



HAL
open science

Effect of paratuberculosis on the diagnosis of bovine tuberculosis in a cattle herd with a mixed infection using interferon-gamma detection assay

Julio Álvarez, Lucía de Juan, Javier Bezos, Beatriz Romero, Jose Luis Sáez, Sergio Marqués, Concepción Domínguez, Olga Mínguez, Baudilio Fernández-Mardomingo, Ana Mateos, et al.

► To cite this version:

Julio Álvarez, Lucía de Juan, Javier Bezos, Beatriz Romero, Jose Luis Sáez, et al.. Effect of paratuberculosis on the diagnosis of bovine tuberculosis in a cattle herd with a mixed infection using interferon-gamma detection assay. *Veterinary Microbiology*, 2009, 135 (3-4), pp.389. 10.1016/j.vetmic.2008.09.060 . hal-00532504

HAL Id: hal-00532504

<https://hal.science/hal-00532504>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Effect of paratuberculosis on the diagnosis of bovine tuberculosis in a cattle herd with a mixed infection using interferon-gamma detection assay

Authors: Julio Álvarez, Lucía de Juan, Javier Bezos, Beatriz Romero, Jose Luis Sáez, Sergio Marqués, Concepción Domínguez, Olga Mínguez, Baudilio Fernández-Mardomingo, Ana Mateos, Lucas Domínguez, Alicia Aranaz



PII: S0378-1135(08)00436-7
DOI: doi:10.1016/j.vetmic.2008.09.060
Reference: VETMIC 4202

To appear in: *VETMIC*

Received date: 22-4-2008
Revised date: 4-9-2008
Accepted date: 15-9-2008

Please cite this article as: Álvarez, J., de Juan, L., Bezos, J., Romero, B., Sáez, J.L., Marqués, S., Domínguez, C., Mínguez, O., Fernández-Mardomingo, B., Mateos, A., Domínguez, L., Aranaz, A., Effect of paratuberculosis on the diagnosis of bovine tuberculosis in a cattle herd with a mixed infection using interferon-gamma detection assay, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2008.09.060

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Effect of paratuberculosis on the diagnosis of bovine tuberculosis in a cattle herd**
2 **with a mixed infection using interferon-gamma detection assay**

3 Julio Álvarez^a, Lucía de Juan^a, Javier Bezos^a, Beatriz Romero^a, Jose Luis Sáez^b, Sergio
4 Marqués^c, Concepción Domínguez^d, Olga Mínguez^c, Baudilio Fernández-Mardomingo^c,
5 Ana Mateos^a, Lucas Domínguez^a, Alicia Aranaz^{a*}

6 ^a Laboratorio Visavet, Departamento de Sanidad Animal, Facultad de Veterinaria,
7 Universidad Complutense de Madrid, 28040 Madrid, Spain.

8 ^b Subdirección General de Sanidad Animal, Dirección General de Ganadería, Ministerio
9 de Agricultura, Pesca y Alimentación, 28071 Madrid, Spain.

10 ^c Dirección General de Producción Agropecuaria, Servicio de Sanidad Animal, Junta de
11 Castilla y León, 47014 Valladolid, Spain.

12 ^d Laboratorio Regional de Sanidad Animal de Castilla y León, 24193 León, Spain.

13 *Corresponding author:

14 Alicia Aranaz, Laboratorio Visavet, Departamento de Sanidad Animal, Facultad de
15 Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain. Phone: +34 91
16 3943721, Fax: +34 91 3943795, e-mail address: alaranaz@vet.ucm.es

