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1 **Ante mortem diagnosis of paratuberculosis: A review of accuracies of ELISA,**
2 **interferon- γ assay and faecal culture techniques**

3
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5
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12
13 **Abstract**

14 Infections with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) can be latent for years without affecting
15 the animal, but the animal may become infectious or clinical at some point. Diagnosis of paratuberculosis
16 can be a challenge primarily in latent stages of the infection, and different diagnosis interpretations are
17 usually required by the variety of decision makers. The objective of this paper was to provide a critical review
18 of reported accuracies of ELISA tests, interferon- γ assays (IFN- γ) and faecal culture (FC) techniques used
19 for diagnosis of three defined target conditions: MAP infected, MAP infectious and MAP affected animals.

20 For each animal species, target condition and diagnostic test-type, sensitivities (Se) and specificities
21 (Sp) were summarised based on a systematic, critical review of information in literature databases. The
22 diagnostic test information often varied substantially for tests of the same type and make, particularly ELISA,
23 which was the most frequently reported test-type. Comparison of the various tests accuracies was generally
24 not possible, but stratification of test-evaluations by target condition improved the interpretation of the test
25 accuracies. Infectious and affected animals can often be detected, but Se for infected animals is generally
26 low. A main conclusion of the review was that the quality of design, implementation and reporting of
27 evaluations of tests for paratuberculosis is generally poor. Particularly, there is a need for better
28 correspondence between the study population and target population, i.e. the subjects chosen for test
29 evaluation should reflect the distribution of animals in the population where the test is intended to be used.

30

31 *Key words: paratuberculosis; sensitivity; specificity; test evaluation*

32

33 **1. Introduction**

34 Paratuberculosis is a chronic infection, which has been of particular concern in ruminants. The infection is
35 caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The major effects of the infection on the
36 animal level can be reduced milk yield (Benedictus et al., 1987; Kudahl et al., 2004), premature culling and
37 reduced slaughter value (Benedictus et al., 1987), and losses due to continued spread of the infection
38 (Kudahl et al., 2007). Not all infected animals will experience losses, which may be because of culling for
39 other reasons than paratuberculosis or because they can resist the infection developing into the debilitating
40 stages. The occurrence of the latter is still poorly understood (Mortensen et al., 2004).

41 Prevalences of the infection vary world-wide (Kennedy and Benedictus, 2001), but most notably the
42 apparent prevalences vary by the test and test strategies used in the prevalence studies conducted. Control
43 of the infection can be obtained via timely detection and culling of infectious animals and reduction of
44 transmission from these animals. Eradication will usually require the detection and isolation of infected
45 animals, as these potentially can become infectious at some point in time. "Isolation" in this regard means
46 that infected animals and their excretions should not be allowed contact with susceptible animals.
47 Eradication is defined as: "The purposeful reduction of specific disease prevalence to the point of continued
48 absence of transmission within a specified area by means of a time-limited campaign" (Yekutieli, 1980). This
49 is to emphasize that complete eradication would require eradication of a microbial agent globally. Control is
50 described as "any effort directed toward reducing the frequency of existing disease to levels biologically
51 and/or economically justifiable or otherwise of little consequence" (Martin et al., 1987).

52 The objective of this report was to conduct a critical review of reported diagnostic test evaluations of
53 ELISA, FC and interferon- γ tests (IFN- γ) used for ante-mortem diagnosis of conditions caused by infection
54 with MAP. To facilitate the comparison across test studies, three target conditions: affected, infectious and
55 infected with MAP were defined and diagnostic sensitivity (Se, probability of correct test positive
56 classification) and diagnostic specificity (Sp, probability of correct test negative classification) reported with
57 respect to these conditions.

58

59 **2. Stages of infection /Conditions detected**

60 *2.1. Pathogenesis*

61 Diagnosis and thereby control of the infection are hampered by a long incubation period. It is generally
62 assumed that infections with MAP occur in young animals, and that some age-resistance occurs. Cattle are
63 thought to be most susceptible from 0 to 4 months of age (Taylor, 1953), although infections have been
64 established in adults feed high dosages of MAP (Doyle, 1953). Similar conditions can be speculated to occur
65 for other animal species. Clinical disease has been observed to most frequently occur among cattle 2 to 5
66 years of age, although cattle from very young to very old (0 to 13 years of age) have been affected (Doyle
67 and Spears, 1951).

68 MAP is an intracellular pathogen. Subsequent to infection, MAP is initially controlled by a
69 predominating T helper 1 (Th1) response. Th1-cells are, among other features, characterised by their
70 production of interferon- γ and some IgG2. Later in the course of infection, a predominant Th2 response may
71 occur, and control of the infection is thought to be lost (Stabel, 2000). During the Th1 response, Map is shed
72 in small numbers, which may be sufficient to elicit a positive result in faecal culture tests (FC). There is
73 correlation between occurrence of IgG and bacterial shedding of MAP (Nielsen and Toft, 2006a), but the
74 time-wise relations between the two events is not fully described. Experimental infections suggest that
75 bacterial shedding decreases 10 to 14 months after inoculation, to increase again later, with sero-conversion
76 occurring around 10 months post-inoculation (Lepper et al., 1989). Waters et al. (2003) demonstrated both
77 cellular and humoral immune responses approximately 100 to 150 days after initial infections with MAP, and
78 Eda et al. (2006) also demonstrated IgG in calves less than 1 year after inoculation. In naturally infected
79 animals, sero-conversion has been shown to occur in 95-98% of cows shedding MAP (Nielsen and Ersbøll,
80 2006). The age at which sero-conversion occurred was from 2.2 to 11.7 years. It is speculated that great
81 variation of the time to occurrence of bacterial shedding and occurrence of antibody responses are caused
82 by variation in infective doses occurring with natural MAP infections. Studies using fixed dosages and known
83 times of infection have also resulted in great variation in the time to occurrence of FC-positivity and ELISA-
84 positivity (Lepper et al., 1989), and the temporal variation in pathogenetic events may be further enhanced if
85 the size and number of dosages varies. In test-evaluations, it is therefore needed to consider the stage of
86 infection. Generally, age can be an indicator of the stage of infection, in that young animals will rarely be
87 expected to shed detectable amounts of bacteria and have IgG1, whereas older animals are more likely to
88 have bacterial shedding, antibodies and clinical disease. The age-distribution among study objects can
89 therefore be of great significance in the evaluation of a diagnostic test. As an example, the probability of
90 detecting infected cows 2 years of age using an antibody ELISA has been estimated to 0.06, whereas the

