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## Transitions in diagnostic tests used for detection of subsp. infections in cattle

S.S. Nielsen

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1       **Transitions in diagnostic tests used for detection of *Mycobacterium avium***  
2                               **subsp. *paratuberculosis* infections in cattle**

3  
4                               S. S. Nielsen

5  
6       *Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen,*  
7                               *Grønnegårdsvej 8, DK-1870 Frederiksberg C, Denmark*

8  
9       Corresponding author:

10       Søren Saxmose Nielsen

11       Grønnegårdsvej 8

12       DK-1870 Frederiksberg C

13       Denmark

14       Phone: + 45 35 33 30 96

15       Fax: +45 35 33 30 22

16       E-mail: ssn@life.ku.dk

17  
18       **Abstract**

19               Diagnosis of infections with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is  
20       difficult due to a long incubation period and lack of tests which can accurately predict the  
21       future status of animals. Early detection of infectious animals is necessary to reduce  
22       transmission of MAP. The objective of this study was to determine the time from first  
23       detection of MAP-antibodies in milk ELISA to start of MAP shedding, for animals with various  
24       shedding patterns.

25               An observational longitudinal study was carried out over 3 years. A total of 24,076 milk  
26       and 10,074 faecal samples were obtained from 1906 cows and tested using ELISA and FC,  
27       respectively. Cows were classified into 5 shedding groups based on their repeated FC: non-

28 shedders (NS; n=1512 cows, 79.3% of total), transient (TS; n=36, 1.9%), intermittent (IS;  
29 n=137, 7.2%), low (LS; 143, 7.5%), and high shedders (HS; 78; 4.1%).

30 Results showed that 5% of TS, 30% of IS, 60% of LS and 70% of HS were ELISA-  
31 positive at the date of first positive FC, and many HS (28%) and LS (14%) were positive  $\geq 1$   
32 year prior to first detection of shedding. FC confirmed shedding within the first year after the  
33 positive ELISA in 10% of 328 cows with fluctuating ELISA compared with 35% of 445 cows  
34 with the last 2 or more ELISAs positive.

35 To conclude, MAP antibodies were generally detected prior to start of bacterial  
36 shedding, with difference between the various patterns of shedding, and a positive ELISA  
37 was useful for predicting that an animal would subsequently become infectious.

38

39 **Key words:** Antibodies; Bacterial shedding; dairy cattle; Faecal culture; milk ELISA;  
40 Paratuberculosis

41 **Introduction**

42 Paratuberculosis in cattle is a chronic infection caused by *Mycobacterium avium* subsp.  
43 *paratuberculosis* (MAP) (Chiodini et al., 1984). Control programmes have been established  
44 in several countries (Kennedy and Nielsen, 2007), because MAP infections can be costly to  
45 the cattle industry (Ott et al., 1999). Most programmes include diagnostic testing, although  
46 purposes of testing can differ with different objectives of the programmes. The requirements  
47 for diagnostic tests ability to differentiate between conditions such as “infectious”, “infected”  
48 and “non-infected” would therefore vary from one type of programme to another. Infectious  
49 animal make up an immediate risk for spread of MAP, whereas the infected animal  
50 constitutes a future risk of spread of MAP. Detection of non-infected animals is of particular  
51 importance in certification schemes. Therefore, a decision maker would need to know the  
52 accuracy of a test relative to the use of a diagnostic test result, and estimates of sensitivity  
53 and specificity should be estimated relative to a given target condition.

54 Timely identification of infectious animals is the primary purpose of testing in the Danish  
55 programme, in order to reduce MAP transmission from these animals to susceptible herd  
56 mates (Nielsen, 2007). Sensitivities of diagnostic tests for ante mortem diagnosis of MAP  
57 infectious animals have been reported in the range 0.21 to 0.94, with a median of 0.46  
58 (reviewed in Nielsen and Toft, 2008). Data from literature do not suggest that one test has a  
59 better accuracy than others, but ELISA is an in-expensive alternative to faecal culture (FC).  
60 As an example, sampling and test costs for one milk ELISA and one FC were estimated to 3  
61 EUR and 27 EUR, respectively, in a herd with whole-herd testing of 120 cows in 2006 in  
62 Denmark (Sergeant et al., 2008).

