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Evaluation of Indirect Fluorescent Antibody Test (IFAT) for the Diagnosis and Screening of Lumpy Skin Disease Using Bayesian Method

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Abstract

The performance of Indirect Fluorescence Antibody Test (IFAT) for serological diagnosis and screening of Lumpy Skin Disease (LSD) was evaluated using methods without gold standard. Virus neutralization test (VNT) was used as the second test and the study sites were selected from two different geographical places in Ethiopia to get different disease prevalence. The analysis of conditional dependent Bayesian model for the accuracy of IFAT showed that sensitivity, specificity, prevalence of the population P_{i1} and the population P_{i2} were 0.92 (0.89- 0.95), 0.88 (0.85- 0.91), 0.28 (0.25- 0.32) and 0.06(0.048- 0.075) respectively. The posterior inferences obtained for VNT sensitivity, specificity and conditional correlation between the tests for sensitivity (ρ_D) and specificity (ρ_{Dc}) were 0.78 (0.74- 0.83), 0.97 (0.95- 0.99), 0.052 (-0.03- 0.15) and 0.019 (-0.01- 0.06) respectively. The interval estimation of conditional correlation for both sensitivity and specificity clusters around zero and thus conditional dependence between the two tests was not significant. Although accuracy measure would not be the only basis for test selection, the result of our study demonstrated that IFAT has a reasonable high accuracy to be used for the diagnosis and sero-surveillance analysis of LSD in the target population.

Keywords: Bayesian model, Cattle, Ethiopia, Lumpy Skin Disease, Sensitivity, Specificity, IFAT

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36 **Introduction**

37

38 Lumpy skin disease (LSD) is an acute to sub acute viral disease of cattle that can cause mild to severe
39 signs including fever, nodules in the skin, mucous membranes and internal organs, skin oedema,
40 lymphadenitis and sometimes death. The disease causes high economic loss as a result of decreased milk
41 production, abortion, infertility, weight loss, poor growth and skin damage (Ali *et al.*, 1990; OIE, 2004).
42 Lumpy skin disease is caused by the strain of Capripox virus which is genetically and antigenically close
43 related to the strain of sheep and goat pox virus and the prototype strain is known as the Neethling Pox
44 Virus (Alexander *et al.*, 1957; Davies, 1982).

45

46 Currently, the distribution of LSD in Africa has increased its horizon from Sub-Saharan countries to
47 Egypt and Western Africa countries (Davies, 1991). Davies (1991) has also emphasized that the
48 epidemiological distribution trend of LSD has posed a considerable risk to extend its range to the
49 Northern Africa countries and Eastern ward of the Egypt to the Middle East countries. In Ethiopia LSD
50 was first observed in the western Ethiopia (South west of Lake Tana) in 1983 and the assumption was that
51 it has been introduced from Sudan (Mebratu *et al.*, 1984). The Ethiopian National Veterinary Service
52 field report from 1999-2006 revealed that the occurrence of LSD outbreak has almost spread to all
53 regions of the country including different agro-climatic zones. According to these reports we noted that
54 the number of outbreaks reported per month increases highly during the wet season that is from June up
55 to October (Ministry of Agriculture and Rural Development Disease Report Database). However, no
56 epidemiological study has been done yet in different regions and ecotypes of the country which is indeed
57 required to give a more realistic epidemiological picture than the one obtained by passive surveillance
58 data.

59 Diagnostic and screening tests are the primary tools for successful such epidemiological study (Greiner
60 and Gardner, 2000(a)). The OIE recommended serological tests used for LSD diagnosis are essentially
61 IFAT (Indirect Fluorescent Antibody Test), ELISA and VNT (Virus Neutralisation Test) (OIE, 2004).
62 Indirect ELISA of recombinant P32 antigen from KS-1 strain has been developed previously but till today
63 it has not been validated to replace the conventional once (Heine *et al.*, 1999). Lack of information on the
64 performance of the available diagnostic test is also one of the limiting factors to conduct large
65 epidemiological studies. Understanding the characteristic of the tests is essential to know how they affect
66 the quality of data obtained from epidemiological research and can be considered as a precursor step
67 (Dohoo *et al.*, 2003). The accuracy of these diagnostic tests should also be evaluated for specific target
68 population of concern (Greiner and Gardner, 2000(b)).

69

70 The availability of a suitable reference test is an important requirement for the performance evaluation
71 study. But it is difficult or sometimes next to impossible to obtain perfect (gold standard) test which can
72 identify the true disease status of the animal (Enoe *et al.*, 2000; Enoe *et al.*, 2001; Dohoo *et al.*, 2003;
73 Biteau-Coroller *et al.*, 2006). However, When gold standard test is not available the performance of two
74 tests can be estimated using latent-class approaches, provided that the error probability of the reference
75 test is known (Enoe *et al.*, 2000). In most cases virus neutralization test (VNT) is considered as reference
76 test which has a strong specificity but less sensitivity for capripox virus (OIE, 2004; Bhanuprakash *et al.*,
77 2006).

78

79 Diagnostic test evaluation is particularly suited to the Bayesian framework (Branscum *et al.*, 2005). The
80 Bayesian analysis for diagnostic test evaluation without gold standard was discussed for conditional
81 independent and conditional dependent tests (Enoe *et al.*, 2000; Gardner *et al.*, 2000; Johnson *et al.*, 2001;
82 Georgiadis *et al.*, 2003; Branscum *et al.*, 2005). Bayesian approach uses prior information knowledge
83 about the parameters of the tests under study either from other similar studies or expert's best guess.
84 Moreover, it has an advantage to provide more stable point estimates and intervals without the necessity
85 of large sample sizes (Enoe *et al.*, 2000). The Bayesian inference is the combination of the beta
86 distribution of the prior information and the maximum likelihood estimates of the observed data (Gardner
87 *et al.*, 2000).

