# Sensitivity and specificity of human point-of-care circulating cathodic antigen (POC-CCA) test in African livestock for rapid diagnosis of schistosomiasis: a Bayesian latent class analysis.

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# **Abstract**

Schistosomiasis is a major neglected tropical disease (NTD) affecting both humans and animals. The morbidity and mortality inflicted upon livestock in sub-Saharan Africa has been largely overlooked, in part due to a lack of validated sensitive and specific tests, which do not require specialist training or equipment to deliver and interpret. Inexpensive, non-invasive, and sensitive diagnostic tests for livestock-use would also facilitate both prevalence mapping and appropriate intervention programmes. The aim of this study was to assess the sensitivity and specificity of the currently available point-of-care circulating cathodic antigen test (POC-CCA), designed for *Schistosoma mansoni* detection in humans, for the detection of intestinal livestock schistosomiasis caused by *Schistosoma bovis* and *Schistosoma curassoni*. POC-CCA, together with the circulating anodic antigen (CAA) test, miracidial hatching technique (MHT) and organ and mesentery inspection (for animals from abattoirs only), were applied to samples collected from 195 animals (56 cattle and 139 small ruminants (goats and sheep) from abattoirs and living populations) from Senegal. POC-CCA sensitivity varied by ruminant group and by location/parasite species: sensitivity was greater in Barkedji (cattle: mean 81% (95% credible interval (CrI): 55%-98%); small ruminants: 49% (29%-87%), where livestock were primarily infected by *S. curassoni*, than in Richard Toll (cattle: 62% (41%-84%); small ruminants: 12% (1%-37%), where *S. bovis* was the main parasite species. Mean POC-CCA specificity across sites in small ruminants was 91% (77%-99%) with little variation between locations/parasites (Barkedji: 91% (73%-99%); Richard Toll: 88% (65% - 99%). Specificity could not be assessed in cattle owing to the low number of uninfected cattle surveyed. Overall, our results indicate that, whilst the current POC-CCA does represent a potential diagnostic tool for animal schistosomiasis, future work is needed to develop a

50    livestock-specific affordable and field-applicable diagnostic tests to enable determination of

51    the true extent of livestock schistosomiasis.

## Keywords

53    Schistosomiasis, One Health, diagnostics, POC-CCA, Bayesian analysis, sensitivity,

54    specificity, NTDs, Livestock, , *Schistosoma bovis, S. curassoni,* Ruminants, Senegal, Africa.

## Author summary

56    Schistosomiasis is a debilitating neglected tropical and zoonotic disease, infecting

57    over 230 million people and multiple millions of animals worldwide, most notably amongst

58    the poorest regions and populations. The potential contribution of livestock schistosomiasis

59    to disease transmission in human populations has implications for the design of effective

60    disease management and elimination programmes. However, our understanding of the true

61    prevalence, transmission and impact of animal schistosomiasis is severely limited, in part due

62    to a lack of inexpensive, accessible, sensitive and specific diagnostic tools. As a point-of-care

63    circulating cathodic antigen (POC-CCA) diagnostic test is now in widespread use to assess

64    intestinal schistosomiasis caused by *Schistosoma mansoni* in humans, we hypothesised that

65    the same test could be used to detect livestock intestinal schistosomiasis caused by

66    *Schistosoma bovis* and *Schistosoma curassoni*. The aim of this study was thus to evaluate the

67    sensitivity and specificity of the POC-CCA for the detection of intestinal livestock

68    schistosomiasis in Senegal. POC-CCA sensitivity varied by ruminant group and by

69    location/parasite species, while POC-CCA specificity in small ruminants, at least, did not

70    vary across sites. We conclude that the currently-available POC-CCA does represent a

71   potential diagnostic tool for animal schistosomiasis, but that the factors determining test

72   performance warrant further investigation.

# Introduction

74   The development and application of sensitive and specific diagnostic techniques for

75   detection of infectious diseases is vital for the monitoring and evaluation of all treatment

76   programs in endemic areas, especially whenever considering elimination and/or drug-

77   resistance pharmacovigilance. Within this, point-of-care diagnostic testing is particularly

78   needed wherever there is a necessity for a fast diagnostic outcome that is independent from

79   sophisticated, time-consuming, labour-intense and/or expensive laboratory procedures [1].

80   This need may be most exemplified for the neglected tropical and zoonotic diseases

81   (NTDs/NZDs), as clearly stressed within the new World Health Organization's (WHO) NTD

82   Roadmap for 2021-2030 [2] and revised WHO Guideline for the control and elimination of

83   human schistosomiasis, most notably that of recommendation 6 [3, 4].