63 Infections with MAP are characterised by long incubation periods, which may be of  
64 variable length. The prevailing hypothesis on the pathogenesis is that some time after  
65 infection, cell-mediated immune responses occur, followed by humoral immune responses  
66 (Stabel, 2000). Cows shedding MAP on average have higher concentrations of IgG  
67 antibodies (Koets et al., 2001), but the patterns of shedding related to immune responses are  
68 poorly described. Because an antibody ELISA test is an indirect measure of infected or

69 infectious status of an individual, the relationship between the detection of antibodies and  
70 commencement of shedding is a key factor in designing an effective control program.  
71 Shedding of MAP is usually defined based on results from an agent detecting test such as  
72 FC in different media or direct PCR. However, various categories of shedding can occur,  
73 depending on the number of bacteria shed and pattern of shedding (transient, intermittent or  
74 continuous). Transient shedding may be either the result of uninfected cows' ingestion of  
75 MAP from the environment with subsequent shedding of MAP in the following days  
76 (Sweeney et al., 1992), or it may simply be a typical pattern seen in the transition from the  
77 infected to the infectious stage of disease. Intermittent and repeated shedding would be  
78 expected to occur as infection progresses. This variable pattern of detection may be due to  
79 either intermittent excretion of MAP or excretion of low levels of MAP around the limits of  
80 detection of the FC system utilised. There is no specific explanation why ELISA positive  
81 results can first be detected in some animals prior to evidence of shedding and after  
82 confirmation in others. Some ELISA positive animals remain FC negative indefinitely even  
83 though they are infected (e.g. confirmed by tissue culture post-mortem).

84 The primary objective of the current study was to estimate the time relationship  
85 between occurrence of antibodies, as determined by ELISA, to first detection of MAP by FC,  
86 for cows with various shedding patterns.

87

## 88 **Materials and Methods**

### 89 *Herds, Animals, and Observations*

90 Data were collected from a non-random sample of 8 Danish dairy herds in the period  
91 Aug. 11, 1999 to Dec. 12, 2002. A brief description of the herds is given in Table 1. Faecal  
92 sampling was performed four times a year on all cows present in each herd at the time of  
93 sampling. Milk samples were collected from all lactating cows via the Danish milk recording  
94 scheme on 11 test-dates per year. The study design was observational; hence each cow  
95 provided variable number of samples.

96 A total of 1906 cows provided both milk (n=24,076) and faecal samples (n=10,074).  
97 The distributions of number of samples per cow were as follow: a) Milk samples: minimum: 1,  
98 1<sup>st</sup> quartile: 6; median: 11; 3<sup>rd</sup> quartile: 18, and maximum 35; b) Faecal samples: minimum: 1,  
99 1<sup>st</sup> quartile: 2; median: 5; 3<sup>rd</sup> quartile: 8, and maximum 18. Information on date of birth and  
100 breed was obtained from the Danish Cattle Database. The breed distribution of the cows  
101 was: 1350 Danish Holsteins, 427 Danish Jerseys, 12 Red Danish, 1 Finnish Ayrshire, 1 Old  
102 Danish, and 115 crossbreds. The age distribution at 1<sup>st</sup> testing of the individuals was:  
103 minimum: 1.74 years; 1<sup>st</sup> quartile: 2.32 years; median: 2.56 years; 3<sup>rd</sup> quartile: 3.44 years,  
104 and maximum 12.49 years. Age distribution at first sampling was: minimum: 2.05 years; 1<sup>st</sup>  
105 quartile: 3.01 years; median: 3.91 years; 3<sup>rd</sup> quartile: 4.91 years, and maximum 10.70 years.

106

#### 107 *Diagnostic tests*

108 Milk samples were tested for presence of antibodies using the ELISA (Nielsen, 2002)  
109 used in the Danish control programme on paratuberculosis (Nielsen, 2007). Sensitivity and  
110 specificity estimates for detection of infected animals vary with age and have been described  
111 in detail in Nielsen and Toft (2006). All samples were tested in duplicate, and the mean result  
112 was used. Samples were retested if the difference in optical density (OD) values was >0.1.  
113 The mean OD-value of a sample was corrected for intra-laboratory variation by subtracting  
114 the mean OD-value of a negative control. Samples with a corrected OD-value (OD<sub>C</sub>) of ≥0.3  
115 were deemed positive, as done in the Danish control programme.