88

89 The objective of this study was to evaluate the performance of IFAT for diagnosis and screening of
90 Lumpy Skin Disease in Ethiopia using VNT as second test for comparison. The Bayesian model and Hui
91 and Walter (1980) model were used to analyse the test performance where there is no gold standard. The
92 parameters used to measure the accuracy are sensitivity, specificity, prevalence and the conditional
93 correlation between the two tests.

94 **Materials and Methods**

95 **Study Area and Study Population**

96 The study was conducted from September 2006- March 2007. Two study areas with different farming
97 system and expected different LSD prevalence were selected. The first study area was in Amhara Region
98 (North Wello, South Wello and Oromia Administrative Zones, in northern part of Ethiopia) where the
99 altitude range from 1,400 to 2,230 meters above sea-level (Figure 1). Livestock production is extensive
100 system whereby animals of different species and age groups share common grazing land and watering
101 point. The breed composition of the subpopulation is predominantly the local zebu breed. At the time of

102 this study, Lumpy Skin Disease re-occurred as an outbreak in these study areas starting from the month of
103 July 2006 after 5-6 years elapse (personal communication with the local veterinary officers).

104

105 The second study area was in Oromia Region (Borena and Guji Administrative Zones in southern part of
106 Ethiopia) which have an agro-pastoral farming system with semi arid and sub-humid climate respectively.
107 Altitude range is from 1,590 to 1,740 meters. There was no reported LSD outbreak since 2005 in the area
108 and no vaccination program was put into place for the last 12 months.

109 **Sampling**

110 The purpose of the sampling design was to obtain different prevalence between the two study populations
111 to hold the Bayesian assumption true where gold standard test is not available. All animals above six
112 months old and both sex groups were subjected to random sampling.

- 113 ▪ Population 1 (P_1): in the northern study area, at the district level, the target Peasant Associations
114 (PA) (it is the lowest rural administrative level in Ethiopia which can hold variable number of
115 villages in it) were those with LSD outbreak history. In 7 PA selected from 3 districts, the herds
116 and animals were randomly selected for sample collection. In all the places, the sampling was
117 carried out before the deployment of vaccination to control the outbreak.
- 118 ▪ Population 2 (P_2): In southern study area, there was no recent evidence of LSD outbreak. The
119 samples were collected by multistage random sampling technique in 5 PA selected from 2
120 Districts. At the district level, the PA's were randomly selected and then the herds and the animals
121 too. Thus the study design applied agrees with the complete verification approach (Greiner and
122 Gardner, 2000(b)).

123

124 Sample size determination was based on Greiner and Gardner (2000) formula using the prior estimates for
125 sensitivity and specificity of IFAT to be 90% and 80% respectively with the desired precision level of
126 0.05. A total of 463 sera that is 263 sera from the northern area (P_1) and 200 sera from the southern area
127 (P_2) were assigned for the study:

128

129 **Serological Tests**

130 Blood samples of 5-7ml were collected in plain vacutainer tube from the jugular vein. The samples were
131 allowed to clot for 2-3 hours at room temperature. Then the serum was extracted by spinning at 2500 rpm
132 and the serum was preserved in -20°C temperature until the test conducted.

133

134 Indirect Fluorescent Antibody Test (IFAT)

135 The IFAT was used to detect serum antibody against Lumpy Skin Disease. Antibodies of capripox virus
136 can be detected from day 2 after the onset of clinical signs and remain detectable for about 7 months, but
137 a significant rise in titre is usually seen between days 21 up to 42 (Lefèvre *et al.*, 2003; OIE, 2004). The
138 serum samples were processed blindly for the test. The antigen used to detect the serum antibody against
139 lumpy Skin Disease was KS1 (Kenyan Sheep pox virus) strain which is recently proved to have
140 genetically identical with Neethling Virus (Gershon and Black, 1988). The KS1 strain was obtained from
141 CIRAD Laboratory and the Lamb testis cell was infected using 50µl of 100_{TCID₅₀} viral suspension per
142 well cultured in 96-well flat bottomed tissue-culture grade microtitre plate. The infected monolayer cells
143 were fixed after 48 hours using 80% acetone. The test serum was diluted in 1/25 in 0,5% Lamb serum
144 blocking buffer (blocking buffer is to avoid the non specific background reaction) and each serum was
145 tested in duplicate wells. The positive and negative control sera were also included in each plate.
146 Fluorescein isothiocyanate conjugated anti-bovine gamma-globuline (IgG) of Rabbit was diluted in 1/40
147 in 0,5% Lamb serum blocking buffer and add to each well (Standard Operating Protocol of CIRAD). The
148 plates were read using Zeiss Fluorescent microscope under 40X magnification. The positive test serum
149 appears bright fluorescence foci where the antibody reacted with the virus and the negative serum appears
150 as dark field or dim gray foci.