84   Schistosomiasis is one of the major debilitating NTDs/NZDs, caused by snail-borne

85   dioecious *Schistosoma* blood-flukes. Approximately 90% of the 230 million people infected

86   worldwide live in sub-Saharan Africa [2]. Animal schistosomiasis is also of major veterinary

87   and socio-economic importance, causing significant mortality and morbidity to livestock, as

88   well as reduced productivity for their owners, although the contribution and consequences of

89   this within sub-Saharan Africa are only just beginning to be realised [2, 5-8]. The main

90   *Schistosoma* species found amongst livestock in Africa are the intestinal *Schistosoma bovis*

91   and *Schistosoma curassoni* in West Africa and *Schistosoma mattheei* in East Africa. These

92   species are phylogenetically close to the human urogenital parasite *Schistosoma*

93   *haematobium* and have been found to regularly form viable hybrids within humans, both

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94    across Africa [8-13], and even within its recent expanse to Europe [14, 15]. Outside of Asia,

95    the contribution of zoonotic schistosomes to human schistosomiasis cases have been largely

96    ignored, despite prevalence levels in humans often remaining unacceptably high following

97    high coverage mass drug administration (MDA) programmes across much of West Africa in

98    particular [8, 16, 17]. Furthermore, recent work combining epidemiological, molecular and

99    mathematical modelling work has demonstrated that the relative role of zoonotic transmission

100   from livestock in Africa is likely to increase as disease control efforts move towards

101   elimination [18].

102       The newly launched WHO NTD 2021-2030 Roadmap and Guideline for the control

103   and elimination of human schistosomiasis therefore poses the question of anti-

104   schistosomiasis treatment of livestock across Africa in order to achieve the new targets of

105   Elimination as a Public Health Problem (EPHP) in all 78 currently-endemic counties and

106   Interruption of Transmission (IoT) in selected African regions by 2030 [2, 3]. Widespread,

107   indiscriminate use of anthelmintics in livestock have increased drug-resistance [19, 20]. This

108   is particularly pertinent for schistosomiasis as there is currently only one efficacious drug,

109   praziquantel (PZQ), available for both humans and livestock. Recent work has highlighted

110   examples of use and misuse of PZQ in livestock, including, but not exclusive to, PZQ tablets

111   donated free via the WHO MDA programmes for school-aged children being used instead for

112   infected livestock, with little knowledge of application or dosage requirements [5, 7].

113   Furthermore, recent surveys and socio-economic analyses have revealed highly important, if

114   also often overlooked, animal welfare, productivity, and financial impacts of animal

115   schistosomiasis, which further jeopardize livelihoods, food security and nutrition amongst

116   neglected communities [5]. Indeed the financial costs incurred to subsistence farmers of

117   infected animals was projected to be significantly greater than would be those of a theoretical

118   test and treat programme [5].

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119    To promote sustainable livestock schistosomiasis prevention and treatment in sub-

120    Saharan Africa (SSA), there is therefore a need to both easily and inexpensively diagnose

121    animal schistosomiasis, and subsequently effectively treat only infected individuals and/or

122    herds, such as through a targeted test-and-treat (TnT) or *T3: Test, Treat, Track* design for

123    livestock schistosomiasis in SSA, which anchors the key recent WHO policy

124    recommendations on diagnostic testing, treatment and surveillance in general.

125    There are, however, currently limited diagnostic techniques that can detect

126    schistosomiasis in livestock with sufficient levels of sensitivity and specificity, as well as

127    logistical ease, that can fully inform disease management decisions [21]. Of those available, a

128    recent systematic review (although data analysed were exclusive to *Schistosoma mansoni* and

129    *Schistosoma japonicum* infections) has recommended the formalin-ethyl acetate

130    sedimentation-digestion with quantitative polymerase chain reaction, as the most promising

131    field-applicable techniques in non-human animal hosts [22], although both are time

132    consuming and/or expensive. A recent extensive field survey of both abattoir and live-

133    sampled livestock within Senegal found some utility with both the Kato-Katz technique and

134    miracidial hatching technique (MHT), but the latter was again labour-intensive and showed

135    significant differences in sensitivities by host and/or parasite species [8].

136    Tests for antigens, rather than antibodies, are preferred in endemic areas due to the lag

137    in clearance of parasite-specific antibodies after infection subsides [23] and therefore

138    immunochromatographic circulating cathodic antigen (CCA) and circulating anodic antigen

139    (CAA) tests for adult worm antigens in urine (or serum) were developed specifically for

140    current *Schistosoma* infections amongst humans [24]. Whilst a high sensitivity laboratory-

141    based lateral flow (LF) test platform utilizing luminescent up-converting reporter particles

142    (UCP) comprises assay formats for quantitative detection of circulating cathodic and anodic

143    antigen detection in urine (respectively, UCCA and UCAA assays) is available [25, 26], rapid

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144    point-of-care testing with visual detection is currently only available for the CCA (POC-

145    CCA, Rapid Medical Diagnostics, Pretoria, South Africa) [27, 28]. Given its utility and field-

146    friendly application, this semi-quantitative (negative, trace, "single positive" +, "double

147    positive" ++ or "triple positive" +++) POC-CCA is now recommended by the WHO for

148    mapping human *S. mansoni* prevalence in endemic areas [29, 30]. The application for

149    detection of human urogenital *S. haematobium* infections with POC-CCA is less efficient i.e.

150    to find the lower worm burden infections [31-34].