116 Faecal samples collected prior to August 2002 were cultured on Löwenstein-Jensen  
117 Medium and samples from September 2002 and onwards were cultured on Herrold's Egg  
118 Yolk Medium, while samples from August and September 2002 were cultured on both media.  
119 The methods are described in Nielsen et al. (2004). IS900 PCR was carried out on positive  
120 cultures to confirm that presumed isolates were MAP. Positive cultures were recorded on an  
121 ordinal scale as follows: 1+: <10 colonies; 2+: 10–49 colonies; 3+: ≥50 colonies.

122

#### 123 *Statistical analysis*

124 Definitions

125 Cows were grouped into MAP shedding groups based on their FC results as follows

126 (illustrated in Fig. 1):

127 Non-shedding: No positive cultures among all bacteriological results from a cow;

128 Transient shedding: Last four samples were MAP negative but at least 1 positive result was  
129 recorded previously;

130 Low shedding: Cows where all samples were FC positive after the first positive sample or  
131 where the last three samples were positive and none of the samples had  $\geq 50$  CFU per test-  
132 tube;

133 High shedding: Cows fulfilling the same criteria as for low shedding, but at least one culture  
134 had  $\geq 50$  CFU per test-tube;

135 Intermittent shedding: Cows with at least one positive FC, but not meeting the criteria of the  
136 other shedding groups.

137 The date of FC positivity (Time0) was defined for all shedding cows as the date of the first  
138 detection of a positive sample. For cows never shedding MAP, Time0 was defined as the  
139 median test-date for the individual cow. The distribution of samples within each shedding  
140 group is shown in Table 2.

141 Cows were also divided into antibody categories based on their antibody profiles  
142 determined by ELISA. The antibody categories were defined as those in the Danish control  
143 programme as follows: A0) a minimum of two milk samples were available, and all samples  
144 had  $OD_C < 0.3$  (negative); A1) only one sample, which was negative; A2) last three samples  
145 were negative, but previously a minimum of 1 sample had been positive ( $OD_C \geq 0.3$ ); A3) last  
146 sample was negative, but some previous samples had been positive (fluctuating response);  
147 A4) last sample was positive, but previous samples were all negative; A5) last two or more  
148 samples were positive (Nielsen, 2007). The date of positivity (Time0) was defined as the date  
149 of first positive ELISA.

150 Descriptive statistics



151 Proportions of positive faecal samples per animal in total and after first positive  
 152 sample were calculated within each MAP shedding group (Table 2). For each of these  
 153 groups, the distribution of OD<sub>C</sub>-values was also described (Table 3). Proportions of positive  
 154 faecal samples in each of the antibody categories overall and after first positive ELISA were  
 155 also calculated (Table 4).

### 156 Analytical statistics

157 The probability of testing positive in ELISA relative to Time=0 for each shedding  
 158 group was estimated using a generalised additive model (Hastie and Tibshirani, 1990) using  
 159 the nonparametric logistic regression model:

$$160 \quad \log \frac{P(\text{OD}_C > 0.3)}{1 - P(\text{OD}_C > 0.3)} = \beta_0 + S(\text{Time})$$

161 where  $P(\text{OD}_C > 0.3)$  was the probability of being ELISA-positive,  $\beta_0$  the baseline probability of  
 162 testing ELISA-positive, and  $S(\text{Time})$  was the smoothing function of the effect of time relative  
 163 to first positive FC (Time0) within a shedding group.

164 A similar logistic regression model was used with FC as the dependent variable and  
 165  $S(\text{Time})$  as the independent variable, for each of the antibody categories. The effect of  
 166 change of faecal culture method was also assessed. For both models, 95% point-wise  
 167 confidence bands were estimated. The generalised additive models were estimated using  
 168 the GAM procedure in SAS v. 9.1 (SAS Institute, Cary, North Carolina, USA).

169

### 170 **Results**

171 Distributions of positive FC among all samples and among samples obtained after the  
 172 first positive FC are summarised in Table 2 for each shedding group. Proportions of FC  
 173 positive samples within each antibody category are shown in Table 4, both overall and after  
 174 1<sup>st</sup> positive ELISA result. The distribution of OD<sub>C</sub>-values within each shedding group is shown  
 175 in Table 3.