91 same probability was 0.50 for cows 5 years of age (Nielsen and Toft, 2006a). The distribution of animals
92 among different stages of the infection can also be of great importance.
93 Different antigens are used in various immunological tests. The antigens are usually derived from MAP or
94 *Mycobacterium avium* subsp. *avium* (MAA). In the paratuberculosis literature, MAP strain 18 is MAA serovar
95 2 (Chiodini, 1993). Different preservations of the antigens are being used based on their biological
96 characteristics (Koets et al., 2001) and availability. Generally, antigens should be immunogenic to be used
97 for diagnostic tests. Cho and Collins (2006) showed that proteins derived from culture filtrates rather than
98 cellular extracts were more likely to be antigenic. The use of antigens from MAA may be as useful as
99 antigens from MAP (Nielsen et al., 2001), but there have been made no extensive comparisons of antigens
100 on field data indicating which antigens are the better. Irrespective of which of the two bacterial species the
101 antigen is derived from, both immunogenicity and cross-reactivity should be considered. So far, the
102 superiority of one specific antigen has not been demonstrated, where both immunogenicity and cross-
103 reactivity have been evaluated. It is likely that this may be specific to specific geographic areas, due to
104 potentially different distribution of bacteria potentially causing cross-reactions, such as MAA, from one area
105 to the other. In herds with high sero-prevalences estimated by two commercial ELISAs (Parachek,
106 CSL/Biocor, Omaha, Nebraska, USA and HerdChek, IDEXX Laboratories Inc., Westbrook, Maine) the
107 prevalence of environmental mycobacteria were higher compared to herds with a low sero-prevalence,
108 suggesting that a high prevalence of environmental mycobacteria may result in many false-positive ELISA
109 results (Roussel et al., 2007). It has also been demonstrated that experimental infections with environmental
110 mycobacteria such as *M. intracellulare*, *M. scrofulaceum* and *M. terrae* can result in significant serological
111 reactions (Osterstock et al., 2007).

112

113 2.2. Target conditions

114 The target condition detected by any diagnostic test is essential in the evaluation of the diagnostic accuracy
115 of the test. Some authors refer to this a “case definition” (e.g. Collins et al., 1991). The choice of target
116 condition for the diagnosis “paratuberculosis” should vary depending on the purpose of testing, i.e. the
117 effects that are of primary interest to decision makers. A schematic presentation of the pathogenesis and the
118 effects is given in Fig. 1. From this, a number of conditions can be identified. The target condition chosen by
119 the evaluators in a given study ideally depends on the decision makers, whom can subsequently make
120 decisions based on the test results. In this report, three target conditions, affected, infectious and infected

121 with MAP, have been defined to classify the test evaluation studies included in the review. These target
122 conditions are considered pivotal.

123

124 2.2.1. Animals affected by MAP.

125 Animals affected by MAP are usually classified based on clinical signs such as diarrhoea (persistent or
126 intermittent), chronic weight loss or reduced milk production. MAP infection should be present, which could
127 be documented via gross pathology, histopathology or cultivation of MAP from tissues or faeces. The animal
128 does not have control of the infection and is affected to a degree, where parameters like milk production and
129 general performance is decreasing due to the infection.

130 This definition can be of use for a decision maker whom wishes to make decisions based on the
131 performance of the cow. Reduction in milk yield is often not recorded and reported systematically in the
132 studies, and this aspect of the infection is therefore rarely included.

133

134 2.2.2. Animals infectious with MAP

135 Infectious animals are defined as those that shed MAP at the time of testing with the test under evaluation
136 and thereby they are a risk for transmission of MAP to susceptible herd-mates. The condition “Infectious”
137 also includes animals which are “Affected”. In principle, the infectious group may also contain non-infected
138 animals, which may be passive shedders of MAP. These animals are hypothesised to ingest MAP from
139 heavy environmental contamination (Sweeney et al., 1992a) without being infected. The “infectious” status of
140 these animals would therefore be considered to be transient.

141 In many study reports, only shedding in faeces is included. The shedding is defined based on one or more
142 tests evaluating the presence of MAP in faeces. Animals which are transmitting the agent in milk and in uteri
143 are thereby not included specifically. Given that animals are infectious via milk and in uteri without shedding
144 bacteria in faeces, they will therefore bias the accuracy estimated in the studies reported.

145

146 2.2.3. Animals infected with MAP.

147 Infected animals carry MAP intracellularly but substantial replication need not take place because the
148 infection can be latent. The condition “Infection” also includes animals which are “Infectious” and “Affected”.
149 The definition of “infection with MAP” is any condition where entrance and persistence of MAP have lasted

150 long enough to give an immune response at any time during their life; i.e. there is no time-specific cut-off for
151 this event to occur. It is assumed that once a cow has an established infection, the infection persists for life.

152

153 2.2.4. Animals not affected, infectious or infected with MAP

154 The diagnostic Se of a test reflects the ability to detect the target condition given it is present and the
155 diagnostic Sp of a test reflects the ability to test negative given the target condition is not present. In many
156 studies, the target conditions mentioned above are mixed. Studies defining one target condition for the
157 evaluation of Se and another for Sp can therefore be subject to peculiar interpretations. The interpretation of
158 “false-positives” can therefore vary.

159 The Sp of the condition “non-affected” has to our knowledge not been systematically assessed in any
160 studies.

161 The Sp of the condition “non-infectious” refers to an animal, which does not shed MAP on the time of testing,
162 but some of these non-infectious animals can be infected. Therefore, false-positive test-results include both
163 infected as well as non-infected (i.e. MAP free) animals. The decision maker will usually have little use of this
164 information, unless documentation exists to show near perfect Sp of the test for non-infected animals. This
165 would mean that all false-positive animals are indeed infected, but not infectious at the time of testing.

166 The Sp of the condition “non-infected” is the situation where an animal is free of the infection. False-positive
167 reactions are due to cross-reactions to other mycobacteria, laboratory errors, vaccination reactions and the
168 like for immunological tests (Houe et al., 2004). False-positive test results could also be a consequence of
169 the “pass-through” phenomenon, where ingested bacteria are shed 1 to 7 days post-ingestion, potentially
170 without being infected (Sweeney et al., 1992a).

171

172 2.2.5. Utility of test-results related to different conditions

173 The utility of the test results related to the three conditions defined above could be as follows:

174 Affected animals: The value of these animals is low because their production is reduced, they loose weight
175 and the value at slaughter is probably reduced. There is a risk they will die from the infection;

176 Infectious: These animals are currently infectious and are a risk to susceptible animals. They should be
177 managed so that transmission to susceptible animals is avoided. These animals constitute both a
178 short-term and a long-term economic burden, as they are likely to have a reduced milk yield or will

179 experience it in the near future. The long term loss will be due to their transmission of MAP to herd-
180 mates;

181 Infection: Infected animals constitute a risk of becoming infectious and thereby transmit the infection to
182 susceptible animals. A population containing infected animals cannot be declared free of infection and
183 proper identification of infected animals is important herd-certification schemes, when trying to
184 establish 'MAP-free' herds/populations or keeping MAP out of certified herds. In economic analyses,
185 these animals may be of interest in long-term planning only, as their effect on the population will often
186 be seen only after a number of years.

187 Theoretically, the conditions may be easily defined. In practice, this may not be the case. Other conditions
188 can be defined, depending of their use, e.g. assuming that the cell-mediated immune reactions are studied, it
189 is necessary to establish a condition "occurrence of cell-mediated immune reactions".

190

191 2.2.6. Effect of age on the condition detected.