151        The POC-CCA has been used to assess *S. mansoni* infection in one non-human-

152    primate study [35]). Given that *S. bovis* and *S. curassoni* (as well as *S. mattheei*) are intestinal

153    schistosomes of livestock, we predicted that the current POC-CCA could also provide a

154    useful rapid and inexpensive diagnostic for animal intestinal schistosomiasis, as it does for

155    human intestinal schistosomiasis. The aim of this study was therefore to evaluate the

156    sensitivity and specificity of the currently available, human-focused, POC-CCA for the

157    detection of intestinal livestock schistosomiasis caused by *S. bovis*, *S. curassoni* and their

158    hybrids in Senegal, by host species, in relation to traditional and novel alternative

159    parasitological and immunological diagnostic methods currently available, employing

160    Bayesian latent class models.

## Methods

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### Study design and sites

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163        Cross-sectional livestock parasitological surveys were conducted from May to August

164    2016 and from October 2017 to January 2018 in two areas in Northern Senegal, West Africa;

165    the town of Richard Toll, in the Senegal River Basin, and villages around the Lac de Guiers

166    (area hereafter referred to as Richard Toll), and the town of Linguere and villages around

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167    Barkedji along the Vallée du Ferlo (area hereafter referred to as Barkedji). Following the

168    construction of the Diama Dam in 1986, situated on the Senegal River over 100 km

169    downstream from Richard Toll, the environment surrounding this location has undergone

170    important permanent alterations. Desalination, creation of irrigation canals and creation of

171    permanent freshwater bodies have facilitated the expansion of *Schistosoma* snail intermediate

172    host and human-livestock water contact points throughout the year, supporting the co-

173    occurrence and interspecific interactions between *S. haematobium*, *S. mansoni*, and other

174    *Schistosoma* spp. of veterinary importance [36-38]. The main livestock schistosome species

175    circulating in Richard Toll is *S. bovis* and cattle is the most affected host species. The

176    Barkedji area presents temporary water sources that disappear during the dry season, leading

177    to important seasonal migration of livestock-keeping communities, and annual interruption in

178    schistosomiasis transmission. Small ruminants are the most important livestock host species,

179    infected by *S. curassoni*. However, both schistosome species and hybrids are present in the

180    two areas and all livestock species can be found infected to a lesser extent. Full details on the

181    infection prevalence amongst definitive and intermediate hosts, together with the habitat of

182    two areas, can be found in [8].

183        Animal sampling was part of a larger survey conducted in these two areas [8]. All

184    animals (cattle, sheep, and goats) routinely slaughtered as part of the normal work of the

185    abattoirs and available for inspection at the time of the surveys were examined post-mortem.

186    Living animals were randomly selected in each village with the initial randomisation carried

187    out at the unit level (owner in this case) and a maximum of five animals of each species

188    (cattle, sheep and goats) then randomly sampled from each selected owner (fewer if the

189    owner had fewer than five animals). Randomisation was carried out using random number

190    generators.

## Diagnostic methods

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192    Whilst adult worms can only be extracted from dead animal (e.g. abattoir or culled

193    animals), infectious status of live animals can be assessed indirectly through parasitological,

194    molecular or immunological methods. Direct and indirect assessment were carried out post-

195    mortem at slaughterhouses and indirect evaluations were applied ante-mortem within the

196    community livestock (see S1). The mesenteric vessels of slaughtered animals were visually

197    inspected for *Schistosoma* adult worms (single males, single females, and paired worms) and

198    those found were stored in RNA-later for molecular analysis (see S2). Faecal, urine, lung,

199    and liver samples were collected post-mortem and were examined for infection using the

200    miracidial hatching technique (MHT) (see S1). Free-swimming miracidia were individually

201    pipetted onto Whatman FTA cards (GE Healthcare Life Sciences, UK) for deoxyribonucleic

202    acid (DNA) storage and subsequent genotyping (see S2). Only faecal and urine samples were

203    obtained from live animals. Faecal samples were assessed for the presence of *Schistosoma*

204    eggs via two Kato-Katz (KK) slides and MHT (see S1 and S2). Animals for which a

205    sufficient volume of urine ($\geq$15 mL) was collected were tested on-site for schistosomiasis

206    with a single point of care-circulating cathodic antigen (POC-CCA) cassette (Rapid Medical

207    Diagnostics, Pretoria, South Africa) and for haematuria with a single Hemastix strip

208    (Siemens Healthcare Diagnostics, Surrey, UK) (see S1). The remaining urine was frozen and

209    transported to Leiden Medical University in the Netherlands for application with the up-

210    converting phosphor-lateral flow (UCP-LF) based assays formats (UCCA and UCAA) for the

211    detection and quantitation of circulating cathodic and anodic antigen in urine (see S1) [39].

212    Definitions of positive results are described in Table S1.

213    # Statistical analyses

214    ## Association between haematuria and POC-CCA results

215    When extracting urine from abattoir animals, some samples became contaminated

216    with blood. As false-positive POC-CCA tests in humans have been associated with

217    haematuria [40], it was of interest to assess whether POC-CCA results were affected by the

218    blood in urine. Hemastix results were dichotomised as negative (score 0) or positive (scores 1

219    and 2) and by means of logistic regression we tested the hypothesis that POC-CCA results

220    were independent of blood-contamination status.