176 Probabilities of testing positive by ELISA relative to Time0 for each of the shedding  
 177 groups are shown in Fig. 2. Among “High shedders” an estimated 70% were ELISA positive

178 on the first detection date of MAP shedding, whereas 60% of cows in the “Low shedding”  
179 group were ELISA positive at Time0. For “intermittent shedders”, 30% were ELISA positive at  
180 the first date of detected shedding. Among “transient shedders” an estimated 7% were  
181 ELISA positive at Time0 with a significant proportion (40%) of animals ELISA positive 2 to 3  
182 years after Time0. The “non-shedder” group showed an estimated 7% ELISA positive at  
183 Time0 and an estimated 27% ELISA positive at 1 year and 60% 2 years after the median  
184 test-date. Approximately 20% of the cows in the “high shedder” group were positive 3 to 4  
185 years prior to detection of shedding, but this estimate was associated with some uncertainty  
186 compared to other estimates. The probability of being ELISA-positive among cows in the “low  
187 shedder” group was 0% 3 to 5 years prior to start of shedding, and cows in all shedding  
188 groups other than the “high shedders” had low probabilities (<4%) of being ELISA-positive 2  
189 to 3 years before shedding was first detected.

190       There was no difference in probability of testing FC-positive with the two different  
191 culture methods ( $p=0.38$ ). Probabilities of testing positive in FC for cows in various antibody  
192 categories are shown in Fig. 3, with Time0 the testing date on which the animal first tested  
193 positive. A cow repeatedly positive by ELISA (antibody category A5) would test positive by  
194 FC in 20% of cases at Time0. The optimal time for confirming the ELISA test result by FC  
195 would be 9 months after the milk sample had been obtained when an estimated 35% of A5  
196 cows would be positive. Cows with only the last ELISA positive (A4 category) showed 17%  
197 FC positive at Time0 rising to 21%. Cows with fluctuating ELISA-responses (A3 category,  
198 e.g. negative-positive-negative-positive-negative results) had an estimated 8% probability of  
199 testing FC positive at Time0 and maximum 12% probability of testing positive approximately  
200 6 months later. The 95% confidence bands for estimates (Fig. 3) for all antibody categories  
201 were generally quite narrow except for those in A4 category (last ELISA positive) where  
202 there was a very marked spread of the confidence bands after Time0 due to a limited  
203 number of observations after Time0 in this category.

204

205 **Discussion**

206 The results in this report show that the ELISA has a high level of predictive ability that  
207 an animal is or will become infectious. Antibodies are generally detectable prior to MAP  
208 shedding, but a positive ELISA result is not necessarily an indicator of shedding on the test-  
209 date. The almost parallel lines in Fig. 2 show an interesting progression of MAP infection.  
210 Cows with “transient shedding” generally do not have a positive ELISA reaction prior to  
211 Time0, while larger proportions of cows are ELISA-positive with progression from “transient”  
212 over “intermittent” and “low” to “high” shedding. Thereby, a continuous progression of MAP  
213 infection appears to occur, and ELISA results are linked to deterioration of shedding. Due to  
214 the complex nature of the interaction between the immune response and MAP, and the long  
215 incubation period, division into “infectious”, “infected” and “non-infected” is not  
216 straightforward, neither based on the immune response, nor based on the detection of MAP  
217 using an agent detecting test such as FC. The advantage of FC is that detection of MAP is a  
218 direct measure of MAP shedding on the test-date. However, the amount of MAP shed need  
219 not constitute an infective dose, or the shedding may cease after a short shedding period  
220 and re-occur later, as demonstrated by Lepper et al. (1989). The advantage of detecting an  
221 immune response is that ELISA is less expensive than FC and results can be available  
222 faster. Frequent testing using ELISA is therefore more attractive to farmers. Antibody profiles  
223 based on frequent testing may subsequently suggest whether there is a high or low risk of  
224 shedding until next test-date. The diagnosis provided is a probability diagnosis, because final  
225 proof of shedding is not provided. Cows with repeated positive ELISA are more likely to be  
226 shedding MAP in the near future than are fluctuating immune responses (Fig. 3). Detection of  
227 animals that are or will become infectious in the time-span until the next test-date is pivotal in  
228 a control programme to avoid transmission. Subsequent decisions on management of test-  
229 positive animals depend on a variety of factors such as milk quota, prevalence of test-  
230 positives, availability of replacement animals, stage in lactation, clinical signs (of MAP  
231 infection or other conditions of priority in the herd), milk yield, facilities for management of the  
232 potentially infectious animals in facilities where they do not make a risk for susceptible