17 **Abstract**

18 Interferon-gamma (IFN- γ) detection assay is being applied as an ancillary test to
19 tuberculin tests in the diagnosis of bovine tuberculosis to detect the maximum number
20 of infected animals. Among possible factors influencing the performance of
21 tuberculosis-diagnostic tests, paratuberculosis, a widespread disease in Spain and other
22 European countries, has been pointed out as a cause of false positive reactions. Still, its
23 effect on the sensitivity of these tests in cattle has yet to be fully characterized. The
24 impact of paratuberculosis in the apparent sensitivity of IFN- γ assay was studied in a
25 bullfighting cattle herd with a mixed tuberculosis-paratuberculosis infection, using
26 culture of *Mycobacterium bovis* and *Mycobacterium avium paratuberculosis* as the gold
27 standard to determine the infection status of every animal. A total of 218 animals were
28 slaughtered and sampled for bacteriology after blood sampling. IFN- γ assay showed a
29 lower apparent sensitivity in animals with a mixed infection (50%) compared to all
30 animals suffering tuberculosis (78.3%). This finding indicates that the presence of
31 paratuberculosis in tuberculosis-infected herds could imply a serious impairment in the
32 sensitivity of IFN- γ detection test.

33 **Keywords:** tuberculosis; paratuberculosis; cattle; IFN- γ ; diagnosis.

34 1. Introduction

35 Bovine tuberculosis is a zoonotic disease affecting cattle worldwide, causing
36 economic losses due to eradication programs and trade limitations (Pollock and Neill,
37 2002). In spite of great efforts the Officially Tuberculosis Free status has not yet been
38 achieved in some countries throughout Europe. This lack of success has been attributed,
39 among other causes, to the insufficient sensitivity of the diagnostic tests under certain
40 field conditions (Monaghan et al., 1994; Morrison et al., 2000).

41 In Spain prevalence of bovine tuberculosis has followed a decreasing trend from
42 11.14% (herd level) and 4.2% (individual level) in 1986 (when the national eradication
43 program was implemented) to 1.76% and 0.42% in 2006 (Anon., 2008). At the moment,
44 detection and removal of the maximum number of *M. bovis* infected animals is the
45 crucial step. Therefore, the increase of the sensitivity of diagnostic tests is a key
46 objective. For this reason the interferon- γ (IFN- γ) detection assay was introduced in the
47 EU legislation (Council Directive 64/432/EEC, amended by (EC) No. 1226/2002 of 8
48 July 2002, Anon. 2002) as an ancillary test and adopted by the Spanish program (Anon.,
49 2008). The conditions for the optimal use of this technique, already demonstrated in
50 several countries (Wood et al., 1990; Gormley et al., 2006), must be determined for the
51 different epidemiological situations in Spain.

52 To our knowledge the sensitivity of the IFN- γ test in cattle naturally co-infected
53 by *M. bovis* and *Mycobacterium avium paratuberculosis* (*Map*) has not been studied
54 under field conditions. A decreased sensitivity was reported by Amadori et al. (2002)
55 and Hope et al. (2005) in cattle previously infected with *M. avium* under experimental
56 conditions, but this might not reflect the field situation of a naturally mixed infection
57 with a *M. tuberculosis* complex member and *Map*. Aranaz et al. found a decreased
58 sensitivity in tuberculosis diagnostic tests in a cattle herd with a dual infection, but the

59 number of animals with positive culture was low, and selection of slaughtered animals
60 could have produced a biased higher sensitivity (Aranaz et al., 2006).

61 The current study was designed to determine the possible effect of natural co-
62 infection by *M. bovis* and *Map* on the performance of IFN- γ detection assay for the
63 diagnosis of tuberculosis in a bullfighting cattle herd.

Accepted Manuscript

64 2. Materials and methods

65 2.1. Herd and design of the study

66 The study was performed in a bullfighting cattle herd with a natural mixed
67 tuberculosis-paratuberculosis infection. The whole herd (n=680) was subjected to
68 analysis by single intradermal comparative cervical tuberculin (SICCT) test, IFN- γ
69 detection assay and an ELISA for the detection of antibodies against *Map*. All animals
70 sent to the slaughterhouse (n=218) because of positive results in any test or for
71 zootechnical reasons were sampled to perform bacteriology.