221    ## Composite reference standard and Bayesian latent class model

222    specification

223    Due to the absence of a gold standard for the diagnosis of schistosomiasis against

224    which we could evaluate the performance of POC-CCA, we derived a composite reference

225    standard (CRS) that was based on three additional diagnostic methods, namely MHT, KK and

226    UCAA. CRS is based on a combination of tests with moderate sensitivity and high specificity

227    [41]. CRS results were considered positive if animals tested positive for either UCAA, MHT

228    or KK. CRS results were assumed to be negative if animals tested negative for all three tests.

229    We then developed a Bayesian latent class model (BLCM) to assess the sensitivity

230    and specificity of POC-CCA for the detection of active schistosomiasis in cattle and small

231    ruminants [42-44] that took account of the imperfect reference diagnostic tests employed.

232    Two latent (i.e. non-observed) classes were assumed, corresponding to either an infected or

233    non-infected status, and these classes were related to the outcomes of POC-CCA, CRS and

234    UCCA by means of a multinomial distribution [42, 45, 46]. We developed a two-test model

235    and a three-test model. The diagnostic tests included in the two-test model were POC-CCA

236    and CRS. The three-test model made use of the UCCA results and comprised POC-CCA,

237    CRS and UCCA outcomes, albeit at the expense of lower sample sizes in each combination

238    of diagnostic tests.

239    **Accuracy model assumptions**

240    Latent class models (LCM), as proposed by Hui and Walter [43], involve three

241    assumptions. Firstly, more than one population needs to be assessed, each with distinct

242    disease prevalence. Secondly, diagnostic test accuracy must remain constant across

243    populations. Thirdly, the accuracy of the tests must be conditionally independent, so that the

244    sensitivity or specificity of one test is independent of the results of a second test [42, 45, 46].

245    In the present study we were planning to model abattoir and live animals as two distinct

246    populations. However, as schistosomiasis observed prevalence in these two populations were

247    similar (see Table S4), it was likely that the assumption of different prevalences amongst

248    populations was not satisfied. Hence, abattoir and live data were combined and a one

249    population approach was adopted for each site and host species group [44]. However, and in

250    order to assess whether test accuracy differed in these two populations, independent abattoir

251    and live estimates were subsequently derived and compared. To help overcome potential

252    identifiability problems that a one population approach could entail and taking into account

253    the assumed high specificity and medium sensitivity of CRS, we employed moderately

254    informative priors for the CRS (see Table S3). Equally, prevalence priors were moderately

255    informative and their values were based on results from a study carried out in the same area

256    [8]. The impact that these moderately informative priors had on the accuracy estimates

257    (sensitivity/specificity) was assessed through a sensitivity analysis (see S6).

258    Conditional independence of POC-CCA and CRS in the two-test model was assessed

259    in two ways. Firstly, the Deviance Information Criterion (DIC) [47] of model variants that

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260     included (dependence) and excluded (independence) covariance terms were estimated and

261     compared. Models with lower DIC were preferred over models with higher DIC. Secondly,

262     95% CrI of covariance parameters were assessed [46], with CrIs intersecting zero indicative

263     of conditional independence. Three-test models included dependence terms for POC-CCA

264     and UCCA only as the two tests measure the same antigen (CCA) and their outcomes may

265     not be independent of each other. Furthermore, the covariances between POC-CCA and CRS

266     had been found not to be relevant (see results section "POC-CCA accuracy").

### Selection of priors and priors' sensitivity analyses

268     The beta distributions used to model the multinomial distribution parameters (Table

269     S3) were parameterized by specifying the mode and the minimum/maximum accuracy value

270     that was believed to be true for each variable, with 95% certainty. Priors for UCAA and

271     UCCA sensitivity and specificity were established based on expert knowledge and published

272     records of high performance [48-51]. Priors for POC-CCA accuracy were non-informative

273     beta (1,1) distributions. Prevalence priors were based on post-mortem examination of abattoir

274     specimens from a previous study [8], where 81% and 82% of cattle were found to be infected

275     in Barkedji and Richard Toll, respectively, and 26% and 16% of small ruminants were found

276     to be infected in Barkedji and Richard Toll, respectively. As the proportion of infected

277     animals were similar at the two sites, only one prior distribution per ruminant group was

278     defined (Table S3). The sensitivity of the results to the prior parameterisations was

279     undertaken by re-estimating POC-CCA accuracy assuming more diffuse priors for prevalence

280     and CRS accuracy (see S6).

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281      **Accuracy comparisons between sites**

282      In order to assess whether test accuracy differed between sites, differences in the

283      posterior distributions of accuracy between sites were calculated and the probability that the

284      accuracy in one location was greater than in the other was calculated (the Bayesian p-value)

285      by means of the JAGS "step" function [52, 53]. When accuracy did not differ between sites,

286      combined accuracy values were calculated.