151 Virus Neutralization Test (VNT)

152 Serial dilution of the test serum was done in 1/5, 1/25, 1/125, 1/625 and 1/3125 dilutions and each serum
153 was tested in duplicate wells. KS1 strain virus in 100 _{TCID₅₀} per wells constant titration was maintained
154 similar for each well. The Vero cell was used for the test and cultured in 96-well flat bottomed tissue-
155 culture grade microtitre plates (OIE, 2004). The reason for Vero cells preferred was the Vero cells are
156 less sensitive to capripox virus and to reduce the problem of “breakthrough” in which the virus dissociate
157 the antibody binding and relapse to infect the cells (OIE, 2004; Bhanuprakash *et al.*, 2006). The plates
158 were incubated at 37°C, 5% carbon dioxide (CO₂) for 9 days. The plates were examined under inverted
159 microscope for the presence of cytopathic effect (CPE) starting from day 4. The final reading was taken
160 on day 9 and the result was recorded from the highest dilution which inhibited the CPE in both or either
161 of the duplicate wells. The test result was recorded as the reciprocal of the log titration. The interpretation
162 of the result is that the wells with no CPE in 1/25 and more dilutions were considered as positive serum.
163 This indicates that the antibody against the LSD virus has reacted with the KS1 virus and inhibited the
164 growth of the virus not to produce CPE.

165 Questionnaire Survey

166 Questionnaire survey included the LSD disease status, potential risk factors and other epidemiological
167 records using questionnaire format which was prepared based on the prior knowledge of the disease in the

168 respective sampled sites. The data was analysed by t-test to compare the disease prevalence between the
169 two sampling areas.

170 **Test evaluation**

171 The laboratory result obtained was cross classified for each population to calculate the test parameters
172 (Table 1). Statistical methods and tools used for evaluation of the test performance under different
173 specific conditions have been discussed (Enoe *et al.*, 2000; Gardner *et al.*, 2000; Johnson *et al.*, 2001;
174 Pouillot *et al.*, 2002; Georgiadis *et al.*, 2003; Orr *et al.*, 2003; Branscum *et al.*, 2005; Kostoulas *et al.*,
175 2006; Van Schaik *et al.*, 2007).

176
177 We applied methods without gold standard to analyse the accuracy since the reference test used was not
178 gold standard in its accuracy. We used comparatively the following methods to analyse our estimates:
179 Maximum Likelihood Estimate (Hui and Walter, 1980) model, conditional independent and dependent
180 Bayesian models (Branscum *et al.*, 2005).

181
182 The maximum-likelihood method assumes three conditions: i) the studied population should consist of
183 two subpopulations with different prevalence, ii) in these subpopulations, the test accuracy should be
184 constant and iii) the two tests should be conditionally independent of each other (Hui and Walter, 1980;
185 Pouillot *et al.*, 2002). We used the spreadsheet model of Hui and Walter (1980) from the web site
186 (www.epi.ucdavis.edu/diagnostictests/).

187
188 The Se and Sp estimates of IFAT (Se_{IFAT} and Sp_{IFAT}), the Se and Sp of VNT (Se_{VNT} and Sp_{VNT}) and the
189 prevalence of the two populations (Pi_1 and Pi_2) were also calculated using Bayesian methods. The study
190 populations have different disease prevalence based on information obtained from the analysis of
191 questionnaire interview data (Table 2) and thus complies with the assumption where gold standard test is
192 not available.

193
194 Tests based on similar biological basis might have correlated errors that cause incorrect estimation of
195 sensitivity and specificity (Gardner *et al.*, 2000; Georgiadis *et al.*, 2003; Orr *et al.*, 2003; Branscum *et al.*,
196 2005). As both IFAT and VNT detect antibodies, it is reasonable to confirm that the tests sensitivity and
197 specificity were indeed conditionally independent on disease status. Then, both conditional independent
198 and dependent Bayesian models for two tests, two populations were applied which allowed us to estimate
199 the Se and Sp conditional correlations (ρ_{D}) and ρ_{Dc} respectively) between the tests and their 95%
200 probability intervals (95% PI). We used the model recently reviewed by Branscum *et al.*, (2005) for both

201 conditionally independent and dependent assumptions, using Winbugs package (for more details see
202 (Enoe *et al.*, 2000; Georgiadis *et al.*, 2003; Branscum *et al.*, 2005).

203 The assumption of equal accuracy of the tests across subpopulations was checked by considering separate
204 analysis of the two populations (Georgiadis *et al.*, 2003). For each population, the model and prior
205 information used were identical to the model and prior used in the two population case.

206 **Prior Information**

207 In Bayesian analysis prior information are often specified for the unknown parameters either from
208 published papers or experts best guess (Enoe *et al.*, 2000; Branscum *et al.*, 2005). Prior information on
209 sensitivity and specificity of these current tests were obtained from scientists working on capripox
210 research in CIRAD and Institute for Animal Health Pirbright (IAH) laboratory. We could not get any
211 scientific publication data relevant to the determination of the accuracy of tests for Lumpy Skin Disease
212 except the general recommendations on the available diagnostic tests currently in use. The prior
213 information of disease prevalence in the two populations (P_{I1} and P_{I2}) were estimated on the basis of the
214 results of the farmers' interviews conducted during the sample collection (Table 2).