72 2.2. Diagnostic methods

73 2.2.1. SICCT test

74 The SICCT test was conducted by the Official Veterinary Services according to
75 European and national legislation (EU Council Directive 64/432/CEE and RD
76 2611/1996) after blood sampling for IFN- γ detection using bovine and avian PPDs (CZ
77 Veterinaria, Porriño, Spain).

78 2.2.2. IFN- γ detection test

79 Heparinized blood samples were collected from every animal and delivered to
80 the laboratory within eight hours of collection at room temperature. Stimulation with
81 avian and bovine PPDs was carried out as previously described (Wood et al., 1990;
82 Liebana et al., 1998), and plasma samples were analysed in duplicate using a sandwich
83 EIA for detection of bovine IFN- γ (Bovigam™ Bovine Gamma Interferon Test,
84 Prionics, Schlieren, Switzerland). Results were interpreted as previously described
85 (Aranaz et al., 2006). Animals were considered positive if the mean optical density
86 (OD) of a sample stimulated with bovine PPD minus the mean OD of nil antigen was
87 greater than 0.05 and greater than the same value of the sample stimulated with avian
88 PPD (interpretation prescribed in the Spanish eradication program).

89 2.2.3. Serology of paratuberculosis

90 Plasma samples were tested in duplicate using the absorbed ELISA kit
91 Parachek™ (Prionics, Schlieren, Switzerland) according to the manufacturer's
92 instructions. Animals were considered positive when its mean OD minus the plate
93 negative control was greater than 0.100 as recommended by the manufacturer.

94 **2.3. Bacteriology**

95 2.3.1. Tissue collection

96 Animals were slaughtered at the abattoir, and samples were taken at the post-
97 mortem inspection for culture of *M. bovis* (retropharyngeal, mediastinal and bronchial
98 lymph nodes, and lung) and *Map* (mesenteric and ileocecal lymph nodes and ileocecal
99 valve). Samples were stored at -20°C until further analysis.

100 2.3.2. *M. bovis* culture and characterization

101 Tuberculosis compatible lesions were selected when present; otherwise, a pool
102 of tissues was used. Culture of samples was performed after decontamination with
103 0.37% hexadecylpyridinium chloride (Corner and Trajstman, 1988) as described
104 elsewhere (Liebana et al., 1998). All isolates were identified by PCR (Wilton and
105 Cousins, 1992) and characterised using spoligotyping (Kamerbeek et al., 1997).

106 2.3.3. *Map* culture and characterization

107 Tissue samples of each animal were pooled and processed as described before
108 (Greig et al., 1997) and inoculated onto four selective culture media (de Juan et al.,
109 2006). Isolates were identified as *Map* based on their mycobactin- dependency and
110 presence of specific elements IS900 and f57 (Coetsier et al., 2000). The type of strain
111 was determined using the PCRs described by Collins et al. (2002) and by Castellanos et
112 al. (2007).

113 **2.4. Evaluation of diagnostic test**

114 The infection status of each animal was determined using their culture results as
115 the gold standard. Results from animals with a mixed infection (both *M. bovis* and *Map*
116 were recovered from their tissue samples) were then compared with all animals infected
117 by *M. bovis* to evaluate the apparent sensitivity of the IFN- γ assay, and with all animals
118 infected by *Map* for the assessment of the apparent sensitivity of the Parachek ELISA.

119 Statistical analysis was performed by means of the software PEPI 4.0 (Computer
120 Program for Epidemiologist), J.H. Abramson and P.M. Gahlinger.

Accepted Manuscript

121 3. Results

122 3.1. Bacteriology results

123 A total of 218 animals were slaughtered and sampled for bacteriology during the
124 study. Ninety-eight were culled based on their positive results in the diagnostic tests for
125 tuberculosis: six animals were positive to the SICCT test, 81 to the IFN- γ assay and 11
126 to both techniques. Eighty-two other animals were slaughtered as they had positive
127 values in the serology of paratuberculosis. The remaining 38 animals were slaughtered
128 for zootechnical reasons (inadequate temper, poor reproductive rate or lameness). *M.*
129 *bovis* and *Map* were isolated from 46 and 111 animals respectively (Table 1). Two of
130 the animals negative to all tests showed lesions compatible with tuberculosis at the
131 abattoir inspection, and both had subsequently *M. bovis* and *Map* positive-culture.