287      **Software**

288      Statistical analyses were carried out in R (BC7) version 4.0.5 (see S3)

289      # **Ethics approval and consent to participate**

290      For all primary data collection activities, the researchers first explained what the study

291      was about, how the data collection would work and the rights of the participants. Following

292      that, each participant was asked to give their written consent. Ethical approval was sought

293      and granted by i) the Clinical Research and Ethical Review Board at the Royal Veterinary

294      College; approval number URN 2019 1899-3, and (ii) the Comité National d'Ethique pour la

295      Recherche en Santé (Dakar, Senegal) approval number SEN15/68 and SEN 19/68. Urine

296      samples were imported from Senegal to the RVC in the UK with the import licence

297      ITIMP19.0161 and from the UK to LUMC in the Netherlands with the import licence

298      VGM_IN17-1416-GvW.

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# Results

## Descriptive statistics

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301    Abattoir surveys were carried out on 89 animals routinely slaughtered, whilst live

302    animal samples were obtained from 106 cattle, sheep and goats from communities within the

303    study area. Given the low number of goats and sheep that were infected in the present study,

304    their data were grouped and analysed as "small ruminants" (see Table 1). POC-CCA

305    diagnostic results were obtained for all 195 animals, 56 of which were cattle and 139 were

306    small ruminants. The distributions of samples across sites and sources of animals (live or

307    abattoir) are shown in Table 1. Barkedji livestock was primarily infected by *S. curassoni*,

308    whilst animals from Richard Toll shed mainly *S. bovis* eggs (see Table S5).

309    **Table 1. Number of live and abattoir livestock animals surveyed in Barkedji and**

310    **Richard Toll.**

| Site | | Total | Bovine | Small ruminants (goat + sheep) |
|---|---|---|---|---|
| Barkedji | Abattoir | 35 | 13 | 22 (19+3) |
| | Live | 76 | 8 | 68 (11+57) |
| | Total | 111 | 21 | 90 (30+60) |
| Richard Toll | Abattoir | 54 | 28 | 26 (25+1) |
| | Live | 30 | 7 | 23 (2+21) |
| | Total | 84 | 35 | 49 (27 +22) |
| Total (both sites) | Abattoir | 89 | 41 | 48 (44 +4) |
| | Live | 106 | 15 | 91 (13 + 78) |

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| | | | |
|---|---|---|---|
| Total | 195 | 56 | 139 (57 + 82) |

## Association between haematuria and POC-CCA results

312    Logistic regression results indicated that abattoir POC-CCA results did not depend on

313    Hemastix outcomes, either in cattle (coefficient z value: -0.36, p-value: 0.719) nor in small

314    ruminants (coefficient z value: 0.773, p-value: 0.444). Consequently, in all subsequent

315    analyses, no distinction was made between blood-positive or negative samples.

## Cross-tabulated results

317    The cross-tabulated results for CRS and POC-CCA are shown in Table 2. The number

318    of small ruminants' discordant pairs (POC-CCA -, CRS +) was relatively large, suggesting

319    that POC-CCA sensitivity in small ruminants might be low. Cattle were CRS negative in 8

320    out of 56 animals, showing that most animals were infected, which limited our ability to

321    estimate POC-CCA specificity in cattle. The number of (+, +) concordant pairs was greater in

322    cattle than in small ruminants and the number of (-,-) concordant pairs were greater in small

323    ruminants than in cattle, suggesting that the test behaved differently in each ruminant group.

324    **Table 2. Cross-tabulated results for POC-CCA and CRS, by site and ruminant group.**

| | | Number of concordant and discordant pairs (POC-CCA, CRS)* | | | |
|---|---|---|---|---|---|
| Site | Ruminant | (+, +) | (+, -) | (-, +) | (-, -) |
| Barkedji | Cattle | 13 | 1 | 3 | 1 |
| | Small ruminants | 19 | 6 | 29 | 35 |
| Richard Toll | Cattle | 17 | 2 | 11 | 4 |
| | Small ruminants | 3 | 2 | 26 | 18 |

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325    * POC-CCA = point-of-care cathodic circulation antigen, CRS = composite reference

326    standard

## POC-CCA accuracy

### Conditional dependence between tests and selection of the best fitting models

330    BLCM results from the two-test dependence and two-test independence models, by

331    site and ruminant group (Table 3), indicate that POC-CCA and CRS are conditionally

332    independent as: 1) the 95% CrIs of the covariance terms in the two-test model intersected

333    zero; and 2) independence models had lower DIC values than the dependence ones. Results

334    from the three-test model indicate that POC-CCA and UCCA sensitivities were conditionally

335    dependent (lower 95% CrI limit was greater than zero) in all cases with the exception of the

336    small ruminants from Richard Toll (where the CrI contained zero) (Tables 3 and 4). By

337    contrast, POC-CCA and UCCA specificities were conditionally independent, their 95% CrI

338    intersecting zero (Tables 3 and 4). Given the DIC of the two-test independence models were

339    the lowest, we adopted these as the best fitting models and the following conclusions on

340    POC-CCA accuracy were derived from them.

### POC-CCA accuracy in cattle

342    POC-CCA sensitivity in Barkedji was 81% (95% CrI: 55% to 98%) and in Richard

343    Toll it was 62% (95% CrI: 41% to 84%). As the probability that the sensitivity in Barkedji

344    was greater than in Richard Toll was high (0.88, see Pr in Table 3), a combined sensitivity

345    value for both sites was not calculated.