215

216 The uncertainty of prior information are often modelled through the use of beta distributions (Enoe *et al.*,
217 2000). The modal value of the prior information was transformed to beta distribution model using
218 Betabuster free software from the website (www.epi.ucdavis.edu/diagnostictests/). For conditional
219 independent Bayesian model the prior information for sensitivity of IFAT was mode 0.90 and the
220 transformed beta (a,b) was beta (130.71, 15.41) with 5th percentile equals to 0.84. Prior mode for
221 specificity of IFAT was 0.85 beta (152.9, 27.8) with a 5th percentile 0.79. The sensitivity prior for VNT
222 was mode 0.75 beta (174.5, 58.8), 5th percentile 0.69 and specificity prior mode was 0.95 beta (99.7, 6.2)
223 and 5th percentile 0.89. The beta prior distributions for prevalence of population1 (π_1) and population2
224 (π_2) were mode 0.24 beta (118.8, 374) and 0.06 (66, 1032) respectively and 95th percentiles of 0.28 and
225 0.075 respectively. For conditional dependent model we used similar prior information as indicated above
226 for Se_{VN} , Sp_{VN} of VNT, π_1 and π_2 . Georgiadis *et al.*, (2003) discussed reparameterization of the second
227 test parameters since prior information is not usually available for the new test during new test validation.
228 However, in this study the prior information obtained from expert's best guess for IFAT sensitivity and
229 specificity were applied instead of reparameterization. Thus we assigned uniform priors for λ_D and γ_D a
230 modal value of 0.90 beta (130.7, 15.4) with 5th percentile 0.84 and in the same way a uniform prior for
231 λ_{Dc} and γ_{Dc} with a mode of 0.85 beta (152.9, 27.8), 5th percentile of 0.79 (Branscum *et al.*, 2005).

232 **Test agreement**

233 McNemar's chi²-test and the Kappa statistic (k) were used to test the level of agreement between the
234 IFAT and the VNT. McNemar's chi² was carried out first to test whether there was test bias (i.e. the
235 difference in proportion positive result in each test) (Dohoo *et al.*, 2003). Kappa and its 95% CI, was used
236 further to measure the degree of agreement between the two tests after taking into account the probability
237 of agreement by chance alone. Strength of agreement based on k was judged according to the following
238 guidelines: <0.2 = slight agreement; 0.2–0.4 = fair; 0.4–0.6 = moderate; 0.6– 0.8 = substantial; >0.8 =
239 almost perfect (Dohoo *et al.*, 2003). The software Intercooler Stata 8.2 (StataCorp LP, College Station,
240 TX) was used for these analyses

241 Statistical and model analysis were computed using STATA 8.0 (Stata Corporation © 1984-2003),
242 Winbugs®, Betabuster and H&W model Excel spread sheet from online at
243 <http://www.epi.ucdavis.edu/diagnostictests/>.

244

245 **Results**

246 **Descriptive Epidemiology**

247 In both study areas LSD occurrence showed to have seasonal pattern and frequently associated with high
248 moisture climate and high insect population dynamics (Figure 2). About 90% respondents replied that the
249 disease occurs from July to November which is the season of high moisture and also extends up to
250 December. In the northern study area the LSD outbreak was commenced in July 2006 and continued up to
251 the end of December 2006 which covered a wide extensive area (in four administrative Zones of Amhara
252 Region). Retrospective data analysis of LSD outbreak pattern from year 1999- 2006 also revealed that the
253 temporal distribution graph peaks high at the end of high rainy season (September) and gradually drops
254 down up to the end of December (National disease outbreak report database) (Data not shown).

255 In the northern study area only 10% herd owners used their own grazing plots but they shared the same
256 watering point with animals in the surrounding community. The farmers are sedentary in their occupation.
257 However, in the southern part all the community in the peasant Association shares the same grazing land
258 and watering point. Moreover, about 50% of the herd owners in the southern part responded that they
259 have transhumant mode of life in which they move their herd seasonally to other grazing places in search
260 of better feed and water for their animals.

261 **Maximum Likelihood Estimates**

262 The Maximum Likelihood Estimates (MLE) of Hui and Walter model highly over estimated the Se_{IFAT} ,
263 Se_{VN} and pi_1 as compared to the estimates of Bayesian models. The point estimates of MLE were not

264 included in the 95% probability interval ranges of the Bayesian posterior inference (Table 3). However,
265 specificity of both tests and π_2 were not significantly different from the estimates of Bayesian models in
266 which the estimates were in the 95% probability interval range of the respective parameters.

267 **Bayesian Conditional Independent and Dependent Models**

268 The posterior inferences obtained by conditional independent and dependent Bayesian models were
269 consistently similar in all estimated parameters. The analysis of conditional correlation between the two
270 tests showed the conditional dependence between the tests were significantly minimum, which was less
271 than 0.1 for both sensitivity (ρ_D) and specificity (ρ_{Dc}) (Table 3). The 95% probability interval of
272 conditional correlation estimate for sensitivity and specificity included zero in which the hypothesis for
273 the conditional dependence could be rejected (Gardner *et al.*, 2000).

274

275 The Sp_{VN} obtained from Bayesian estimates was nearly perfect for LSD diagnosis which is in the contrary
276 to its low sensitivity estimate 0.78 (0.74- 0.83). In our finding, the Se_{IFAT} was found to be high at 0.92
277 (0.89- 0.95) as expected. Similarly the specificity was also fairly good at 0.88 (0.85- 0.91) as it was
278 considered to have lower specificity due to the possible cross reactions of parapox and orthopox virus
279 with capripox virus.

280

281 The Se and Sp estimates calculated separately for each population showed that one-population analysis
282 were consistent with the second population analysis and with the two-population case, indicating that our
283 assumption of similar accuracy of the tests across the two populations was valid (Table 4). The precision
284 of point estimates for sensitivity and specificity of both tests were within the range of 0.03 and 0.01
285 respectively.