132 The 46 *M. bovis* isolates belonged to six spoligotypes, from which two were
133 much more prevalent: SB0298 (n=29), SB0339 (n=12), SB0121 (n=2), and SB0120,
134 SB1313 and SB1321 (n=1). Regarding the *Map* isolates, 80 (72.1%) belonged to the
135 type II and 31 (27.9%) were type III.

136 3.2. Impact of infection status in tuberculosis diagnosis

137 Animals were classified based on their culture results according to the criteria
138 detailed above. Proportion of positive animals and apparent sensitivity were determined
139 and compared in each group. Compulsory skin test was performed according to the
140 eradication program; however the results are not analyzed in detail in this study because
141 the dangerous handling of this bullfighting herd may have impaired the carrying out of
142 the test, therefore making difficult the interpretation of results.

143 Regarding IFN- γ assay, a low apparent sensitivity of 78.3% (36 out of the 46
144 animals with *M. bovis*-positive culture) was observed, though a detailed analysis of the
145 results revealed it could be mostly attributed to the animals with a mixed infection

146 (Table 2). In fact, among these animals only eight of the 16 (50%) were positive
147 reactors in the IFN- γ assay (Table 2), although when their avian PPD readings were
148 ignored the number of detected animals raised to 11 (68.8%) (Table 3). All of the false
149 negative reactors to the IFN- γ test in this group (n=8) were also negative in the SICCT
150 test. Remarkably, six out of these eight negative animals were positive in the Parachek
151 ELISA, while most IFN- γ reactors (six out of eight) showed negative results in that
152 ELISA (Table 3). None of these IFN- γ negative animals presented disseminated
153 tuberculous lesions which could indicate anergy due to advanced progression of
154 infection.

155 **3.3. Impact of infectious status in paratuberculosis diagnosis.**

156 The ELISA for detection of *Map* antibodies detected a lower proportion of *Map*
157 infected animals in the group with a mixed infection (eight out of 16, 50%) compared to
158 all *Map* infected animals (81 out of 111, 73%) (Table 2).

159 No association was observed between the type of *Map* strain infecting an animal
160 and the probability of being detected by any method.

161 **4. Discussion**

162 In spite of investing great efforts in the fight against tuberculosis, achievement
163 of complete eradication has been inconsistent in certain regions or countries (Neill and
164 Pollock, 2000). Wildlife reservoirs (Corner, 2006) and dissemination due to movement
165 of infected animals (Johnston et al., 2005; Collins, 2006) have been pointed out as
166 possible causes of this failure. Other major factors hampering eradication programs are
167 limitations of routine diagnostic tests (mainly skin tests), in particular concerning their
168 sensitivity (Monaghan et al., 1994; Morrison et al., 2000). Diagnostic accuracy is a key
169 issue in the test-and-slaughter programs applied in Spain and many other countries,
170 especially at the present low-prevalence stage, when detection of all infected animals is
171 crucial (Pollock et al., 2001).