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346    This study was not able to estimate POC-CCA specificity in cattle with precision due

347    to the low number of animals that were not infected. This is shown by the wide credible

348    intervals obtained in the analyses (see Table 3).

349    **Table 3. Cattle Bayesian latent class models results from two-test and three-test models**

350    **showing parameter means (95% CrI) for POC-CCA sensitivity (Se %) and specificity**

351    **(Sp %) and for covariance (cov), model deviance information criterion (DIC) and**

352    **Bayesian p-values (Pr) comparing accuracy results between sites. The best fitting model**

353    **was the two-test independence model. "B & R" rows shows the pooled results for**

354    **Barkedji (B) and Richard Toll (R).**

| Site | Variable | Two-Test Independence | Two-Test Dependence | Three-Test |
|---|---|---|---|---|
| B | Sensitivity | 81 (55, 98) | 77 (52, 97) | 72 (48, 94) |
| | Specificity | 55 (5, 98) | 51 (3, 97) | 61 (13, 96) |
| | Se cov | n.a. | 0.04 (-0.04, 0.14) | 0.13 (0.01, 0.22) |
| | Sp cov | n.a. | 0 (-0.12, 0.12) | -0.03 (-0.3, 0.14) |
| | DIC | 47.96 | 49.38 | 66.98 |
| R | Sensitivity | 62 (41, 84) | 58 (38, 82) | 60 (41, 80) |
| | Specificity | 70 (16, 98) | 63 (10, 98) | 82 (40, 99) |
| | Se cov | n.a. | 0.03 (-0.06, 0.11) | 0.19 (0.1, 0.23) |
| | Sp cov | n.a. | 0.01 (-0.1, 0.14) | 0.04 (-0.09, 0.19) |
| | DIC | 54.27 | 54.87 | 76.05 |
| B & R | Sensitivity | -- | 66 (49, 86) | 64 (48, 80) |
| | Specificity | -- | 61 (8, 98) | 71 (31, 97) |
| | Se cov | n.a. | 0.03 (-0.04, 0.1) | 0.19 (0.12, 0.23) |

| Site | Variable | Two-Test Independence | Two-Test Dependence | Three-Test |
|---|---|---|---|---|
| | Sp cov | n.a. | 0.01 (-0.11, 0.14) | 0.05 (-0.06, 0.17) |
| | DIC | 54.40 | 55.23 | 77.89 |
| | Pr(Se B > Se R) | 0.88 | 0.86 | 0.78 |
| | Pr(Sp B > Sp R) | 0.36 | 0.38 | 0.26 |

355  n.a. = not applicable; Pr = probability

## POC-CCA accuracy in small ruminants

357  POC-CCA sensitivity in Barkedji was 49% (95% CrI: 29% to 87%) and in Richard

358  Toll it was 12% (95% CrI: 1% to 37%) (see Table 4). The probability that the sensitivity in

359  Barkedji was greater than in Richard Toll was 0.99 (see Pr in Table 4). As this difference was

360  large, overall sensitivity across sites was not estimated.

361  POC-CCA specificity in Barkedji was 91% (95% CrI: 73% to 99%) and in Richard

362  Toll it was 88% (95% CrI: 65% to 99%). The probability that the specificity in Barkedji was

363  greater than in Richard Toll was 0.60. The overall specificity was 91% (95% CrI: 77% to

364  99%).

365  **Table 4. Small ruminants Bayesian latent class models results from two-test and three-**

366  **test models showing parameter means (95% CrI) for POC-CCA sensitivity (Se %) and**

367  **specificity (Sp %) and for covariance (cov), model deviance information criterion (DIC)**

368  **and Bayesian p-values (Pr) comparing accuracy results between sites. The best fitting**

369  **model was the two-test independence model. "B & R" rows shows the pooled results for**

370  **Barkedji (B) and Richard Toll (R).**

| Site | Variable | Two-Test Independence | Two-Test Dependence | Three-Tests |
|---|---|---|---|---|
| | Sensitivity | 49 (29, 87) | 42 (7, 85) | 41 (25, 66) |
| | Specificity | 91 (73, 99) | 85 (58, 99) | 91 (75, 99) |
| B | Se cov | n.a. | 0 (-0.1, 0.1) | 0.09 (0, 0.16) |
| | Sp cov | n.a. | 0.03 (-0.03, 0.17) | 0.02 (-0.03, 0.12) |
| | DIC | 55.92 | 55.94 | 107.55 |
| | Sensitivity | 12 (1, 37) | 13 (1, 40) | 26 (8, 68) |
| | Specificity | 88 (65, 99) | 89 (65, 99) | 93 (74, 100) |
| R | Se cov | n.a. | -0.02 (-0.13, 0.03) | 0.12 (-0.01, 0.21) |
| | Sp cov | n.a. | 0.02 (-0.03, 0.11) | 0.01 (-0.05, 0.1) |
| | DIC | 46.53 | 55.92 | 92.92 |
| | Sensitivity | n.a. | 30 (3, 69) | 34 (19, 65) |
| | Specificity | 91 (77, 99) | 86 (61, 99) | 92 (80, 99) |
| B | Se cov | n.a. | -0.01 (-0.1, 0.07) | 0.12 (0.04, 0.19) |
| & | Sp cov | n.a. | 0.03 (-0.02, 0.16) | 0.02 (-0.01, 0.09) |
| R | DIC | 54.01 | 54.51 | 106.18 |
| | Pr(Se B > Se R) | 0.99 | 0.91 | 0.80 |
| | Pr(Sp B > Sp R) | 0.60 | 0.41 | 0.45 |

