; only non-informative priors were used (2.3.1)
	25	Report any adverse events from performing the of the tests under evaluation	Not applicable
Discussion	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Discussion (4)
	27	Implications for practice, including the intended use and clinical role of the tests under evaluation in relevant settings (clinical, research, surveillance etc.)	Discussion (4)
Other information	28	Registration number and name of registry	Not applicable

	29	Where the full study protocol can be accessed	Data availability (11) and included in manuscript
	30	Sources of funding and other support; role of funders	Funding (8)

Appendix S3: R PROGRAMMING CODE

This appendix shows the R programming code for a Bayesian latent class model evaluating three diagnostic tests for *Coxiella burnetii* in ticks. Ticks were collected from wildlife and cattle located in wildlife conservancies in Northern Kenya between 2011 and 2019 (model n = 169 tick samples). Evaluated tests included a conventional PCR (cPCR), a PCR High Resolution Melting (PCR HRM) technique for *C. burnetii* and using the Biomeme *C. burnetii* Go-strip qPCR assays and thermocycler (referred to herein as “Biomeme”).

```
#####  
# COMPARISON OF TEST PERFORMANCE OF A CONVENTIONAL PCR AND TWO FIELD-FRIENDLY  
# TESTS TO  
  
# DETECT COXIELLA BURNETII DNA IN TICKS USING BAYESIAN LATENT CLASS ANALYSIS.  
  
# R programming code for Kamau et al. 2023  
  
# Code derived and adapted from:  
  
# Cheung, et al. Bayesian latent class analysis when the reference test is imperfect. Rev Sci Tech. 2021  
# Jun;40(1):271-286. doi: 10.20506/rst.40.1.3224.  
  
# Salgado et al. Bayesian latent class analysis to estimate the optimal cut-off for the MiLA # ELISA for the  
# detection of Mycoplasma bovis antibodies in sera, accounting for repeated # measures. Prev. Vet Med. 2022  
# Aug; 205:105694. doi: 10.1016/j.prevetmed.2022.105694  
  
#####  
####  
  
#Estimating Diagnostic Sensitivity (Se) & Specificity (Sp) for 3 tests in 1 population  
  
#####  
  
# Coxiella in ticks, 3 tests: Test 1=cPCR, Test 2=Biomeme, Test 3=PCR_HRM  
  
  
# Assumptions:  
  
# 1 population, all tests are independent.  
  
  
#read dataset from working directory  
coxiella <- read.csv("coxiellaData.csv")
```



```

# load required libraries

library(R2OpenBUGS) #for driving OpenBUGS from R

library(coda) #for reading and diagnostics of MCMC chains

library(mcmcplots) #for plotting

#####

# Assign a flat prior distribution for all model parameters

#variables below are called later in the code

pi1.s1 <- 1; pi1.s2 <- 1

cPCR.se.s1 <- 1; cPCR.se.s2 <- 1

cPCR.sp.s1 <- 1; cPCR.sp.s2 <- 1

biomeme.se.s1 <- 1; biomeme.se.s2 <- 1

biomeme.sp.s1 <- 1; biomeme.sp.s2 <- 1

PCRhrm.se.s1 <- 1; PCRhrm.se.s2 <- 1

PCRhrm.sp.s1 <- 1; PCRhrm.sp.s2 <- 1

#####

# Specify the model:

model=paste0("model{

#Multinomial Model for the Data

x1[1:2,1:2,1:2] ~ dmulti(p1[1:2,1:2,1:2], n1)

# 1=positive, 2=negative

# +++ All tests are positive

p1[1,1,1] <- (pi1* (Se1*Se2*Se3)) + ((1-pi1)* ((1-Sp1)*(1-Sp2)*(1-Sp3)))

#-++,-+-,++- All combinations of 1 test negative

p1[2,1,1] <- (pi1* ((1-Se1)*Se2*Se3)) + ((1-pi1)* (Sp1*(1-Sp2)*(1-Sp3)))

p1[1,2,1] <- (pi1* ((Se1*(1-Se2)*Se3))) + ((1-pi1)* ((1-Sp1)*Sp2*(1-Sp3)))