172 In the present study the proportion of *M. bovis* infected animals detected by the
173 IFN- γ assay seems to be greatly affected by the presence of a concomitant *Map*
174 infection; the apparent sensitivity of the test in this herd was 78.3%, but in the group of
175 animals with a mixed infection it dropped to 50%. The impact of paratuberculosis
176 compromising the specificity of tuberculosis diagnostic tests has been demonstrated
177 (Cagiola et al., 2004; Dunn et al., 2005); however, its effect on the sensitivity in cattle
178 had not been determined in natural conditions. Nevertheless, paratuberculosis could be
179 of great importance, as it is widespread in certain areas of the European Union (Nielsen
180 and Toft, 2007) and other parts of the world (Kennedy and Benedictus, 2001). A
181 statistically significant relationship (C.I. 95%) was found between the recovery of *Map*
182 from tissue samples and response to the IFN- γ test in *M. bovis* infected animals. While
183 93.3% (28 out of 30) of animals with *Map* negative culture results did respond to the
184 assay, only 50% of cattle yielding *Map* by culture was detected. Furthermore, among
185 the ten false negative reactors found in the whole herd, eight of them (80%) were

186 suffering a concomitant *Map* infection.

187 These results corroborate the findings of a previous report in a goat flock
188 (Alvarez et al., 2007), where paratuberculosis seemed to have a major effect on the
189 sensitivity of tuberculosis diagnostic tests, especially on skin test, as the IFN- γ assay
190 was less affected. On the other hand, in the present study the ability of IFN- γ test to
191 detect tuberculosis-infected animals is more influenced by paratuberculosis; these
192 differences could be due to the host species or the strains present in each herd.

193 In the group of cattle with dual infection, 50% (8/16) of the animals were false
194 negative reactors in the IFN- γ assay, although this apparent sensitivity increases to
195 68.8% if interpretation is performed overlooking values obtained with avian PPD (Table
196 3). Further studies would be needed in order to disclose the advisability of adapting
197 interpretation criteria of the IFN- γ assay under these circumstances.

198 Interestingly, 75% of these dually infected animals with no response in the IFN-
199 γ assay were positive in the serology of *Map*, in opposition to what happened to most
200 animals positive in the IFN- γ test in the same group (that were seronegative for *Map*)
201 (Table 3). The negative association between results in IFN- γ assay and serology of *Map*
202 in animals with a mixed infection was also observed in the previous report in goats
203 (Alvarez et al., 2007) as from eight false negative reactors to the IFN- γ assay found in
204 that study, seven (87.5%) had high titers of antibodies against *Map* (unpublished data).

205 Regarding the ELISA for detection of antibodies against *Map*, it is remarkable
206 that a lower apparent sensitivity was observed in the group of animals with a mixed
207 infection compared to the whole group of animals infected with *Map*. However, this fact
208 would have no consequences under field conditions because animals would be culled
209 due to positive response to the tuberculosis diagnostic tests.

210 In conclusion, our results would suggest that both *M. bovis* and *Map* influence

211 the overall immune response developed in the dually-infected animals, and may
212 compromise the reliability of diagnostic tests that are based in the measurement of
213 specific response generated against each pathogen. Differences in the proportion of *M.*
214 *bovis*-infected animals reacting to the IFN- γ assay were observed depending on their
215 paratuberculosis status: animals with dual infection were more likely to produce false
216 negative reactions to this test. This fact highlights the importance of ascertaining the
217 paratuberculosis status of a herd when implementing a strategic use for the IFN- γ assay,
218 not only because its effect on the specificity but also for its impact on the sensitivity.
219 More studies should be performed with larger numbers of animals representing the
220 actual bovine population in order to confirm this finding.

221 **Acknowledgements**

222 This research was funded by project AGL2006-06206 of the Spanish Ministry of
223 Science and Technology, ParaTBTools (STREP 23106) of the European Union, and the
224 Spanish Ministry of Agriculture, Food and Fisheries. J. Álvarez was the recipient of a
225 predoctoral grant assigned by the Spanish Ministry of Education and Culture. The group
226 is a partner of the coordination action “Veterinary European Network on Mycobacteria
227 (VENoMYC)” funded by the European Union.

