Albanian j. agric. sci. 2015;14 (4): 395-403 *Agricultural University of Tirana*

RESEARCH ARTICLE (Open Access)

Bayesian Assessment of Accuracy Properties of Rose Bengal Test in REV-1 Vaccinated Small Ruminants

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Abstract

The live *Brucella melitensis* Rev-1 strain is considered the best vaccine available for the prevention of brucellosis in small ruminants. In Albania, vaccination of small ruminants with REV-1 has been used under various regimes since 2004. The effectiveness of the vaccination campaigns is monitored through a post vaccination monitoring and surveillance system based on testing vaccinated animals with Rose Bengal Plate Test (RBPT). Regrettably the test accuracy properties such as the sensitivity and specificity have not been validated in vaccinated small ruminants. The lack of knowledge on these properties hampers the correct evaluation of the true sero-conversion rate of the post vaccination monitoring at national and at flock scale. This study addressed this issue by using a Bayesian modelling framework to estimate two serological tests the RBPT which is the standard serological test used in the post vaccination monitoring and Complement Fixation Test (CFT). Serum samples from 191 reportedly vaccinated small ruminants were tested in parallel with RBPT and CFT. The estimates of sensitivity and specificity values of RBPT were 91% (95% CrI: 82 -98) and 89% (95% CrI: 70 - 98). For CFT the sensitivity resulted 86% (95% CrI: 70 - 95) and the specificity 95% (95% CrI: 80 - 99). The good sensitivity and acceptable specificity of RBPT support its utilization as screening test for post vaccination monitoring. The interpretation of the post vaccination data with RBPT are well acceptable at national level but not well suited for flock status interpretation. In terms of disease diagnosis, especially for a latter phase of the brucellosis control strategy in Albania, both tests could justify their use in association.

Keywords: Brucellosis, Small Ruminants, Bayesian modelling, Screening test, Sensitivity, Specificity

1. Introduction

Brucella melitensis is the main causative agent of caprine and ovine brucellosis. Brucellosis is readily transmissible to humans, causing acute febrile illness – undulant fever – which may progress to a more chronic form and can also produce serious complications affecting the musculo–skeletal, cardiovascular, and central nervous systems [1]. While there is no treatment of choice for brucellosis in animals, vaccination in sheep and goats is one of the most effective procedures to reduce the incidence of brucellosis in animals and as a result also in humans. *Brucella melitensis* strain Rev.1 remains the reference vaccine to immunise sheep and goats at risk of infection from *B. melitensis*. In Albania, vaccination with REV-1 of 3 to 6 months old replacement breeding stock commenced in a number of districts in 2004 and was extended nationally in 2005. This program continued until 2011 and was carried out jointly with test and slaughter of adult animals. The strategic approach has changed to mass vaccination of small ruminants during 2012-2013 and then reverted back to replacement stock vaccination during 2014 to 2015 [2].

In principle it is sufficient that animals are adequately immunised by vaccination, then within herd transmission of brucellosis will stop and consequently it will stop also the between herds transmission. However, if vaccination is inadequate (e.g. poor cold chain conditions, wrong reconstitution of vaccine, or insufficient number of animals correctly vaccinated), spread may continue, especially if other supplementary measures, such as movement restrictions, are inexistent.

To address these potential drawbacks the post vaccination monitoring and surveillance system (MOSS) is an essential component of the vaccination programme. The aim of this MOSS is to determine efficiency of the vaccination strategy by identifying the proportion of animals of the target population with significant titres of brucella specific antibodies. The specific objectives of the

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(Accepted for publication on December 15, 2015)
***ISSN**: 2218-2020,* *© Agricultural University of Tirana*

MOSS are to asses: i) the proportion of flocks - and animals within flocks - which have been vaccinated i.e. the coverage; ii) the rate of sero-conversion in vaccinated animals; and iii) to some extent estimate the performance of Private Veterinary Practitioners (PVP) [3].

From 2012 to 2015, Food Safety and Veterinary Institute (ISUV), was contracted to monitor the efficiency of the vaccination programme by monitoring sero-conversion rate among vaccinated animals using Rose Bengal Plate Test (RBPT). The RBPT is recommended for screening of samples to determine flock prevalence since the test is deemed highly sensitive [4]. However the test properties such as the sensitivity and specificity have not been validated in vaccinated small ruminants. The lack of knowledge on these properties of RBPT hampers the correct evaluation of the true sero-conversion rate of the post vaccination monitoring at national and at flock scale.