371  n.a. = not applicable; Pr = probability

372     **Prior sensitivity analysis and comparison of accuracy in abattoir and**

373     **live populations**

374     The results of the prior sensitivity analyses indicate that the baseline prior models

375     adopted were robust and that the priors were not exerting an unduly effect on the POC-CCA

376     accuracy estimates (see S6).

377     Mean POC-CCA accuracy in abattoir and live populations were similar (not shown),

378     although their 95% CrI were wider than those of pooled samples.

379     # Discussion

380     In multi-host, multi-parasite systems, all reservoir hosts must be considered in order

381     to achieve disease elimination. Accurate detection of *Schistosoma* infection in animals would

382     provide not only critical information to guide surveillance and inform control [2-4], but also

383     help to improve livestock-keeping communities wellbeing, finances, and animal welfare [5,

384     7]. As WHO therefore calls for consideration of the need to treat livestock within Africa in

385     order to minimize zoonotic transmission to humans, as well as for improved diagnostics in

386     general [2-4], accurate diagnosis of schistosomiasis at both the individual and population

387     levels is required for sustainable control programmes as well as assessing, and mitigating

388     against, changes in drug efficacy. This study thereby evaluated the clinical performance of

389     the commercially-available POC-CCA, a diagnostic test routinely used for the detection of

390     the human intestinal parasite, *S. mansoni*, for intestinal *S. bovis, S. curassoni* and hybridized

391     schistosomiasis infections within ruminant livestock of Senegal, West Africa.

392     We compared the accuracy results obtained in this study with those of the routinely-

393     employed diagnostic techniques, MHT and KK, reported from Senegal by ruminant and

394     parasite species [8], and found that POC-CCA sensitivity was better than that of MHT and

21

395     KK in Barkedji, where *S. curassoni* was the main parasite species, and similar or inferior in

396     Richard Toll, where *S. bovis* was the main parasite. We are not aware of other studies in the

397     literature reporting accuracy results for the detection of CCA in *S. bovis* or *S. curassoni*

398     infections in livestock. However, CCA has been reported as being strongly associated with *S.*

399     *bovis* worm and faecal egg counts in goats [54, 55] and CAA were detected in *S. mattheei*

400     infected cows [56].

401         The test manufacturers have found POC-CCA accuracy to vary by parasite species,

402     indicating that the test was particularly useful for the detection of human intestinal

403     schistosomiasis caused by *S. mansoni*, and less so in the diagnosis of *S. haematobium* [57].

404     Kittur *et al.* suggested that *S. haematobium* and *S. mansoni* worms may produce different

405     amounts of CCA or that *S. haematobium* may metabolise it more efficiently [58]. It is

406     plausible that *S. bovis* and *S. curassoni* similarly have different patterns of CCA excretion

407     and metabolic pathways, and thus respond differently in POC-CCA accuracy. For instance, if

408     the maximum number of adult worms a host can carry falls beneath the test's minimum level

409     of detection, infected hosts would be misdiagnosed as uninfected. These maxima may differ

410     with parasite species, resulting in differential misclassification. To our knowledge, the

411     respective maximum number of worms each host can carry, by parasite species and ruminant

412     group, has not been determined. The test manufacturers have also found that, in the case of *S.*

413     *haematobium*, CCA levels vary by region. Moreover, a study comparing POC-CCA

414     performance across five countries found that *S. mansoni* results varied between countries

415     [29]. The present study could not discern between the effects of location and parasite species,

416     as *S. curassoni* was the predominant parasite in Barkedji whilst *S. bovis* was primarily

417     present in Richard Toll. Hence, differences in test accuracy between the two locations could

418     have arisen due to differences in parasite species/hybrid distributions, as well as local factors

419     determining intensity of infection such as intermediate snail distributions. The disparity

420    between each host species' test performance might be attributable to the factors such as

421    consistency of urine, rate of metabolising CCA, or cross-reaction with other infections.

422          Our study also found that there were no differences in POC-CCA specificity in small

423    ruminants across sites and parasite species, and that POC-CCA specificity was not affected

424    by haematuria. This contrasts with results in humans, where POC-CCA specificity has been

425    found to be affected by the host's age (in pre-school aged children < 5 years), their pregnancy

426    status, and whether or not they have haematuria or a urogenital infection [40, 59].

427          POC-CCA accuracy has been found to vary depending on production batch, raising

428    questions regarding production quality control and calls for the optimisation and

429    standardisation of production [60-62]. This is of particular relevance if POC-CCA is to

430    become reliable a tool with which to determine whether to treat human and livestock

431    populations [2-4].