192 Considering the chronic nature of the infection, with many disease stages, it could be speculated that older
193 animals are of greater risk of having a given condition than younger animals. However, this does not
194 necessarily imply that it is easier to detect the condition in older animals. Higher diagnostic Se for detection
195 of infection has been demonstrated for older cows relative to younger cows (Nielsen and Toft, 2006a), but
196 age does not seem to influence detection of infectious and affected cattle (Nielsen and Toft, 2006a; 2006b),
197 although this has not been formally tested.

198

199 **3. The ideal test evaluation**

200 Very few if any perfect test evaluations have yet been performed. The lack of a 100% accurate reference test
201 and a variable incubation time seem to be primary obstacles in doing so. The effect of choice of reference
202 test on accuracy estimates was demonstrated by McKenna et al. (2005), where accuracy estimates of ELISA
203 based on use of tissue culture as reference test was different from accuracy estimates based on faecal
204 culture as reference test. "Infection" may be established if a thorough microbiological examination of the
205 animal is performed at slaughter, but it is insufficient to sample tissues only from ileum and the ileocecal
206 lymph nodes, because this sampling procedure will fail in detecting of many infected animals (Whitlock et al.,
207 1996). The authors state that up to 100 sites per animal are required sampled to establish the true infection
208 status of the animal. A study containing an adequate number of animals would therefore become quite

209 expensive, since the prior infection status should not be known when carrying out the study, in order to
210 ascertain a distribution of infection stages that is representative of the distribution in the target population.

211

212 Greiner and Gardner (2000) provide an extensive check list, which should be used as a starting point for any
213 epidemiological validation of diagnostic tests. From their recommendations and the discussion of target
214 conditions in the previous sections, we suggest that any test evaluation must at least include the following
215 components:

- 216 a) Data must be from an observational study and the study population should be representative of the
217 target population in which the test is to be used, i.e. the variations in incubation period, exposure
218 dosages and age-distribution of the target population should be reflected in the study population
- 219 b) The target condition should reflect the intended purpose of the test. The same target condition
220 should be used for evaluation of both the Se and Sp. The interpretation of the test-information should
221 be made in concordance with the target condition, and the interpretation of false-positives needs to
222 be clear. As an example, the target condition "Infected" can be studied by classical test-evaluation
223 methods, where animals are classified into truly infected, not infected based on microbiological
224 examination of multiple tissues per animal. An alternative is to use latent class analyses (e.g.
225 Nielsen et al., 2002), where tests that are biologically unrelated are studied, e.g. FC for detection of
226 MAP and antibody ELISA for detection of humoral immune reactions.
- 227 c) A calculated sample size which reflects the purpose of the test evaluation, i.e. the choice of precision
228 in the estimates should depend on the specific purpose of the test evaluation.

229

230 **4. Frequent mistakes in test evaluations performed**

231 A number of mistakes are usually made in evaluation of diagnostic tests. Some of these can be avoided by
232 thorough planning of the study, whereas others are difficult to avoid while still making the study practically
233 and economically feasible. Frequent mistakes include:

- 234 1) Selection of animals for the evaluation is performed by use of the test being evaluated, or a test
235 measuring the same response. Examples include animals selected from herds classified free of
236 infection based on negative humoral immune responses, where the test evaluated detects the same
237 responses.

- 238 2) Variable case definitions used to classify animals across a study. All study objects should be subject
239 to the same classification procedures. An example is the study by Sockett et al. (1992b), in which not
240 all infected animals were subject to the same set of classification procedures, since only sero-
241 positive and FC-negative animals were subject to the defining histopathological procedures. This
242 would result in an over-estimation of the Se , because animals in early-stages of infection could be
243 missed by the reference test. However, the study could be included for inference on infectious
244 animals, given that FC was used to define the reference status, because all animals had been
245 subject to the FC.
- 246 3) Another frequent, but to some extent unavoidable mistake is the use of a study population from a
247 region historically known to be free of MAP to estimate Sp and sample the non-free target population
248 to estimate Se . These two populations should be geographically comparable, because the
249 environmental flora can be expected to give rise to different cross-reactive responses in serological
250 tests.

251

252 **5. Review of test-evaluations in literature**

253 A review of test-evaluations was carried out by searching the available databases by November 14, 2006.

254 These included: Agricola (1970 to Sept. 2006); Agris (1975 to Sept. 2006); Biological Abstracts /RPM (R)

255 (1989 to 2003); BIOSIS Previews (2004- Nov. 11, 2006); Biological Abstracts (1990 to 2000), CAB Abstracts

256 (1973 to Sept. 2006) and Medline through Pubmed (1970 to Nov. 14, 2006). The search terms were:

257 paratuberculosis, Johnes or Johnes combined with diagnosis, diagnostic accuracy, Se , Sp , validation or

258 diagnostic performance.

259 This search generated 2137 hits including duplicate records. After exclusion of duplicate records and

260 non-peer-reviewed publications, 312 publications remained. These were further reduced to 102 publications

261 by exclusion of studies, where the abstract indicated that a test-evaluation was not an objective of the

262 publication or if the language was not English. In cases of uncertainty on whether a test-evaluation had been

263 performed, the studies were included for further assessment of the full paper. The remaining 102

264 publications were further evaluated for study objective and data quality.

265

266 *5.1. Data extraction*

267 Data were extracted from the publications in a standard form including the following items:

- 268 a) Animal species;
- 269 b) Type of test, i.e. serum antibody ELISA, milk antibody ELISA, FC and IFN- γ ;
- 270 c) Test system, i.e. name of test for commercial tests and in-house for non-commercial tests;
- 271 d) Antigen used in serological tests;
- 272 e) Study design: Observational (case-control; cohort; cross-sectional) or experimental; longitudinal or
273 instantaneous;
- 274 f) Data origin: field data or serum-bank or similar;
- 275 g) Conditions detected: “infectious” if animals were shedding bacteria; “infected” if animals were
276 deemed infected by a reference method stated; “affected” if cows were clinical, had reduced
277 production or were in some other way deemed to be affected by the infection (“shedding” alone was
278 not considered affected); “from free region” if this had been justified (herds that had once been FC
279 negative were not from “a free region”); “non-infectious” were from non-shedding animals);
- 280 h) Sample size, i.e. the number of animals assessed among animals with condition (for Se) and without
281 condition (for Sp);
- 282 i) The number of test-positives among animals with condition and the number of test-negatives among
283 animals without the condition;
- 284 j) Uncertainty measures, e.g. 95% confidence interval (95% CI) for classical methods or 95% credibility
285 posterior intervals for Bayesian methods;
- 286 k) Cut-off used for discriminating between positive and negative animals in tests that are not
287 dichotomous;
- 288 l) Age distribution of the study population(s);
- 289 m) Arguments for inclusion and exclusion of the data from the study;
- 290 n) Authors and year of publication;
- 291 o) Year of study;
- 292 p) Geographical origin of data.