In principle diagnostic test are validated by comparing the outcomes with the gold standard. The gold standard for the diagnosis of brucellosis is isolation and identification of the organism [1]. The isolation *per se* is not an easy procedure to perform and the situation is even more complicated because for vaccinated animals the isolated strains have to be typed in order to discriminate the vaccine strain against the wild ones. In the absence of a gold standard, simultaneous estimation of true sero-conversion and diagnostic test properties can be performed successfully when applying multiple diagnostic tests using a Bayesian approach which combines test results and external information [5].

The objective of this study is to evaluate the performance and diagnostic characteristic of two serological tests namely RBPT and complement fixation test (CFT) in vaccinated small ruminants.

2. Material and Methods

2.1 Study design

During the 2013 mass vaccination campaign as part of the post vaccination monitoring programme have been collected 5303 blood samples from reportedly vaccinated animals, deriving from a two stage random sample of 433 selected flocks. From these samples, a subset of 191 animals has been selected for the evaluation of test properties. This subset of samples was taken from 10 flocks which had different apparent sero-conversion rates. The original idea was to derive different sub population to test the behaviour of diagnostic tests in flocks with different status in relation to the 80% sero-conversion rate which is considered the benchmark for a successful vaccination. Since the sample size of the intended groups would have been quite small all data were pulled in a single population as indicated in the summary of table 1.

Table 1. Selected flocks and apparent sero-conversion rate based on RBPT.

Flock ID	No. of animals	Apparent rate	
		RBPT	CFT
Flock 1	20	100%	85%
Flock 2	18	83%	78%
Flock 3	13	77%	46%
Flock 4	12	100%	100%
Flock 5	29	79%	79%
Flock 6	24	58%	58%
Flock 7	23	65%	57%
Flock 8	13	0%	0%
Flock 9	14	0%	0%
Flock 10	25	32%	28%
Summary	**191**	**61%**	**55%**

2.2 Serological tests

All blood samples were tested in parallel by RBPT, and CFT, in the serology laboratory of the animal health department at ISUV. All reagents used in these tests have been imported from certified producers in European Union. To reduce human related bias, laboratory tests were performed by the same experts and results have been assigned according to the diagnostic kit producer guidelines. The procedures have been performed in line with ISUV approved laboratory standard operating procedures which have been adopted from the World Animal Health Organization (OIE) Manual of

Diagnostic Tests and Vaccines for Terrestrial Animals 2013 [1]. Results were dichotomised and recorded in standardised forms and digitized into a spreadsheet.

2.3 Statistical analysis

Each test sensitivity and specificity were estimated using a Bayesian approach implemented through Markov Chain Monte Carlo algorithms applied in the software's WinBUGS version 1.4.3 [6], and R version 3.2.0 [7]. Specific packages used in R were R2WinBUGS [8] and epiR beta buster [9]. True prevalence together with positive and negative predicted values have been estimated for some of the flocks using the Epi Tools Calculator [10].

The model used is an adaptation of a Bayesian model which has been developed by Branscum in 2005 to estimate the respective covariance of the sensitivities and specificities of two tests for one population [11]. An important consideration in the evaluation of diagnostic tests is whether or not the tests can be assumed conditionally independent of each other. Tests are supposed to be independent given that an animal is vaccinated or not, the probability of positive or negative outcome for the first test is the same no matter what the outcome is for the other test [12]. Both tests, RBPT and CFT, used in this study are based on the same biological process because they detect the same brucella antibodies. Therefore they are consequently expected to be conditionally dependent. This influences the choice of the model, which has to account for the covariance structure between the two tests, see box 1 in the Addendum.

The main advantage of Bayesian framework is to combine prior information from the literature and experts' advice with field data. Prior information for both tests have been taken from a thorough literature review were 8 articles have been selected to be used as prior information for the model. According to previous studies, RBPT sensitivity average is 89.2% and mean values ranging from 96.1% to 76.4%. Average specificity is 95.9% and mean values ranging from 100% to 68.4%. For CFT the sensitivity average is 90.3% with mean values from 100% to 81.9% and specificity average of 95.6% with mean values from 100% to 65.5%, see table 2.