432

## Limitations

434          Due to the absence of a gold-stand diagnostic test for schistosomiasis, this study

435    derived a composite reference standard (CRS) that was based on KK, MHT and UCAA

436    results. The sensitivities of KK and MHT are relatively low, partly due to the technical

437    difficulties associated with the management of large volumes of faecal material. These

438    methods are highly specific, although misclassification of eggs and miracidia can occur. On

439    the other hand, UCAA assays had been labelled as ultrasensitive for human schistosomiasis

440    detection [51, 63], but have not been optimised for livestock. By combining these tests into a

441    CRS we aimed to obtain a composite measure that was highly specific and moderately

442    sensitive. However, we found eight cases where UCAA was negative and KK or MHT were

443    positive, indicating that the high sensitivity UCAA test may not detect all the infections

23

444     despite its lower limit of detection of 0.6 pg/mL CAA [63]. Given the variable quality of the

445     urine samples, it was unfortunate that paired serum samples were not available to validate

446     urine testing. Following recommendations in the literature for human schistosomiasis and in

447     order to identify the most infections [64], the present study considered POC-CCA trace as a

448     positive result. The relatively low sample sizes for goats, sheep and infected cattle had an

449     impact on the precision (wide 95% CrI) of our estimates and our ability to analyse goat and

450     sheep data separately. Lastly, we were not able to assess the interactions between location,

451     ruminant group and parasite species. It is likely that POC-CCA accuracy in Barkedji and

452     Richard Toll can be assimilated to accuracy for *S. curassoni* and *S. bovis* detection,

453     respectively. However, this need to be further investigated. The observed high probabilities

454     of sensitivity differences point to novel evidence of variation between host and parasite spp.

455     that can inform the design and analyses of future diagnostic evaluations.

456        **Implications for practice**

457        Overall, our results indicate that POC-CCA represents a potential diagnostic tool for

458     schistosomiasis in ruminant livestock populations. However, in order to move towards the

459     interruption of transmission, the elimination of this zoonotic transmitted disease [2, 3] and to

460     safeguard the welfare of livestock and the livelihoods of the communities that depend on

461     them [5], it is of great importance to develop inexpensive livestock-specific POC-CCA tests

462     that enable us to formulate accurate assessments of disease prevalence. Furthermore, the

463     observed variation in test performance across sites and parasite species has implications for

464     the applicability of this diagnostic method, as it may hinder our ability to establish

465     universally valid thresholds for disease prevalence that inform control programmes. Hence,

466     the factors that determine test performance need to be investigated further so that region-

467     specific guidelines could be derived if needed. Lastly, manufacturers quality control must be

24

468    a foremost priority if POC-CCA diagnostic tests are implemented for the assessment of

469    schistosomiasis, both in humans and animals.

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491    # Availability of data and materials

492        All data generated or analysed during this study, together with the coding developed,

493    are included in this published article [and its supplementary information files]. Other datasets

494    used and/or analysed can be made available by the corresponding author on reasonable

495    request.

496    # Consent for publication

497        Not applicable.

498    # Competing interests

499        We declare no competing interests.

500    # Authors' contributions

501        JPW conceptualized the study; EL, ND, AB, MS and JPW performed field work

502    and/or facilitated access to farmers; CJDD, PLAMC and GVD performed CAA validation,

503    BC-U performed statistical analyses with input or guidance from IG, AB, MW and JPW.

504    Original draft preparation was performed by BC-U, EL, IG and JPW, with all authors

505    contributing and approving the final manuscript.

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# Supporting information Captions

750     **S1. Diagnostic tests**

751     **S1 Text. Description of diagnostic techniques**

752     **S2. Molecular analyses for *Schistosoma* species determination**

753     **S2 Text. Description of molecular analyses for the determination of *Schistosoma***

754     **species**

755     **S3. Statistical analysis**

756     **S3 Text. Latent Class Models**

757     **S3 Table. Baseline priors used in multivariate modelling.**

758     **S4. Observed prevalence**

759     **S4 Table. Summary statistics by diagnostic method: number of infected**

760     **animals/number of animals examined (empirical prevalence), per ruminant group R: C**

761     **= cattle, SR = small ruminants), site (column S: B = Barkedji, R = Richard Toll) and**

762     **animal population (column P: A = abattoir, L = live animals).**

763     **S5. Distribution of *Schistosoma* species**

764     **S5 Text. Distribution of *Schistosoma* species**

765     **S5 Table. Number of animals genotyped and number of animals in each**

766     **combination of *Schistosoma* species.**

767      **S6. BLCM sensitivity**

768     **S6.1 Table. Priors adopted in the sensitivity analysis, modifying CRS priors (A)**

769     **and prevalence priors (B).**

770     **S6 Text**

771        **S6.2 Table. BLCM results from 2-tests independence analyses showing**

772    **parameter means (95% CrI) and models' deviance information criterium (DIC), for**

773    **POC-CCA sensitivity (Se, %) and specificity (Sp, %), comparing baseline priors, CRS**

774    **modified priors and prevalence modified priors.**

775        **S7. Models JAGS code**

776        **S7.1 Text. JAGS code for two-test independence/dependence models [20].**

777        **S7.2 Text. JAGS code for three-test model [21].**

778        **S 8. Appendix references**