228 We are grateful to F. Lozano and N. Moya for technical help; to the Official
229 Veterinary Services for the IDTB testing and collection of blood samples; and to C.
230 García, V. Bielsa, and A. Zabaleta [abattoir staff of Leganes (Madrid)] for assistance in
231 collection of tissue samples. We would like to thank C. Escribano, L. Carbajo, and J. L.
232 Paramio (Dirección General de Ganadería, Spanish Ministry of Agriculture, Fisheries
233 and Food) for their continuous encouragement. The willingness and cooperation of
234 landowner is greatly appreciated. We acknowledge Matthew Gilmour and Devin J.
235 Morey for careful revision of the manuscript.

236 **References**

- 237 Alvarez, J., De, Juan L., Bezos, J., Romero, B., Saez, J. L., Gordejo, F.J., Briones, V.,
238 Moreno, M.A., Mateos, A., Dominguez, L., Aranaz, A., 2007. Interference of
239 paratuberculosis with the diagnosis of tuberculosis in a goat flock with a natural mixed
240 infection. *Vet. Microbiol.* 128, 72-80.
- 241 Amadori, M., Tagliabue, S., Lauzi, S., Finazzi, G., Lombardi, G., Telo, P., Pacciarini,
242 L., and Bonizzi, L., 2002. Diagnosis of *Mycobacterium bovis* infection in calves
243 sensitized by mycobacteria of the *avium/intracellulare* group. *J. Vet. Med. B Infect.*
244 *Dis. Vet. Public Health* 49, 89-96.
- 245 Anon., July 9, 2002. *Off. J. Eur. Union* L179, 13–18.
- 246 Anon., 2008. Programa nacional de erradicacion de tuberculosis bovina presentado por
247 España para los años 2008-2010. Subdirección General de Sanidad Animal, Dirección
248 General de Ganadería, Ministerio de Agricultura, Pesca y Alimentación. [http://](http://rasve.mapa.es/Publica/Programas/Normativa.asp)
249 rasve.mapa.es/Publica/Programas/Normativa.asp.
- 250 Aranaz, A., de Juan, L., Bezos, J., Alvarez, J., Romero, B., Lozano, F., Paramio, J.L.,
251 Lopez-Sanchez, J., Mateos, A., and Dominguez, L., 2006. Assessment of diagnostic
252 tools for eradication of bovine tuberculosis in cattle co-infected with *Mycobacterium*
253 *bovis* and *M. avium* subsp. *paratuberculosis*. *Vet. Res.* 37, 593-606.
- 254 Cagiola, M., Feliziani, F., Severi, G., Pasquali, P., and Rutili, D., 2004. Analysis of
255 possible factors affecting the specificity of the gamma interferon test in tuberculosis-
256 free cattle herds. *Clin. Diagn. Lab. Immunol.* 11, 952-956.
- 257 Castellanos, E., Aranaz, A., Romero, B., De, Juan L., Alvarez, J., Bezos, J., Rodriguez,
258 S., Stevenson, K., Mateos, A., and Dominguez, L., 2007. Polymorphisms in *gyrA* and