Table 2. Mean value sources used for estimation of diagnostic test characteristics.

Sensitivity %		Specificity %		Source
RBPT	**CFT**	**RBPT**	**CFT**	
90	100	100	100	Aparico 94 [13]
76.4	81.9	99.7	99.4	Nielsen 04 [14]
95	92.7	100	100	Ferreira 03 [15]
81.1	74.7	68.4	65.5	Pfeiffer 08 [16]
90.4	98.8	99.6	100	Minas 05 [17]
92.5	88.6	100	100	Blasco 94 [18]
96.1	93.3	100	100	Blasco 94 [19]
92.5	92.6	99.9	99.9	EFSA 06 [20]

To construct beta prior distributions for the parameters like sero-conversion rate, sensitivities and specificities, is used the most probable value of the parameter and a lower limit which is the value for which the general opinion is 95% sure that the parameter will be larger. As a median sero-conversion value in the population has been set 60% based on the RBPT apparent sero-conversion rate and the lower limit was set to 40% by guesstimate. Alpha and beta priors were determined by modelling sero-conversion and the literature derived parameters as beta distributions using epiR beta buster package in R. The estimated alpha and beta values have been incorporated into the Bayesian framework as informative priors for the sensitivity and specificity of the RBPT and CFT together with sero-conversion to inform posterior estimates, see Box 1 in the Addendum.

To obtain posterior estimates the model was set to run using three chains, a burn-in period of 100.000 iterations and 10.000 burning with a thinning factor of 5. Convergence of chains after the initial burn-in was assessed by visual inspection of time-series plots like trace, autocorrelation and running means. The parameters were checked by Gelman-Rubin diagnostic plots using the three sample chains.

3. Results

Based on diagnostic plots the model showed good mixing and convergence. The sero-conversion rate, sensitivities and specificities estimated from the model are given in table 4 and the full posterior distributions plotted in figure 1. Figure 2 and figure 3 shows the plotted values of true sero-conversion rate and apparent sero-conversion rates in conjunction with positive and negative predictive values over a variable number of positive test results for each of the tests.

Table 3. Parameter estimates with 95% Bayesian Credibility Interval.

Parameter	Estimates	median (95% CrI)
True prevalence	Pr	63% (50 - 70)
RBPT	SeRBPT	91% (82 -98)
	SpRBPT	89% (70 - 98)
CFT	SeCFT	86% (70 - 95)
	SpCFT	95% (80 - 99)
Dependencies	CovDp	0.3% (0 - 1)
	CovDn	0.7% (0 - 1.5)

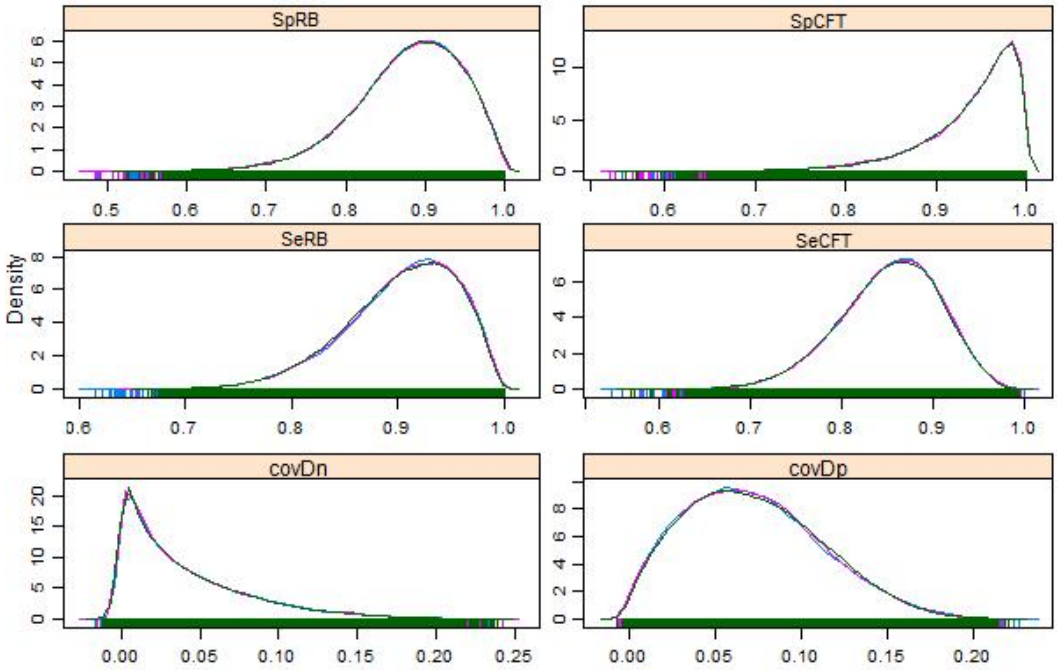

Figure 1. Posterior distributions of test parameters.