293

294 5.2. Assessment of study quality

295 Many studies included some sort of bias, and it was not possible to avoid these biases generally. Therefore,
296 it was decided to include studies if the following criteria were fulfilled:

- 297 a) One of the objectives of the study should be related to test evaluation;

- 298 b) A unique target condition should be defined for animals with the condition and animals without the
299 condition. The target condition could differ between the estimates for Se and the estimates for Sp. If
300 a condition could not be determined or varied across study objects, the study was excluded;
- 301 c) Animals were classified by the same criteria within the study;
- 302 d) The study population was not selected or defined by use of the test (or a related test) under
303 evaluation, e.g. selection of herds that had previously been shown to be negative by serological
304 methods could not be used for evaluation of a serological test;
- 305 e) Random inclusion of study objects was used, e.g. studies where study objects were included due to
306 being suspect were excluded from the current study. Given that the selection of study objects was
307 not described sufficiently, these studies were also excluded;
- 308 f) Infections were natural, i.e. results from experimental infections were excluded, but results of natural
309 infections were included if these were given separately in a given publication;
- 310 g) The test-result of a given test under evaluation was based on a single test, i.e. repeated test-results
311 could be used for defining a given condition but could not be used for the test evaluated;

312

313 Some test-results were confirmed by re-testing, but the original data were used. The argument for doing so
314 is that by sufficient re-testing, the required result can usually be obtained if enough re-tests are carried out,
315 and the same set of procedures should be applied to all samples.

316

317 *5.3 Data analysis*

318 The recorded data from each of the studies were tabulated in separate tables for different animal species.
319 The target condition detected, age and test-characteristics were included in the tables, and the range of
320 estimates was extracted.

321

322 **6. Test evaluation summaries**

323 The 102 publications evaluated in detail contained a total of 153 test-evaluations. A test-evaluation is here
324 defined as the evaluation of one test, but some publications contained more than one test. In some
325 publications, more than one test or more than one condition was evaluated. In Table 1, the distribution of 153
326 test-evaluations is given by target condition and animal species. A total of 68 studies were excluded, as they
327 did not fulfil the inclusion criteria. The remaining studies included 58 studies from cattle, 15 studies from

328 goats, 11 studies from sheep and 1 study from deer. Four of these studies were conducted using latent class
329 analyses: 1 on cattle, 2 on goats and 1 on sheep. The distribution among tests used was as follows: 64 serum
330 antibody ELISA (SELISA) studies, 6 milk antibody ELISA (MELISA) studies, 4 IFN- γ studies and 8 studies on
331 FC. An overview of these is given in the subsequent sections, with division into animal species, test-type and
332 condition detected. The test-evaluations did not cover all types of tests FC, SELISA, MELISA and IFN- γ for
333 all animal species, and studies on those not mentioned have not been reported or did not fulfil the inclusion
334 criteria set.

335

336 Although data should have been extracted as specified section 5.1, some information was consistently not
337 available from most publications. Therefore, only parameters shown in Tables 2 and 3 are reported further,
338 as these were almost consistently reported. Age of the study groups was also included, although this
339 information was only reported in some cases. Unless otherwise stated, it is assumed that adult, mature
340 animals comprised the target population.

341

342 *6.1. Cattle – Faecal Culture*

343 Usually, the Sp of FC is considered to be almost 100%, if the isolates obtained at culture are confirmed to be
344 MAP by molecular methods such as confirmatory IS900 PCR. However, due to the potential pass-through
345 phenomenon (Sweeney et al., 1992a), it is possible that testing of non-infected animals on contaminated
346 premises can lead to false-positive reactions. A latent-class approach to evaluation of the Sp of FC for
347 detection of non-infected animals would therefore seem appropriate. Nielsen et al. (2002) estimated the Sp
348 to be 98% in a population, where the non-infected animals were subject to contamination from infected herd-
349 mates.

350 The Se from the test-evaluations in the present study are given in Table 2, for the three different target
351 conditions. The Se of FC to detect affected animals have been estimated in one study only, resulting in an
352 estimated of 0.70, which is similar to the Se of 0.74 estimated for detection of infectious animals. The Ses of
353 FC for detection of infected animals were in the range 0.23 to 0.29, except for the study by Billman-Jacobe et
354 al. (1992). However, in that study the infection status was determined at necropsy of cull cattle, many of
355 which were clinically ill or gave strong positive reactions in serological tests. Therefore, their estimate must
356 be considered biased to a degree which suggests it should be excluded from the review or that the true
357 target condition of the study differ from their reported target condition.

358

359 *6.2. Cattle - Serum antibody ELISA*

360 The serum antibody ELISA for cattle is the test most frequently evaluated. The test evaluations include
361 studies on a number of commercial ELISAs and in-house ELISAs, a variety of antigen preparations and
362 different age-groups of animals. In Table 3, the Se and Sp is given for each study. The test used is classified
363 into groups based on the producer and the antigen used. The antigen preparations are in most cases not
364 comparable. Therefore, the names in the groups indicate only the source of the antigen. It is assumed that
365 the antigen in the Parachek[®] (Prionics AG, Schlieren-Zurich, Switzerland) test is from the MAP VRI 316
366 strain, but this has not been confirmed. The producer did not respond to requests of the test specification,
367 but the test should apparently be based on the test described originally by Milner et al. (1990). IDEXX
368 Laboratories, Inc. (Westbrook, Maine, USA) being the provider of the IDEXX HerdChek *Mycobacterium*
369 *paratuberculosis* test, cannot share the specification of their antigen preparation either, and it is unknown
370 whether the both the HerdChek and the Parachek test have remained the same over the years. Other test-
371 names were categorised as "Various" because they often do not have a name. For antigen preparations, the
372 variety of preparations gives reason to the same "Various" group. The test names and antigen preparations
373 are therefore not suitable for further subdivision of the data. In most studies, the age-groups studied are
374 incompletely characterised. For those studies in which they were given, it is clear that the studies are hardly
375 comparable, but it is assumed that the majority of animals in each study are a random collection of parturient
376 animals, except if stated otherwise.

377

378 *6.3. Cattle - Milk antibody ELISA*

379 The constitution of milk antibody ELISAs has been less variable than the serum antibody ELISA. Only two
380 different antigens have been used: a lipo-arabinomannan preparation (LAM) and a commercially available
381 antigen from Allied Monitor (Fayette, Missouri, USA). The Se and Sp still vary, which may be primarily due to
382 the choice of cut-off used in the different studies. A summary is given in Table 4. The test has not been
383 evaluated for affected animals and very few studies have been conducted on infected animals. Therefore,
384 the most studies have been conducted for diagnosis of infectious animals, and the Se varies from 0.29 to
385 0.61, with Sp in the range of 0.83 to 1.00.

386

387 *6.4. Cattle – interferon- γ*

388 The IFN- γ has only been evaluated in two studies in cattle (Paolicchi et al., 2003; Huda et al., 2004), and on
389 a limited data material. The results are summarised in Table 5, and the Se for detection of infectious animals
390 varied from 0.13 to 0.85. Unfortunately, the results may not be representative of the populations in general,
391 but so far the studies are the only information available. In Paolicchi et al. (2003), the data material is based
392 on only one herd, in which clinical paratuberculosis had occurred. In Huda et al. (2004), the negative
393 reference herds were chosen among herds with low prevalence of antibody positive cows. It is uncertain
394 whether both publications should be excluded based on these weaknesses. An advantage of the study by
395 Huda et al. (2004) is the separation into three age-groups (Table 5). Neither of the studies assessed the Se
396 of the test for detection of infected animals.