```

```

p1[1,1,2] <- (pi1 * ((Se1*Se2*(1-Se3))) + ((1-pi1)* ((1-Sp1)*(1-Sp2)*Sp3))

# --+,-+,-+ All combinations of 2 tests negative
p1[2,2,1] <- (pi1 * ((1-Se1)*(1-Se2)*Se3)) + ((1-pi1)* (Sp1*Sp2*(1-Sp3)))
p1[2,1,2] <- (pi1 * ((1-Se1)*Se2*(1-Se3))) + ((1-pi1)* (Sp1*(1-Sp2)*Sp3))
p1[1,2,2] <- (pi1 * (Se1*(1-Se2)*(1-Se3))) + ((1-pi1)* ((1-Sp1)*Sp2*Sp3))

# --- All 3 tests are negative
p1[2,2,2] <- (pi1 * ((1-Se1)*(1-Se2)*(1-Se3)) ) + ((1-pi1)* (Sp1*Sp2*Sp3 ))

#####

# apply flat prior distributions to all parameters

##### Priors for main parameters
##### Assign a flat prior distribution (1,1) for all model parameters
pi1 ~ dbeta("pi1.s1," , "pi1.s2,")
Se1 ~ dbeta("cPCR.se.s1," , "cPCR.se.s2,")
Sp1 ~ dbeta("cPCR.sp.s1," , "cPCR.sp.s2,")
Se2 ~ dbeta("biomeme.se.s1," , "biomeme.se.s2,")
Sp2 ~ dbeta("biomeme.sp.s1," , "biomeme.sp.s2,")
Se3 ~ dbeta("PCRhrm.se.s1," , "PCRhrm.se.s2,")
Sp3 ~ dbeta("PCRhrm.sp.s1," , "PCRhrm.sp.s2,")

})

#write to temporary text file
write.table(model, file="model.txt", quote=FALSE, sep="", row.names=FALSE, col.names=FALSE)

#####

#Data

```

```

coxiella.dat <- na.omit(coxiella)

## organize variables as binary factors with 1 listed first, then zero
T1 <- as.factor(coxiella.dat$cPCR)
T1 <- factor(T1,levels=c(1,0))

T2 <- as.factor(coxiella.dat$biomeme)
T2 <- factor(T2,levels=c(1,0)) # in orig data, 1 = pos, 0 = neg
T3 <- coxiella.dat$PCRhrm
T3 <- factor(T3,levels=c(1,0)) # in orig data, 1 = pos based on cutoff identified from unexposed cohort of 20
goats
n1 <- length(T1)
x1 <- table(T1,T2,T3)

#set data inputs to BUGS
dat <- list("x1","n1")

#Set parameters desired to monitor
paras1<- c("Se1","Sp1","Se2","Sp2","Se3","Sp3","pi1")

paras_names<-c("cPCR Se", "cPCR Sp", "Biomeme Se", "Biomeme Sp", "PCR-HRM Se", "PCR-HRM Sp",
"Proportion")

#Initialising values for 3 chains
inits1<-list(
  list(Se1=0.50, Sp1=0.95, Se2=0.8, Sp2=0.95, Se3=0.85, Sp3=0.97, pi1=0.15),
  list(Se1=0.45, Sp1=0.97, Se2=0.60, Sp2=0.98, Se3=0.7, Sp3=0.95, pi1=0.1),
  list(Se1=0.40, Sp1=0.90, Se2=0.75, Sp2=0.95, Se3=0.8, Sp3=0.97, pi1=0.13)
)

#####

```

```
### run model in OpenBUGS ###
```

```
niterations=30000
```

```
#final model
```

```
bug.out <- bugs(data=dat, inits=inits1, parameters.to.save=paras1, n.iter=niterations+1, n.burnin=0, n.thin=1,  
n.chains=3, model.file="model.txt", debug=T)
```

```
#####
```

```
### model diagnostics and outputs ###
```

```
# preliminary output to check neffective and rhat
```

```
bug.out
```

```
# Diagnostics are available model output is converted into an
```

```
#MCMC object with this command:
```

```
bug.mcmc <- as.mcmc(bug.out)
```

```
# html files with trace, density, and autocorrelation plots
```

```
dir.name<-"mcmcout"
```

```
if(!dir.exists(dir.name)){dir.create(dir.name)}
```

```
mcmcplot(bug.out, title="Diagnostic plots",
```

```
  filename = "_MCMCoutput",
```

```
  dir = paste0("./",dir.name,"/"),
```

```
  extension = "html")
```

```
# Here, we consider convergence to be achieved after 10000 iterations
```

```
# therefore, burn-in was set to so set to 10000
```

```
burnin <- 10000
```

```
#####  
# Final estimates  
k<-seq(burnin+1,niterations,1)  
# combine chains post-burnin  
est<-data.frame(rbind(bug.out$sims.array[k,1,],bug.out$sims.array[k,2,]))  
res<-t(apply(est,2,quantile,probs=c(0.5, 0.025, 0.975)))  
round(res, 3)  
res
```