The estimate of the sensitivity of the RBPT from this model is 91% with a specificity of 89%. The RBPT shows to be more sensitive than CFT which has a sensitivity of 86%, on the other hand the CFT is more specific with an estimated specificity of 95%. Distributions shown in figure 2 indicate that the estimates of sensitivity and specificity for both tests are congruent with some of the literature data described in table 2. There is a slight positive covariance between tests for both positive 0.3% (CovDp) and negative 0.7% (CovDn), which indicate the conditional dependence of these tests. Apparent sero-conversion rate evaluated from RBPT was 61.2% (95% CL*: 54.2 - 67.9) meanwhile the apparent sero-conversion rate from CFT was 55.5% (95% CL: 48.4 - 62.4). The estimated true sero-conversion rate for the 191 vaccinated animals involved in this study is 62.8% (95% CL: 54 - 71.1).

*In this article CL = confidence limits, are the numbers at the upper and lower end of a 95% confidence interval. Meanwhile CrI = Credibility Interval where the value of interest lies with a 95% probability in the interval.

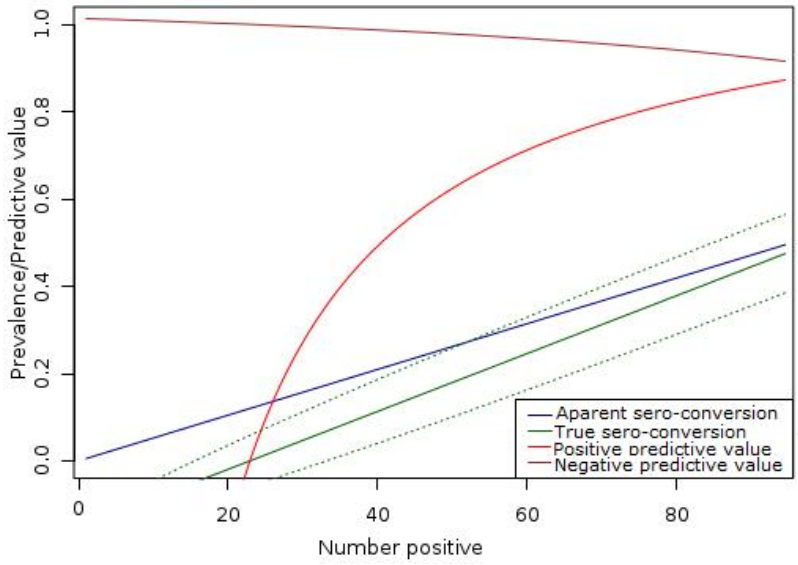

Figure 2. True sero-conversion rate and predictive values for RBPT (*doted lines are 95% Rogan-Gladen CL for the estimated true prevalence*).

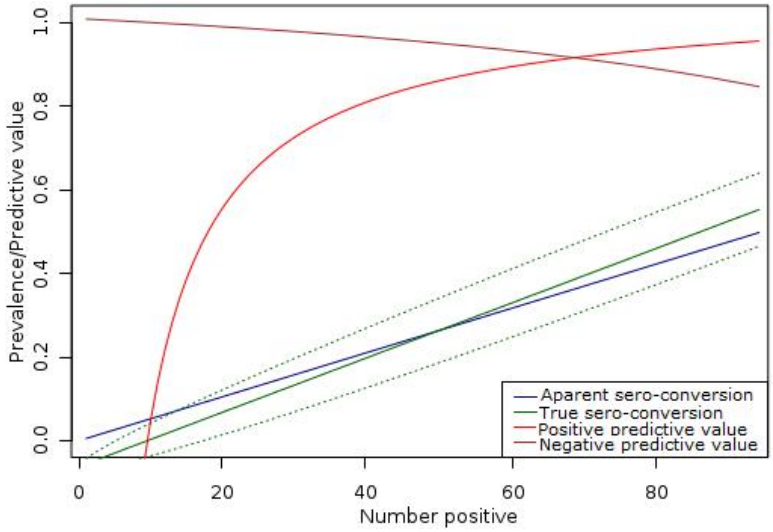

Figure 3. True sero-conversion rate and predictive values for CFT (*doted lines are 95% Rogan-Gladen CL for the estimated true prevalence*).