397

398 6.5. Goats – Faecal Culture

399 Kostoulas et al. (2006) have conducted the only study reporting on the accuracy of FC in goats. The
400 condition studied was infected animals, and Se and Sp were assessed using latent class methods on data
401 derived from animals > 1 year of age. The Se was estimated to 0.08 (95% credibility posterior interval (95%
402 CPI): 0.02; 0.17) and the Sp was estimated to 0.98 (95% CPI: 0.95; 1.0), based on data from 368 goats.

403

404 6.6. Goats - Serum antibody ELISA

405 As in cattle, the most widely assessed test in goats has been the serum ELISA for detection of antibodies.
406 However, contrary to cattle, the most widely condition detected has been affected and infected animals,
407 whereas infectious animals has rarely been the condition detected (Table 6). Nonetheless, it appears that
408 ELISA is more accurate in detection of all conditions than in cattle, with Se ranging from 0.82 to 1.0 for
409 affected animals and 0.63 to 0.84 for infected animals. The corresponding Sp generally range from 0.92 to
410 1.0, although in one study with a very high Se of 0.91 the corresponding Sp was 0.79 only (Dimareli-Malli et
411 al., 2004).

412

413 6.7. Sheep – Faecal Culture

414 FC has only been evaluated for the condition infected in sheep, and in one study only. The Se was estimated
415 to 0.16 (95% CPI: 0.02; 0.48) and the Sp to 0.97 (95% CPI: 0.95-0.99) based on data from 368 animals
416 evaluated in a latent class analysis (Kostoulas et al., 2006).

417

418 *6.8. Sheep - Serum antibody ELISA*

419 Ten serum antibody ELISAs have been evaluated for use in sheep (Table 7). In the evaluation of 5 tests, the
420 Se were evaluated for detection of affected sheep and in the evaluation of 5 tests, infected animals were the
421 target condition. All studies were based on detection of non-infected animals for estimation of Sp. The most
422 widely used test was Parachek, and in 7 of the 10 studies, the antigen used was apparently MAP VRI316.
423 For affected animals, the Se varied from 0.36 to 0.85, and for infected animals, the Se were 0.16 to 0.44. Sp
424 ranged from 0.95 to 0.99 (Table 7).

425

426 *6.9. Deer – Serum antibody ELISA.*

427 In clinically affected deer, two non-commercial serum antibody ELISAs have been evaluated (Griffin et al.,
428 2005). The ELISA based on PPDj antigen from CIDC (Lelystad, The Netherlands) had a Se of 0.83 (95% CI:
429 0.74; 0.89) (among 102 clinically affected deer) and a Sp of 1.00 (exact 95% CI: 0.9906; 0.9998) among
430 animals from herds with no MAP infection history or clinical signs associated with MAP. An ELISA based on
431 PPA3 antigen from Allied Monitor (Fayette, Missouri, USA) had a Se of 0.85 (95% CI: 0.77; 0.91) with a Sp
432 of 0.98 (95% CI: 0.96; 0.99).

433

434 *6.10. Overall summary of sensitivity and specificity*

435 An overall summary of Se and Sp for each animal species, test and target condition is shown in Table 8,
436 based on information in Sections 6.1 to 6.9. Both FC and ELISA generally have medium to high Se for
437 detection of affected and infectious adult cattle, with Sp of 1.0 by definition. The range of Se of ELISA for
438 detection of infectious cattle is huge, which is partly a reflection of the number of test-evaluations included in
439 this group. The Se of FC for detection of infected cattle are in the range 0.23 to 0.29, which may be slightly
440 better than Se of ELISA (range 0.07 to 0.39). However, a given Se of ELISA needs to be evaluated in
441 combination with Sp, and it cannot be determined which of the tests that are the better. The variation in test-
442 accuracy within test-evaluations for IFN- γ is huge, but basically only two studies have been performed, with
443 significant differences in the results. It cannot be determined which of the results that are the more reliable.

444 In deer, only one study including two ELISAs used for clinically affected animals have been reported,
445 with promising results. However, the lack of studies on infected and infectious deer prompts for studies on
446 these conditions.

447 ELISA used for infected, infectious and affected goats indicates that this test can have utility because
448 of generally high Se, irrespective of target condition. The Se of FC for detection of infected goats is,
449 however, not promising with a Se of 0.08 and a Sp of 0.98, in the one study reported. Further studies are
450 needed to draw reasonable conclusions. However, the Se of FC is comparable to that of sheep, indicating
451 that the estimate may be valid for the particular test. The FC method evaluated for both sheep and goats
452 originate from the same study, and other FC methods may prove to be superior. IFN- γ and milk ELISA have
453 not been evaluated in deer, goats and sheep. Results of test-evaluations for serum ELISA for sheep show
454 considerable variation for both affected and infected animals, but the Se for serum ELISAs used for infected
455 animals appear to be lower than serum ELISAs used for infected animals, as would be expected.

456

457 **7. Discussion**

458 This report summarises Se and Sp estimates for detection of animals infected by, infectious with or affected
459 by MAP, obtained through a critical review of literature. The report is the first to divide animals into the three
460 target conditions (infected, infectious and affected), but these can be very useful in the processes where
461 decision makers have to make decisions related to different conditions. The division also appears to provide
462 estimates which are more homogenous for a given test than had the estimates been reported for one group
463 only. As an example, the Sp of FC were 0.96, 0.98 and 0.97, for cattle, goats and sheep respectively. Serum
464 antibody ELISA used to detect infected cattle had Se in the range from 0.07 to 0.22, whereas serum
465 antibody ELISAs for detection of infected goats were in the range 0.63 to 0.84. These ranges are much
466 narrower than could be expected. The narrow range is of course partly a function of the low number of test-
467 evaluations per group, but it still appears to be narrower irrespective hereof.

468 Division of the target conditions into affected, infectious and infected animals to some extent reduced
469 the variation of Se within a group, as would be expected. For a decision maker, estimates of Se and Sp that
470 are specific for a given target condition, should be preferred, because the decision maker can then report the
471 probability of having the given condition based on the test result. If no distinction between target conditions is
472 made, a Se and Sp is for an average of infected, infectious and affected animals will have to be assumed. In
473 Collins et al. (2006), such an average must have been assumed, as we were unable to identify in literature
474 the Se and Sp estimates reported as “assumptions for test Se and Sp”. As an example, they reported a Se of
475 0.60 ± 0.05 for FC in cattle, given the best test is used. This figure is twice the size of the Se reported in
476 literature for detection of infected animals. The Sp of FC was reported to 0.999 ± 0.001 , which would be

477 applicable only for non-infected animals in non-infected herds, not a randomly selected animal. The
478 estimates from literature suggest the Sp to be 0.98 for cattle, which is supported by the estimates of 0.97
479 and 0.98 for sheep and goats, respectively. The target conditions and basis for the accuracies reported by
480 Collins et al. (2006) as “consensus recommendations” were not given. Differences in target condition and the
481 choice of “best test” could be the reason for differences between their figures and the estimates reported in
482 literature.