286 **Analysis of Bayesian Model Sensitivity**

287 We used three sets of prior information for model sensitivity analysis (Table 3). (1.) Non informative
288 priors for all parameters of the two tests showed that the posterior inferences for Se_{IFAT} and Se_{VN} were
289 largely over-estimated while the rest parameters were remained almost similar estimation (result not
290 shown). (2.) Using informative priors for the two prevalences only, the median estimate for Se_{VN} was
291 still over-estimated although its interval estimate included the true value and the remaining estimates
292 were seemed not significantly affected (Table 3 Model 5). (3.) Additional model sensitivity analysis using
293 informative priors for π_1 , π_2 , Se_{VN} and Sp_{VN} showed that the model estimates were not distinctly
294 different from the analysis obtained using prior information for all parameters (Table 3 model 4). But the
295 conditional correlation for specificity resulted significant test dependence. The model converged fairly for

296 all parameters in model 4 and 5 (Table 3) prior information than when non informative priors were
297 assigned for all the parameters.

298 The convergence of the Bayesian models were analysed by observing kernel density and trace plots of the
299 model visually and the plots stabilized consistently for all the parameters. The first 5000 iterations were
300 discarded as burn-in phase and the posterior inferences were based on 100,000 iterations.
301 Autocorrelations were also checked and there was no meaningful autocorrelation observed.

302 **Test Agreement**

303 The difference in the proportion positive tests calculated for McNemar's X^2 test showed significant
304 difference (McNemar's $\chi^2= 33.62$, $p< 0.000$) between the tests. Test agreement between the two tests
305 using Kappa statistics was $Kappa= 0.70$ (0.61- 0.78) showing that the two tests have substantial
306 agreement according to the interpretation of Kappa result (Dohoo *et al.*, 2003).

307 **Discussion**

308 In both study areas we noted that extensive livestock production system allows maximum chance for
309 different herd mixing during utilization of communal grazing lands and watering points. Under this
310 prevailing system it is likely to speculate that the introduction and spread of LSD infection could have
311 favourable environment. Uncontrolled cattle movements due to trade, pastoralism, vector insects
312 population and dynamic, wet climate which favours insect multiplications and other reasons of cattle
313 movement from place to place could render potential risk factors for the transmission of the disease from
314 herd to herd and from place to place as it is true for other infectious disease too (Toma *et al.*, 1999).

315 Seasonal characteristics of LSD occurrence implies that the transmission of the disease might linked with
316 the optimum season for the development of vector insects population (Kitching and Mellor, 1986;
317 Chihota *et al.*, 2001, 2003). However, there are still little hard evidences for the specific insect vectors
318 incriminated in the transmission of LDSV and may deserve further study to elaborate the principal
319 vectors.

320 The immune response against LSD involves predominantly cell mediated immune response and the
321 humoral immune system would last short period of life mostly for 7 months (Lefèvre *et al.*, 2003; OIE,
322 2004). Hence studies based on serological detection of the disease should take into consideration the short
323 lifespan of detectable antibody in the blood. For sample collection, we selected the natural infected
324 population under active disease outbreak situation as P1 and the other population with unknown disease
325 status but which could have had exposure to the infection as P2. This approach has greatly enabled to get

326 significantly different prevalence between the two sub-populations which might be the ideal assumption
327 for epidemiological approach of diagnostic test evaluation for Lumpy Skin Disease.

328 An optimum consideration was taken during the laboratory techniques to limit the possible cross reaction
329 of parapox and orthopox virus with LSD virus and the information obtained through epidemiological
330 disease investigation records was also used to understand the clinical disease situation in the study
331 population. The members of Capripox virus Genus are antigenically very close related, which makes not
332 possible to distinguish them by serological tests (Davies and Otema, 1981). However, capripox virus is
333 highly host-specific under natural environment (Capstick and Coackley, 1961) and there has not been
334 recorded incidence of Lumpy Skin Disease occurrence from sheep pox or goat pox disease outbreak. This
335 has been clearly evidenced that the Republic of South Africa had LSD but having huge number of sheep
336 and goats there was no incidence of Sheep and goat pox disease (Capstick and Coackley, 1961). In
337 Middle East countries where sheep pox is endemic, LSD incidence has never been reported except the
338 case reported in Israel and eradicated soon in 1989 (Yeruham *et al.*, 1995). In Kenya sheep was found
339 infected where the first outbreak of LSD occurred, which was the first in kind (Davies, 1991). But in an
340 other study this Kenyan sheep and goat pox strain was proved to have more genetic similarity to
341 Neethling virus than classical sheep pox or goat pox virus which maybe due to some genetic mutation
342 enabled for adaptation to cattle (Gershon and Black, 1988). In our study sheep pox did not occur
343 concurrently with LSD in the outbreak areas and it had never been noted to occur as a multi-host outbreak
344 in the same place unless they coincided due to accidental overlap (personal communication with vet
345 officers in study area).

346 Cross reaction of cowpox virus was observed to occur with LSD virus at lower dilution ($\leq 1/8$) (Davies
347 and Otema, 1981). But we diluted the test sera for IFAT at 1/25 concentration that might help us to reduce
348 the possibility of cross reacting globulins and non specific background reactions. As a result it might have
349 contributed to get better specificity test result which was 0.88 (0.85- 0.91). However, the cross reaction of
350 cowpox with LSD virus observed in IFAT had not been demonstrated in VNT (Davies and Otema, 1981)
351 which is in congruent with the high specificity estimated for VNT in our finding.