Predictive values are functions of prevalence and the test sensitivity and specificity. As prevalence increases so does positive predictive value, the opposite is true for negative predictive values. This is more evident for the CFT predictive values, see fig 3.

4. Discussions

It is generally recommended that diagnostic test characteristics be validated carefully before being applied to real monitoring data, especially in the case where the test is used in a national monitoring program. Sero-conversion rate is the most important parameter in practical terms because it is likely to vary substantially among flocks. Conditional probability relationships exist between sero-conversion rate and test characteristics and as it can be seen from Figure 2 the RBPT which has a lower specificity tends to overestimate the apparent prevalence, especially when the number of positive animals is small. Since both tests sensitivities and specificities are suboptimal, the apparent prevalence will be a result of both the false negative and false positive results. Thus at higher rates of sero-conversion it is expected that the apparent sero-conversion will be underestimated which is visibly manifested in figure 3 for CFT that have a lower sensitivity.

The post vaccination monitoring programme relay on RBPT testing of randomly selected flocks. The interpretation of results at flock level is important because the performance of private veterinary practitioners is assessed on flock bases. Figure 4 shows a comparison of RBPT true and apparent sero-conversion rates for some of the flocks involved in

this study. Flocks with 0% and 100% sero-conversion rates have been omitted because result are not plausible.

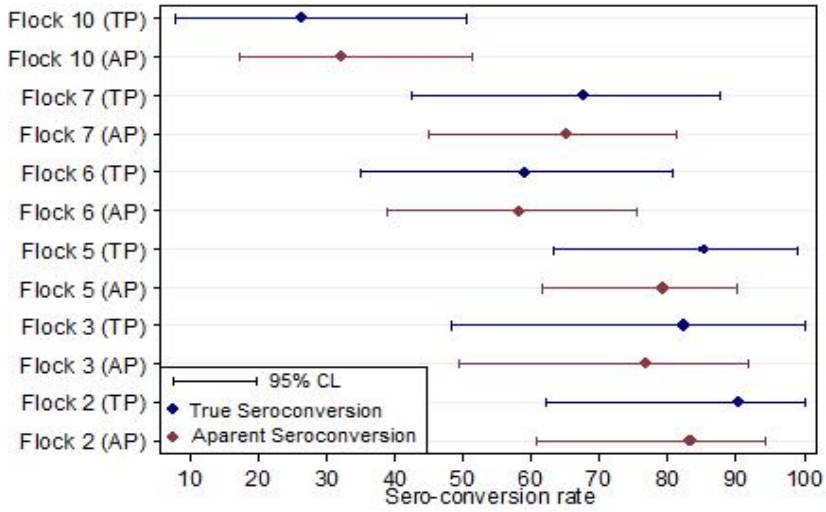

Figure 4. Flock point estimates and 95% confidence limits for true and apparent sero-conversion rates (*CL are based on Wilson Score).*

As expected at lower sero-conversion rates the apparent rate is overestimated and substantially underestimated at higher sero-conversion rates above 60%. This implication has a great impact on the performance of PVPs especially those which achieved border lining rates near to 80%, this value is used as a threshold for contracted veterinary work payment [3]. As an example flock 3 and flock 5 have apparent rates of 76.9% (95% CL: 49.7 - 91.8) and 79.3% (95% CL: 61.6 - 90.2) respectively. The true sero-conversion which is the proportion of animals that have been successfully immunized in the flocks is 82.4% (95% CL: 48.4 - 100) for flock 3 and 85.4% (95% CL: 63.3 - 98.9) for flock 5. The large confidence limits demonstrate a high variability of both true and apparent sero-conversion estimates which hampers the interpretations of results. Such high variability pinpoint the small sample size, a factor which has significant influence on the probability of identifying vaccination status at a small scale of aggregation.