483 Combining the estimates within groups of animals and conditions into one estimate with associated
484 uncertainty estimates would have been preferred. With the current approach, it is also problematic that there
485 is a uniform weighting of test-evaluations irrespective of sample size and year of publication. The latter is
486 due to potential improvement of tests with time. A formal meta-analysis was not performed, primarily due to
487 the differences in test protocols used, particularly differences in antigen formulations and chosen cut-offs,
488 making it hard to justify comparisons across studies.

489 The numbers given in the present report may be useful not only to decision makers, but also as input
490 parameters in simulation studies. There are still a number of test-target condition combinations, which have
491 not been evaluated, particularly in deer. There is therefore still a need for further well-planned diagnostic
492 test-evaluations. Also, the quality of test-evaluations was inadequate for across-study comparisons also
493 because very few studies report the target and the study population. Improvements in planning, conducting
494 and reporting test-evaluations are generally required, not only for test evaluations related to MAP infection..
495 It is recommended to follow the guidelines given by Greiner and Gardner (2000). The ideal test-evaluation
496 for a set of diagnostic paratuberculosis data have still to be published for several reasons. The complicated
497 nature of the long, slowly developing MAP infections and the lack of good reference tests will most likely
498 introduce selection bias in traditional test-evaluations. Therefore, while it is relatively simple to include
499 covariate effects such as animal or laboratory effects using traditional methods, the resulting accuracy
500 estimates will most likely be biased. As an alternative, multivariable test-evaluations without selection bias
501 can be performed use of some latent class methods such as Bayesian analyses. However, such methods
502 are not always as computationally simple to perform, and the interpretation of target conditions is not always
503 straight forward when these models are used. For the target condition “infection”, latent-class models are
504 probably the best alternative, whereas for conditions “infectious” and “affected”, traditional methods may be
505 better used, because these conditions generally are easier detected. An ‘ideal’ test-evaluation could include
506 a longitudinal study design over the entire lifetime of the animals studied. The animals would have to be

507 tested regularly with an agent detecting test (e.g. FC), a test detecting cell-mediated immune responses (e.g.
508 IFN- γ) and antibody reactions (e.g. serum ELISA), ultimately ending with a post-mortem histopathological
509 evaluation of up to 100 tissues per animal to determine the infection status of the animal. Such a study would
510 be extremely expensive and perhaps not even provide the necessary information. However, longitudinal
511 studies and/or more frequent use of latent class methodology could provide better test-evaluations than is
512 currently seen. Latent class models with inclusion of covariates have been used to demonstrate the
513 improved accuracy obtained when several tests are used, i.e. reduced milk production combined with FC
514 and milk antibody ELISA (Wang et al., 2006). Such an approach would reduce the need for division into
515 different conditions as suggested here. The approach needs to be developed further to include repeated test
516 data over time. At the current stage, division into detection of different conditions will however be beneficial,
517 particularly for decision makers.

518

519 **9. Conclusion**

520 The Se and Sp of diagnostic tests for various stages of MAP infections varied significantly, but formal
521 comparison of the different tests cannot be justified. The main reasons are variations in study design, test
522 components and target conditions. Stratification of target conditions into those relevant for decision makers
523 can decrease the variation of each test in each animal species, thereby improving the interpretation.
524 However, the accuracies of the various test types appear to vary from species to species for different target
525 definitions, therefore interpretation of diagnostic test information should be made by species, target condition
526 and test. There is still a profound lack of reliable test evaluations, and future assessments should be
527 conducted more stringently to allow appropriate interpretation and comparison across populations.

528

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532

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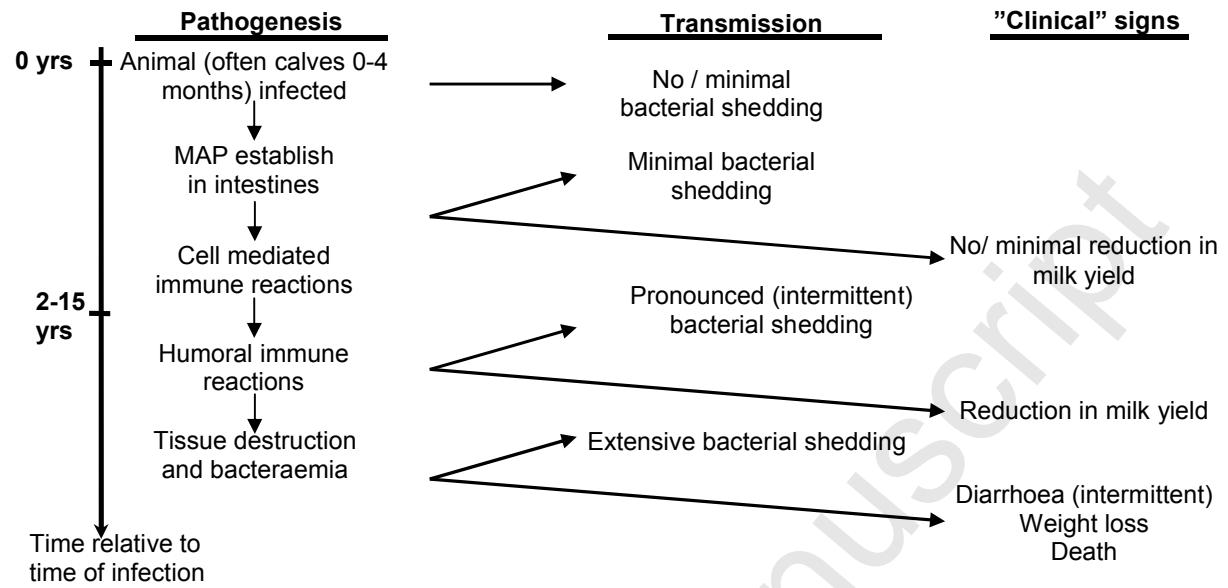


Fig. 1. Schematic presentation of various stages of infection and their effects. This presentation may represent the typical picture, but deviations are likely to occur

Table 1. Overview of 102 publications on evaluation of diagnostic test for paratuberculosis in animals, divided into three target conditions and animal species

Condition	Cattle	Goats	Sheep	Llamas & alpacas	Deer	Water buffaloes	Total
Affected	4	8	5	0	1	0	18
Infectious	36	2	0	0	0	0	38
Infected	18	5	6	0	0	0	29
Excluded	45	9	12	1	0	1	68
Total	103	24	23	1	1	1	153

Table 2. Sensitivity of faecal culture methods used for diagnosis of affected, infectious and infected cattle.