352 Maximum likelihood estimate (MLE) over-estimated three parameters out of six which did not fall in the
353 95% probability interval of the Bayesian estimates. The 95% confidence interval of MLE was also wider
354 than the interval estimates of Bayesian models. The variation in the point estimates and the wider range
355 for the interval estimates might reveal the uncertainty of MLE that assumes large sample size. Thus the
356 method might not be applicable in our case due to small sample size (Enoe *et al.*, 2000; Orr *et al.*, 2003).

357 As both tests measure the same biological factor we expected certain degree of dependence between the
358 two tests. However, both conditional independent and dependent models had similar estimates for all

359 parameters which indicated that the two tests are conditionally independent. The 95% probability interval
360 of conditional correlation estimates were clustered around zero for both sensitivity and specificity which
361 showed that the dependence of the tests were not significant (Georgiadis *et al.*, 2003). This implies also
362 the two tests could be used in series and parallel test combinations for a maximum test efficiency
363 (Gardner *et al.*, 2000; Greiner and Gardner, 2000; Dohoo *et al.*, 2003). Although the estimates we found
364 from conditional independent and dependent models did not vary, we preferred to use the conditional
365 dependent model for discussion to elucidate the information regarding the magnitude of test dependence
366 (Enoe *et al.*, 2000; Gardner *et al.*, 2000; Branscum *et al.*, 2005).

367 The posterior inferences estimated across the two populations separately and jointly showed insignificant
368 difference (Table 4). The point estimates of each parameter obtained from separate analysis of each
369 population lies within the 95% probability interval ranges of respective parameter in the combined
370 population analysis. This supported the model assumption for similar test accuracy in the two
371 populations. The slight variation observed in the precision of point estimates of Se_{IFAT} and Se_{VN} in
372 population 2 might be due to small sample size coupled with lower disease prevalence in this population.
373 The results could also reveal that Bayesian method has superior approximation to give reasonable
374 posterior inference even under a small sample size condition (Branscum *et al.*, 2005)..

375 In the analysis of model sensitivity using non informative priors for all the parameters, we found that the
376 Se_{IFAT} and Se_{VN} estimates were unlikely over estimated which might be due to non-identifiable model
377 where the unknown parameters are greater than the degree of freedom. Whereas the posterior median
378 obtained by using informative prior for π_1 , π_2 , Se_{VN} and Sp_{VN} (Table 3, model 4) and for π_1 , π_2 (Table
379 3, model 5) resulted better estimation except the overestimated value of Se_{VN} in the latter model analysis.
380 This indicates that the availability of prior information for the prevalences and the accuracy of one test
381 would be necessary to get optimum posterior inferences (Georgiadis *et al.*, 2003; Branscum *et al.*, 2005).
382 In general we can conclude that the posterior inferences of the Bayesian models did not vary distinctly for
383 the changes in the prior information which indicates that the models were not significantly influenced by
384 the prior information.

385 The substantial agreement between the tests observed from kappa statistics was not supported by
386 McNemar's X^2 test. The difference in the proportion positive test results was significantly different for the
387 two tests (McNemar's $\chi^2 = 33.62$, $p < 0.000$). This significant difference in the proportion of positive
388 test results might be explained by the low sensitivity in Virus neutralization test and a minimum
389 conditional dependence between the two tests (Dohoo *et al.*, 2003). But in reality this empirical difference
390 might not justify the presence of a test bias from a biological point of view since it reflects the existence
391 of significant difference in the sensitivity estimates of the two tests.

392 Conclusion

393 In this study we observed that the accuracy of Indirect Fluorescent Antibody test was fairly good in both
394 sensitivity and specificity parameters indicating that it can be used for LSD diagnosis and screening with
395 low misclassification. Its capacity to run large number of samples per plate (45 samples per plate) could
396 be also taken as an advantage to use for large epidemiological studies of LSD. However, to undertake
397 similar epidemiological study on sheep pox and goat pox, we suggest further evaluation study to
398 determine the accuracy of these tests for sheep pox and goat pox diseases. Test accuracy may vary
399 according to the target population of concern and extrapolating directly the result of current study on LSD
400 might lead to unwise conclusion for sheep pox and goat pox diseases.

401 The conditional correlation estimates between the two tests revealed that the tests are conditionally
402 independent on the disease status of the animal. This implies that the two tests could be used especially in
403 parallel test combinations with maximum sensitivity efficiency.

404 The drawback in using IFAT is that the test requires longer time and may be more costly as compared to
405 ELISA technique. We recommend more efforts and studies should be done towards the development and
406 validation of ELISA test which may outmatch the limitations of the currently in-use diagnostic and
407 screening tools.