5. Conclusions

This study is the first in Albania to estimate test accuracy for two dependent dichotomous screening tests in reportedly brucellosis REV-1 vaccinated small ruminants. The Bayesian approach used here has been implemented in other studies as well [5], [12] and [21], however none of the studies has examined the test properties under circumstances of a high sero-conversion rate.

The results demonstrate that RBPT is more sensitive than the CFT, but somewhat less specific. These findings are consistent with some of previous results available in the literature. These data support that the post vaccination technical monitoring based on RBPT is valid to estimate the overall success of the vaccination campaign at national level. The interpretation of the post vaccination data with RBPT are well acceptable at a district level data aggregation but not well suited at flock level. Here is clearly demonstrated that true sero-conversion rate based on RBPT properties and in conjunction with the current sampling protocol cannot be used to evaluate appropriately the performance of PVPs. Under the light of such findings this practice should be discouraged and more robust methods need to be developed.

In terms of disease diagnosis, especially for a latter phase of the brucellosis control strategy in Albania, both tests could justify their use in association. Even though the results shows a modest degree of covariance it is expected that it will not significantly affect the results when the tests are used in combination. The vaccination with REV-1 is an induced infection and meta populations with different infection rates can be created. Bayesian framework can be used to calculate estimates of sensitivities and specificities of multiple test in multiple populations [21] and [11]. It is therefore recommended that National Veterinary Epidemiology Unit (NVEU) focuses its future work to determine the clinical usefulness of these and other diagnostic test and their related combinations.

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7. Addendum

WinBUGS 1.4 code to accompany the manuscript entitled " Bayesian assessment of accuracy properties of Rose Bengal test in REV-1 vaccinated small ruminants".

```
function() {
x[1:4] ~ dmulti(p[1:4], n)
p[1] <-pr*(SeRB*SeCFT+covDp) + (1-pr)*((1-SpRB)*(1-SpCFT)
+ covDn)
p[2] <-pr*(SeRB*(1-SeCFT)-covDp)+(1-pr)*((1-SpRB)*SpCFT -
covDn)
p[3] <-pr*((1-SeRB)*SeCFT-covDp)+(1-pr)*(SpRB*(1-SpCFT) -
covDn)
p[4] <-pr*((1-SeRB)*(1-SeRLK)+covDp)+(1-pr)*(SpRB*SpCFT
+ covDn)
ls <- (SeRB-1)*(1-SeCFT)
us <- min(SeRB,SeCFT) - Serb*Serlk
lc <- (SpRB-1)*(1-SpCFT)
uc <- min(SpRB,SpCFT) - Sprb*Sprlk
pr ~ dbeta(10.902, 7.6013) #M=0.60, 95% > 0.40
SeRB ~ dbeta(16.2869,2.8894) #M=0.89, 95% > 0.7
SpRB ~ dbeta(8.447, 1.3919) #M = 0.95, 95% > 0.65
SeCFT ~ dbeta(10.0828, 2.4786) #M = 0.86, 95% > 0.6
SpCFT ~ dbeta(7.3523, 1.4781) #M=0.93, 95% > 0.6
covDn ~ dunif(lc, uc)
covDp ~ dunif(ls, us)
rhoD <- covDp / sqrt(SeRB*(1-SeRB)*Serlk*(1-SeCFT))
rhoDc<-covDn / sqrt(SpRB*(1-SpRB)*SpCFT*(1-SpCFT))
}
#cross tabulation of RBPT and CFT results
#PozPoz (p1) = 106
#PozNeg (p2) = 11
#NegPoz (p3) = 0
#NegNeg (p4) = 74
list(n=191, x=c(106,11,0,74))

# primary data
list(pr=0.6, Serb=0.88, Sprb=0.96, Serlk=0.86, Sprlk=0.93)

#Annotations
#pr = true sero-conversion rate
#SeRB = RBPT sensitivity
#SpRB = RBPT specificity
#SeCFT = CFT sensitivity
#SpCFT = CFT specificity
#covDp = positive animal covariance between tests
#covDp = negative animal covariance between tests
# rhoD = correlation of negative subpopulation between CFT
and RBPT
# rhoDc = correlation of negative subpopulation between CFT
and RBPT
```

Box 1. Bayesian framework model of two dependent tests, one population and no gold standard, adapted from Branscum 2005 [11].