- 259 *gyrB* genes among *Mycobacterium avium* subsp. *paratuberculosis* type I, II, and III
260 isolates. J. Clin. Microbiol. 45, 3439-3442.
- 261 Coetsier, C., Vannuffel, P., Blondeel, N., Deneff, J.F., Cocito, C., and Gala, J.L., 2000.
262 Duplex PCR for differential identification of *Mycobacterium bovis*, *M. avium*, and *M.*
263 *avium* subsp. *paratuberculosis* in formalin- fixed paraffin-embedded tissues from cattle.
264 J. Clin. Microbiol. 38, 3048-3054.
- 265 Collins, D.M., De Zoete, M., and Cavaignac, S.M., 2002. *Mycobacterium avium* subsp.
266 *paratuberculosis* strains from cattle and sheep can be distinguished by a PCR test based
267 on a novel DNA sequence difference. J. Clin. Microbiol. 40, 4760-4762.
- 268 Collins, J.D., 2006. Tuberculosis in cattle: Strategic planning for the future. Vet.
269 Microbiol. 112, 369-381.
- 270 Corner, L.A. and Trajstman, A.C., 1988. An evaluation of 1-hexadecylpyridinium
271 chloride as a decontaminant in the primary isolation of *Mycobacterium bovis* from
272 bovine lesions. Vet. Microbiol. 18, 127-134.
- 273 Corner, L.A.L., 2006. The role of wild animal populations in the epidemiology of
274 tuberculosis in domestic animals: how to assess the risk. Vet. Microbiol. 112, 303-312.
- 275 de Juan, L., Alvarez, J., Romero, B., Bezos, J., Castellanos, E., Aranaz, A., Mateos, A.,
276 and Dominguez, L., 2006. Comparison of four different culture media for isolation and
277 growth of type II and type I/III *Mycobacterium avium* subsp. *paratuberculosis* strains
278 isolated from cattle and goats. Appl. Environ. Microbiol. 72, 5927-5932.
- 279 Dunn, J.R., Kaneene, J.B., Grooms, D.L., Bolin, S.R., Bolin, C.A., and Bruning-Fann,
280 C.S., 2005. Effects of positive results for *Mycobacterium avium* subsp *paratuberculosis*
281 as determined by microbial culture of feces or antibody ELISA on results of caudal fold

- 282 tuberculin test and interferon-gamma assay for tuberculosis in cattle. J. Am. Vet. Med.
283 Assoc. 226, 429-435.
- 284 Gormley, E., Doyle, M.B., Fitzsimons, T., McGill, K., and Collins, J.D., 2006.
285 Diagnosis of *Mycobacterium bovis* infection in cattle by use of the gamma-interferon
286 (Bovigam) assay. Vet. Microbiol. 112, 171-179.
- 287 Greig, A., Stevenson, K., Perez, V., Pirie, A.A., Grant, J.M., and Sharp, J.M., 1997.
288 Paratuberculosis in wild rabbits (*Oryctolagus cuniculus*). Vet. Rec. 140, 141-143.
- 289 Hope, J.C., Thom, M.L., Villarreal-Ramos, B., Vordermeier, H.M., Hewinson, R.G.,
290 and Howard, C.J., 2005. Exposure to *Mycobacterium avium* induces low-level
291 protection from *Mycobacterium bovis* infection but compromises diagnosis of disease in
292 cattle. Clin. Exp. Immunol. 141, 432-439.
- 293 Johnston, W.T., Gettinby, G., Cox, D.R., Donnelly, C.A., Bourne, J., Clifton-Hadley,
294 R., Le Fevre, A.M., McInerney, J.P., Mitchell, A., Morrison, W.I., and Woodroffe, R.,
295 2005. Herd-level risk factors associated with tuberculosis breakdowns among cattle
296 herds in England before the 2001 foot-and-mouth disease epidemic. Biol. Lett. 1, 53-56.
- 297 Kamerbeek, J., Schouls, L., Kolk, A., van, Agterveld M., van, Soolingen D., Kuijper, S.,
298 Bunschoten, A., Molhuizen, H., Shaw, R., Goyal, M., and van, Embden J., 1997.
299 Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for
300 diagnosis and epidemiology. J. Clin. Microbiol. 35, 907-914.
- 301 Kennedy, D.J. and Benedictus, G., 2001. Control of *Mycobacterium avium* subsp.
302 *paratuberculosis* infection in agricultural species. Rev. Sci. Tech. 20, 151-179.
- 303 Liebana, E., Aranaz, A., Urquia, J. J., Mateos, A., and Dominguez, L., 1998. Evaluation
304 of the gamma-interferon assay for eradication of tuberculosis in a goat herd. Aust. Vet.
305 J. 76, 50-53.