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417

418

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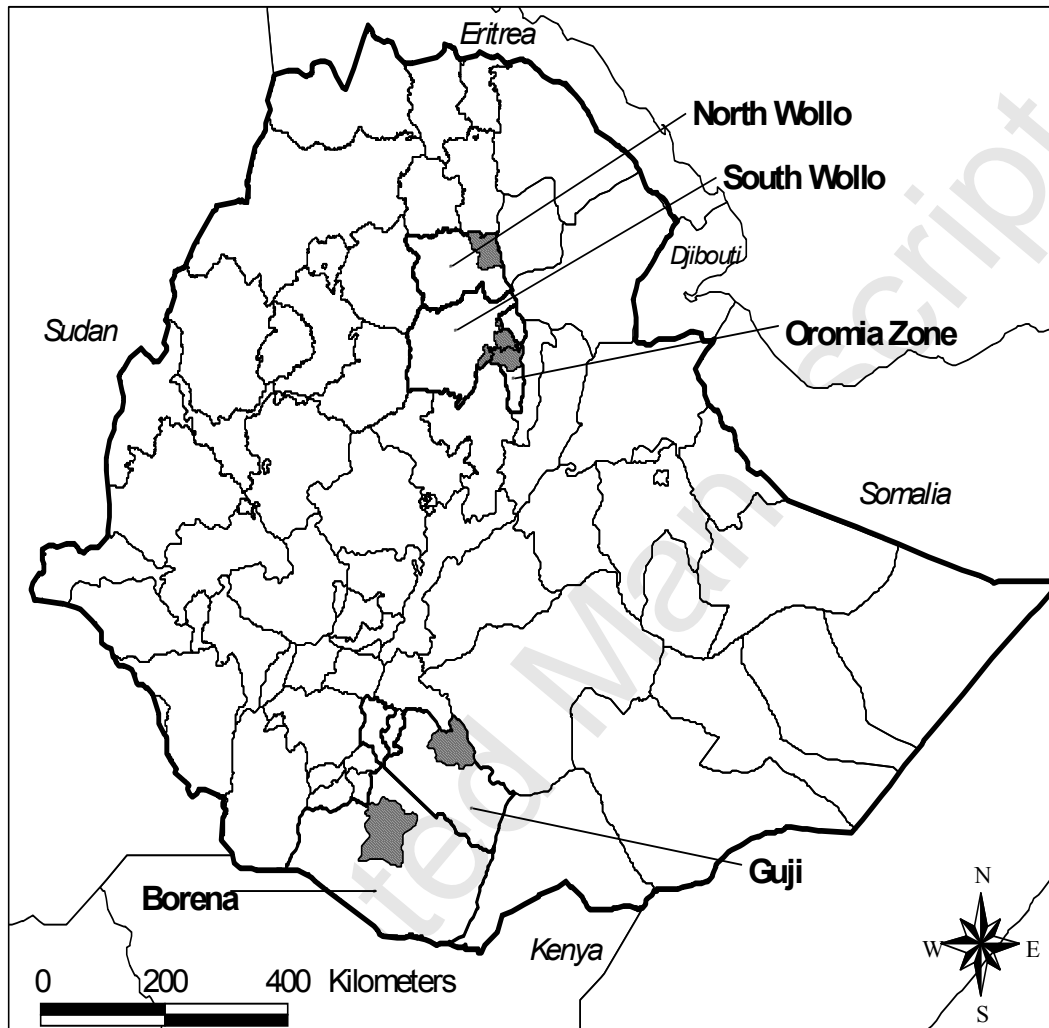


Figure 1: Map of Ethiopia with the locations of the study areas (shaded areas) (Source: International Food Policy Research Institute, Atlas of the Ethiopian Rural Economy, 2006)

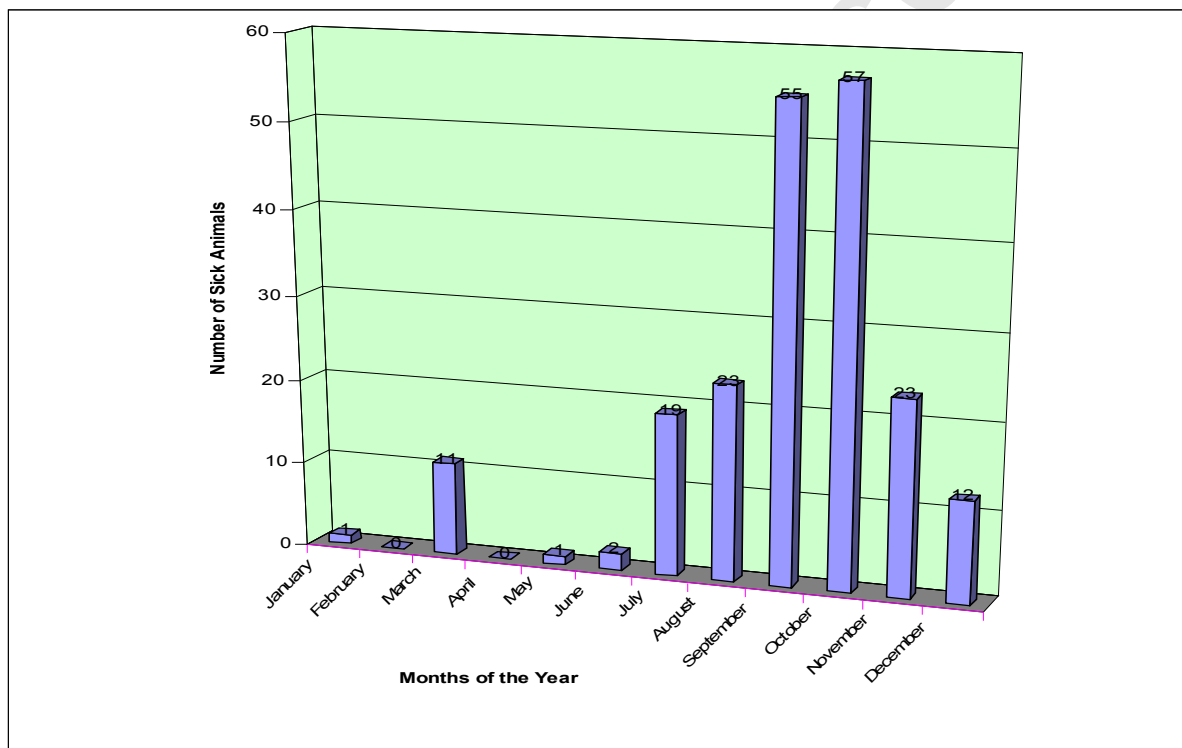


Figure 1: Seasonal occurrence of LSD in the studied areas based on the data from questionnaire interview