Condition	Test medium [#]	No. with condition	No. test-Positive	Sensitivity (95% C.I.)	Age	Reference
Affected	HEYM	56	39	0.70 (0.56, 0.81)	?	Egan et al., 1999
Infectious	HEYM	111	82	0.74 (0.65, 0.82)	> 2 yrs	Sockett et al., 1992b
Infected	HEYM	177	86	0.49 (0.41, 0.56)	?	Billman-Jacobe et al., 1992
Infected	TREK	160	36	0.23 (0.16, 0.30)	?	McKenna et al., 2005
Infected	HEYM	321	79	0.25 (0.20, 0.30)	All cattle in herd, >0 yr	Whitlock et al., 2000
Infected	HEYM	232	67	0.29 (0.23, 0.35)	All parturient cows	Whitlock et al., 2000

[#]) HEYM = Herrold's Egg Yolk Medium; TREK = TREK ESP culture system, TREK Diagnostics, Cleveland, Ohio, USA

Table 3. Sensitivity and specificity estimates from studies of serum antibody ELISAs for detection of affected (A), infectious (I) and infected (E) cattle

Condition for Test			Antigen	Sample size [#]		Test outcome ^a		Se ^s	Sp ^s	Age	Reference
Se ^s	Sp ^s			C+	C-	T+ C+	T- C-				
A	NA	Various	ATCC 19698	40		20		0.50		≥12 mo.	Bech-Nielsen et al., 1992
A	NA	HerdChek	IDEXX	62		54		0.87		?	Sweeney et al., 1995
A	NA	Parachek	VRI316	56		43		0.77		?	Egan et al., 1999
I	I	Various	ATCC 19698	134	62	64	62	0.48	1.00	≥12 mo.	Bech-Nielsen et al., 1992
I	I	HerdChek	IDEXX	373	2383					Cows	Berghaus et al., 2006
I	I	HerdChek	IDEXX	72	617	53	540	0.74	0.88	?	Hendrick et al., 2005
I	I	HerdChek	IDEXX	44	607	11	569	0.25	0.94	?	Stabel et al., 2002
I	I	HerdChek	IDEXX	174		62		0.36		?	Sweeney et al., 1995
I	I	HerdChek	IDEXX	41	263	13	258	0.32	0.98	?	Sweeney et al., 1995
I	I	Various	LAM	102	65	61	54	0.60	0.83	?	Sweeney et al., 1994
I	I	Various	PPA3	67	513	42	460	0.63	0.90	≥ 2 yr	Klausen et al., 2003
I	I	Various	PPA3	8	16	5	11	0.63	0.69	?	Paolicchi et al., 2003
I	I	Various	PPA3	177	196	71	187	0.40	0.95	?	Sockett et al., 1992a
I	I	Various	Various	60	44	37	18	0.62	0.41	?	Abbas et al., 1983
I	I	Various	Various	60	44	50	39	0.83	0.89	?	Abbas et al., 1983
I	I	Various	Various	36	156	34	129	0.94	0.83	?	Colgrove et al., 1989
I	I	Various	Various	14	210	10	174	0.71	0.83	> 6 mo	Spangler et al., 1992
I	I	Parachek	VRI316	170	1751	40	1719	0.24	0.98	≥ 2 nd lact.	Lombard et al., 2006
I	I	Parachek	VRI316	177	196	61	194	0.34	0.99	?	Sockett et al., 1992a
I	I	Various	VRI316	60	304	22	287	0.37	0.94	?	Eamens et al., 2000
I	E	HerdChek	IDEXX	415	359	127	342	0.31	0.95	?	Collins et al., 2005
I	E	HerdChek	IDEXX	198	346	48	345	0.24	1.00	>2 yr	Kalis et al., 2002
I	E	SVANOVA	LAM	15	100	6	91	0.40	0.91	2 to 15 yr	Glanemann et al., 2004
I	E	Various	PPA3	64	68	51	61	0.80	0.90	?	Nielsen et al., 2001
I	E	IDEXX Scand	Various	198	346	66	322	0.33	0.93	>2 yr	Kalis et al., 2002
I	E	IDEXX Scand	Various	198	346	51	335	0.26	0.97	>2 yr	Kalis et al., 2002
I	E	Pourquier	Various	415	359	116	359	0.28	1.00	?	Collins et al., 2005
I	E	SERELISA	Various	301	359	134	304	0.45	0.85	?	Collins et al., 2005
I	E	Various	Various	156	200	126	200	0.81	1.00	>15 mo.	Yokomizo et al., 1991
I	E	Parachek	VRI316	126	196	71	194	0.56	0.99	?	Collins et al., 1991
I	E	Parachek	VRI316	415	359	118	358	0.28	1.00	?	Collins et al., 2005
I	E	Parachek	VRI316	64	68	51	61	0.80	0.90	?	Nielsen et al., 2001
I	NA	Various	Various	106	341	50	340	0.47	1.00	?	Reichel et al. 1999
I	NA	Parachek	VRI316	106	341	33	334	0.31	0.98	?	Reichel et al. 1999
E	E	HerdChek	IDEXX	160	834	14	814	0.09	0.98	?	McKenna et al, 2005
E	E	Various	LAM	22	378	4	363	0.18	0.96	?	McNab et al., 1991
E	E	SVANOVA	Various	160	834	27	757	0.17	0.91	?	McKenna et al, 2005
E	E	Parachek	VRI316	160	834	11	801	0.07	0.96	?	McKenna et al, 2005
E	NA	Parachek	VRI316	1188		265		0.22		>2 yrs	Jubb et al., 2004
NA	E	Parachek	VRI316		15566		15467	0.99	?	?	Holmes et al., 2004
NA	E	Parachek	VRI316		5588		5579	1.00	?	?	Jubb and Galvin, 2004
NA	E	Parachek	VRI316		1049		1028	0.98		Cows	Pitt et al., 2002

^s) Se= Sensitivity; Sp=Specificity; A=Affected; I=Infectious; E=Infected; NA=Not available.

[#]) Sample sizes for animals with condition (C+) and without condition (C-)

^a) No. of test-positive (T+) or test-negative (T-) given the condition (C)

Table 4. Sensitivity and specificity estimates from studies of milk antibody ELISAs for detection of infectious (I) and infected (E) cattle

Condition for	Test	Antigen	Sample size [#]		Test outcome [‡]		Se [§]	Sp [§]	Age	Reference	
			C+	C-	T+ C+	T- C-					
I	I	Antel [§]	Allied	72	617	44	584	0.61	0.95	?	Hendrick et al., 2005
I	I	Various	Allied	67	513	36	487	0.54	0.95	≥ 2 yr	Klausen et al., 2003
I	I	Antel [§]	Allied	170	1751	36	1724	0.21	0.98	≥ 2 nd lact.	Lombard et al., 2006
I	I	Various	LAM	102	65	61	54	0.60	0.83	?	Sweeney et al., 1994
I	E	Antel [§]	Allied	364	352	105	351	0.29	1.00	?	Collins et al., 2005
E	E	Various	Allied	2662 comb.				0.39	0.96	Cows	Nielsen et al., 2002

[§]) Se= Sensitivity; Sp=Specificity; I=Infectious; E=Infected.