- 306 Monaghan, M.L., Doherty, M.L., Collins, J.D., Kazda, J.F., and Quinn, P.J., 1994. The
307 tuberculin test. *Vet. Microbiol.* 40, 111-124.
- 308 Morrison, W.I., Bourne, F.J., Cox, D.R., Donnelly, C.A., Gettinby, G., McInerney, J.P.,
309 and Woodroffe, R., 2000. Pathogenesis and diagnosis of infections with *Mycobacterium*
310 *bovis* in cattle. Independent Scientific Group on Cattle TB. *Vet. Rec.* 146, 236-242.
- 311 Neill, S.D. and Pollock, J.M., 2000. Testing for bovine tuberculosis--more than skin
312 deep. *Vet. J.* 160, 3-5.
- 313 Nielsen, S.S., and Toft, N., 2007. Review of prevalences of paratuberculosis in farmed
314 animals in Europe. In: Proceedings of the 9th international colloquium on
315 Paratuberculosis, Tsukuba, Japan, October 29-November 2, pp. 70.
- 316 Pollock, J.M. and Neill, S.D., 2002. *Mycobacterium bovis* infection and tuberculosis in
317 cattle. *Vet. J.* 163, 115-127.
- 318 Pollock, J.M., Buddle, B.M., and Andersen, P., 2001. Towards more accurate diagnosis
319 of bovine tuberculosis using defined antigens. *Tuberculosis (Edinb.)* 81,65-69.
- 320 Wilton, S. and Cousins, D., 1992. Detection and identification of multiple
321 mycobacterial pathogens by DNA amplification in a single tube. *PCR Methods Appl.* 1,
322 269-273.
- 323 Wood, P.R., Corner, L.A., and Plackett, P., 1990. Development of a simple, rapid in
324 vitro cellular assay for bovine tuberculosis based on the production of gamma
325 interferon. *Res. Vet. Sci.* 49, 46-49.

326

Table 1.

Mycobacterium bovis and *Mycobacterium avium* subsp. *paratuberculosis* culture results of slaughtered animals (n=218)

Culture results	<i>Map</i> +	<i>Map</i> -	Total
<i>M. bovis</i> +	16	30	46
<i>M. bovis</i> -	95	77	172
Total	111	107	218

Accepted Manuscript

327

Table 2.

Apparent sensitivities with confidence intervals (95%) of the IFN- γ detection test and the ParachekTM ELISA in the groups formed based on culture results

Diagnostic test	Tbc+ animals (n=46)^a	Ptb+ animals (n=111)^b	Tbc+/Ptb+ (n=16)^c
IFN- γ detection test	78.3 (63.6-89)	-	50 (24.6-75.3)
Parachek ELISA	-	73 (63.7-81)	50 (24.6-75.3)

328 ^a Animals with *M. bovis* positive isolation (regardless their *Map* culture results).

329 ^b Animals with *Map* infection (regardless their *M. bovis* culture results).

330 ^c Animals with positive cultures of both *M. bovis* and *Map*.

331

Table 3.

Results of IFN- γ assay and Parachek ELISA in animals suffering a tuberculosis-paratuberculosis dual infection (n=16)

Animal	Bovine IFN- γ^a	Avian IFN- γ^b	Parachek ELISA ^c
1	0.635	0.132	-0.012
2	0.529	0.080	0.008
3	0.314	0.263	-0.025
4	0.212	0.069	-0.025
5	0.161	0.044	0.384
6	0.157	0.036	-0.020
7	0.115	0.036	-0.026
8	0.051	0.016	0.126
9	0.035	0.077	1.048
10	0.136	0.139	0.883
11	0.239	0.888	0.400
12	0.070	0.141	0.242
13	0.007	0.005	0.213
14	0.007	0.017	0.210
15	0.018	0.024	0.013
16	0.017	0.002	-0.007

332 Optical densities above the cut-off point (positive animals) are presented in bold letters.

333 ^a Results after the in-vitro stimulation with bovine PPD minus the value obtained after
334 stimulation with PBS.

335 ^b Results after the in-vitro stimulation with avian PPD minus the value obtained after
336 stimulation with PBS.

337 ^c Results in the ELISA for detection of *Map* antibodies.