1 Table 1: Cross classification of the IFAT and VNT results from serum samples in two cattle populations
2 with expected high prevalence in P_1 and low prevalence in P_2

3

		Virus neutralization test				Total
		P1		P2		
		+	-	+	-	
IFAT	+	82	29	12	20	143
	-	3	149	3	165	320
Total		85	178	15	185	463

4

5 Table 2: Lumpy Skin Disease prevalence estimation based on farmers' opinions collected in the two study
6 areas.

7

Studied Population	Northern study area	Southern study area	Total
Number of investigated herds	99	53	152
Number of sampled animals	615	1 097	1 712
Number of LSD diseased cattle	150	66	216
Estimated Prevalence	24.4% (CI: 21, 27.8%)	6% (CI: 4.6, 7.4%)**	
LSD Mortality	2.8% (CI: 1.3, 4.3%)	1.8% (CI :1, 2.6%)	

8 ** Significantly different at $p < 0.05$

9 Note: Herd in this context is defined as cattle possessed by one farmer or a group of relatives which are
10 managed together in a similar manner.

11

12

13

14 Table 3: The sensitivity and specificity estimates of Indirect Fluorescent Antibody Test (IFAT) (Se_{IFAT} and Sp_{IFAT}) and Virus Neutralization Test (VNT) (Se_{VNT}
 15 and Sp_{VNT}), for the detection of antibodies against Lumpy Skin Disease in serum samples by the Hui and Walter and the Bayesian models with prevalence estimates
 16 for the two study populations and conditional correlation estimates.

17

Parameters	Median (%) and (95% Probability interval)				
	Model 1 ^a	Model 2 ^{b,d}	Model 3 ^{c,d}	Model 4 ^{c,e}	Model 5 ^{c,f}
Pi_1	0.36 (0.27– 0.44)	0.28 (0.25- 0.32)	0.28 (0.25- 0.32)	0.27 (0.24- 0.31)	0.265 (0.23- 0.30)
Pi_2	0.07 (0.03– 0.10)	0.06 (0.05- 0.08)	0.06(0.048- 0.075)	0.058 (0.045- 0.07)	0.058 (0.05- 0.07)
Se_{IFAT}	0.99 (0.93– 1.0)	0.93 (0.89 – 0.96)	0.92 (0.89- 0.95)	0.89 (0.75- 0.98)	0.95 (0.84- 0.99)
Se_{VN}	0.87 (0.72– 1.0)	0.79 (0.74- 0.83)	0.78 (0.74- 0.83)	0.77 (0.71- 0.82)	0.94 (0.79- 0.99)
Sp_{IFAT}	0.90 (0.85– 0.95)	0.88 (0.85- 0.91)	0.88 (0.85- 0.91)	0.85 (0.8- 0.90)	0.85 (0.80- 0.89)
Sp_{VN}	0.98 (0.96– 1.0)	0.97 (0.96- 0.99)	0.97 (0.95- 0.99)	0.94 (0.91- 0.97)	0.96 (0.91- 0.99)
RhoD			0.052 (-0.03- 0.15)	0.46(-0.04-0.90)	0.38(-0.016-0.88)
RhoDc			0.019 (-0.01- 0.06)	0.43(0.17-0.61)	0.28(-0.015-0.55)

18 ^a Model 1 is a Maximum Likelihood Estimate (MLE) based on the Hui and Walter (1980) model.

19 ^b Model 2 is based on the assumption of conditional independent model.

20 ^c Models 3, 4 and 5 are based on the assumption of conditional dependent model.

21 ^d Models 2 and 3 used the following priors: Se_{IFAT} : 0.90, $\beta(130.7,15.4)$; Sp_{IFAT} : 0.85, $\beta(152.9,27.8)$; Se_{VN} : 0.75, $\beta(174.5,58.8)$;

22 Sp_{VN} : 0.95, $\beta(99.7,6.2)$; Pi_1 : 0.24, $\beta(118.8,374)$; Pi_2 : 0.06, $\beta(66,1032)$

23 ^e Model 4 used non-informative priors $\beta(1;1)$ for se_{IFAT} , sp_{IFAT} , the other parameters are the same priors as in models 2 and 3.

24 ^f Model 5 used non-informative priors $\beta(1;1)$ for se_{IFAT} , sp_{IFAT} , se_{VN} , sp_{VN} and the same priors as in model 2 and 3 for Pi_1 and Pi_2 .

25

26 Table 4: Estimates of one population-two tests and combined two populations-two tests Bayesian
 27 Analysis for the evaluation of test accuracy similarity across the two populations.

28

Parameters	P_1	P_2	$P_1 + P_2$
Se_{IFAT}	0.93 (0.89- 0.96)	0.90 (0.85- 0.94)	0.92 (0.89- 0.95)
Se_{VNT}	0.78 (0.74- 0.83)	0.75 (0.70- 0.80)	0.78 (0.74- 0.83)
Sp_{IFAT}	0.86 (0.82- 0.90)	0.87 (0.84- 0.91)	0.88 (0.85- 0.91)
Sp_{VNT}	0.97 (0.94- 0.97)	0.97 (0.94- 0.98)	0.97 (0.95- 0.99)
P_i	0.28 (0.25- 0.32)	0.061 (0.049- 0.076)	

29 In parenthesis, 95% probability interval

30

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