[#]) Sample sizes for animals with condition (C+) and without condition (C-)

[‡]) No. of test-positive (T+) or test-negative (T-) given the condition (C)

[§]) Antel Biosystems Inc., Lansing, Michigan, USA

Table 5. Sensitivity and specificity estimates from studies of interferon- γ tests for detection of infectious (I) and infected (E) cattle

Condition for		Test	Anti- gen	Sample size [#]		Test outcome ^a		Se ^s	Sp ^s	Age	Reference
Se ^s	Sp ^s			C+	C-	T+ C+	T- C-				
I	I	Various	PPDa	8	16	1	14	0.13	0.88	?	Paolicchi et al., 2003
I	E	Bovigam	PPDj	8	53	4	50	0.50	0.94	1-2 yrs	Huda et al., 2004
I	E	Bovigam	PPDj	13	65	11	50	0.85	0.94	2-3 yrs	Huda et al., 2004
I	E	Bovigam	PPDj	28	65	21	14	0.75	0.95	> 3 yrs	Huda et al., 2004

^s) Se= Sensitivity; Sp=Specificity; I=Infectious; E=Infected.

[#]) Sample sizes for animals with condition (C+) and without condition (C-)

^a) No. of test-positive (T+) or test-negative (T-) given the condition (C)

Table 6. Sensitivity and specificity estimates from studies of serum antibody ELISAs for detection of affected (A), infectious (I) and infected (E) goats

Condition for	Test	Antigen	Sample size [#]	Test outcome ^a				Se ^s	Sp ^s	Age	Reference
				C+	C-	T+ C+	T- C-				
A	A	HerdChek	IDEXX	44	62	36	59	0.82	0.95	?	Dimareli-Malli et al., 2004
A	A	Various	Various	44	62	40	49	0.91	0.79	?	Dimareli-Malli et al., 2004
A	A	Various	Various	44	62	38	57	0.86	0.92	?	Dimareli-Malli et al., 2004
A	A	Various	Various	44	62	40	57	0.91	0.92	?	Dimareli-Malli et al., 2004
A	A	Parachek	VRI316	15	11	13	11	0.87	1.00	?	Milner et al., 1989
A	I	Various	Various	16	63	14	59	0.88	0.94	?	Molina et al., 1991
A	I	Various	PPA3	17	63	15	60	0.88	0.95	?	Molina et al., 1991
A	E	Various	PPA3	35	61	35	57	1.00	0.93	?	Molina Cabellero et al., 1993
I	E	HerdChek	IDEXX	35	123	19	123	0.54	1.00	?	Burnside & Rowley, 1994
I	NA	Various	Various	36		33		0.92		?	Tripathi et al., 2006
E	E	Pourquier	Various	36	945	28	945	0.78	1.00	> 1 yr	Gumber et al., 2006
E	E	HerdChek	IDEXX	368 combined				0.63	0.95	> 1 yr	Kostoulas et al., 2006
E	E	Parachek	VRI316	19	1000	16	997	0.84	1.00	?	Whittington et al., 2003
E	E	HerdChek	IDEXX	47	1000	39	995	0.83	1.00	?	Whittington et al., 2003

^s) Se= Sensitivity; Sp=Specificity; A=Affected; I=Infectious; E=Infected; NA=Not available.

[#]) Sample sizes for animals with condition (C+) and without condition (C-)

^a) No. of test-positive (T+) or test-negative (T-) given the condition (C)

Table 7. Sensitivity and specificity estimates from studies of serum antibody ELISAs for detection of affected (A), infectious (I) and infected (E) sheep

Condition for Test		Antigen	Sample size [#]	Test outcome ^a		Se ^s	Sp ^s	Age	Reference		
Se ^s	Sp ^s			C+	C-					T+ C+	T- C-
A	E	Parachek VRI316	32	43	20	42	0.63	0.98	2-5 yr	Clarke et al., 1996	
A	E	Various	Various	12	10	10	10	0.83	1.0	2-4 yr	Gwozdz et al., 1997
A	E	Parachek VRI316	12	10	10	10	0.83	1.0	?	Gwozdz et al., 1997	
A	E	Parachek VRI316	59	253	50	252	0.85	1.0	?	Hilbink et al., 1994	
A	E	Various	Various	59	252	21	247	0.36	0.98	?	Hilbink et al., 1994
E	E	Parachek VRI316	120	1137	53	1125	0.44	0.99	> 1 yr	Hope et al., 2000	
E	E	Various	Various	368 combined				0.37	0.97	> 1 yr	Kostoulas et al., 2006
E	E	Parachek VRI316	2465 combined				0.16	0.98	> 1 yr	Robbe-Austerman et al., 2006	
E	E	Various	VRI316	224	1748	93	1661	0.42	0.95	> 1 yr	Sergeant et al., 2003
E	E	Various	VRI316	224	1748	49	1731	0.22	0.99	> 1 yr	Sergeant et al., 2003

^s) Se= Sensitivity; Sp=Specificity; A=Affected; I=Infectious; E=Infected; NA=Not available.

[#]) Sample sizes for animals with condition (C+) and without condition (C-)

^a) No. of test-positive (T+) or test-negative (T-) given the condition (C)

Table 8. Summary (range) of reported sensitivities and specificities of faecal culture (FC), serum antibody ELISA (SELISA), milk antibody ELISA (MELISA) and interferon- γ tests for diagnosis of three stages of infection with *Mycobacterium avium* subsp. *paratuberculosis* in cattle, deer, goats and sheep.

	Sensitivities (range)				Specificities (range)			
	FC	SELISA	MELISA	IFN- γ	FC	SELISA	MELISA	IFN- γ
Cattle								
Affected	0.70 ^{#1}	0.50; 0.87 ^{#3}	None	None	1.0 [§]	None	None	None
Infectious	0.74 ^{#1}	0.24; 0.94 ^{#30}	0.21; 0.61 ^{#5}	0.13; 0.85 ^{#4}	1.0 [§]	0.40; 1.0 ^{#15}	0.83; 0.99 ^{#2}	0.88 ^{#1}
Infected	0.23; 0.29 ^{3#}	0.07; 0.22 ^{#5}	0.39 ^{#1}	None	0.98 ^{#1}	0.85; 1.0 ^{#19}	0.96; 1.0 ^{#2}	0.94; 0.95 ^{#3}
Deer								
Affected	None	0.83; 0.85 [#]	None	None	1.0 [§]	0.98; 1.0 ^{2#}	None	None
Infectious	None	None	None	None	1.0 [§]	None	None	None
Infected	None	None	None	None	None	None	None	None
Goats								
Affected	None	0.82; 1.0 ^{#8}	None	None	1.0 [§]	0.79; 1.0 ^{#5}	None	None
Infectious	None	0.54; 0.92 ^{#2}	None	None	1.0 [§]	0.94; 0.95 ^{#2}	None	None
Infected	0.08 ^{1#}	0.63; 0.84 ^{#4}	None	None	0.98 ^{#1}	0.93; 1.0 ^{#6}	None	None
Sheep								
Affected	None	0.36; 0.85 ^{#5}	None	None	1.0 [§]	None	None	None
Infectious	None	None	None	None	1.0 [§]	None	None	None
Infected	0.16 ^{#1}	0.16; 0.44 ^{#5}	None	None	0.97 ^{#1}	0.95; 1.0 ^{#10}	None	None

[#]) Number given is the number of test-evaluations included in the summary. One study (Billman-Jacobe et al., 1991) was excluded from group FC, Infected, Cattle because of selection procedure of animals.

[§]) Specificity is 1.0 by definition