233 animals etc. The farmer's ultimate goal of intervention is also important, while different goals  
234 often require different decisions. Possible decisions could include: a) follow-up testing; b)  
235 culling based on one ELISA result only; c) "isolation", i.e. measures should be taken to avoid  
236 transmission of MAP from test-positive to susceptible animals; and d) do nothing. The ideal  
237 evaluation of such decision processes should be through simulation models (e.g. Kudahl et  
238 al., 2007), where cost and impact of false-positive and false-negative test-results can be  
239 accounted for. Costs related to misclassification can vary greatly from one herd to another.

240 Follow-up testing with ELISA, FC or other tests is an option in the decision making  
241 process. The test-history may be part of the decision process, and multiple previous positive  
242 test results may suggest that re-testing is not needed. Two or more ELISA-positive results  
243 suggest that a cow has a higher probability of shedding MAP, but the basis for making a  
244 decision will vary from one decision maker to another. It should be noted that probabilities  
245 presented in this paper should be read as one diagnostic test result at a given time point, as  
246 the observations were not independent. Hence, the cumulative probability leading up to a  
247 time point is greater than the single estimated probability at that time point but less than the  
248 sum of the probabilities for the series of time points. Most cows shedding MAP will be ELISA-  
249 positive at some point in their lifetime (Nielsen and Ersbøll, 2006). Should a decision maker  
250 wish to confirm MAP shedding predicted by an ELISA-result, the highest chance of  
251 confirmation by FC is  $\frac{1}{2}$  to  $\frac{3}{4}$  year after the ELISA was performed (Fig. 3).

252 ELISA-results may in some cases be false-positive. However, considering that most  
253 cows in the pre-shedding phase in the present study were ELISA-negative, it is unlikely that  
254 antibodies to non-MAP infections caused the high probabilities of testing ELISA-positive seen  
255 in cows that were never shedding MAP. Data presented here suggest that specificity of the  
256 ELISA was >97.5%, based on the early ELISA-responses in all shedding groups, excluding  
257 ELISA responses of High shedders (Fig. 2). Repeated testing with ELISA could increase  
258 specificity, although not to 100%. A high specificity is primarily required for herd-classification  
259 purposes, but while FC is not appropriate for confirmatory purposes in infected but non-  
260 infectious animals, other methods could be used for herd classification, e.g. the Rogan-

261 Gladen estimator for prevalence estimation (Rogan and Gladen, 1978) as exemplified in  
262 Sergeant et al. (2008).

263 The probability curves (Fig. 2) suggest that occurrence of antibodies was a good  
264 indicator of progression of MAP infection, but many non-shedding animals will test positive.  
265 The high proportions of ELISA-positive animals among non-shedding and transient shedding  
266 animals suggest that many of these animals were in an early phase of infection. “Transient  
267 shedding” may not be “passive shedding” in the meaning described by Sweeney et al.  
268 (1992), where un-infected cows fed high dosages of MAP shed MAP after a few days. It  
269 appears that “transient” as defined in the current study may merely be a precursor of  
270 intermittent, low and high shedding. The occurrence of the phenomenon “passive shedding”  
271 cannot be confirmed with the current study design.

272 The current study has a number of limitations. Firstly, the herds may not be  
273 representative of the cattle population. A complete random inclusion of animals would be  
274 preferred, and the animals should then be followed for their entire lifetime with follow-up post  
275 mortem examinations to verify their infection status. Such a design was not possible, partly  
276 for practical reasons, partly because it would be extremely expensive. Secondly, the ELISAs  
277 available world-wide appear to vary greatly in sensitivity and specificity (Nielsen and Toft,  
278 2008), and few ELISAs have been appropriately compared. Therefore, caution should be  
279 made if conclusions from this study are transferred to other ELISAs. Lastly, caution should  
280 be made when interpreting point estimates in the graphs, when wide 95% confidence  
281 intervals are present. Wide confidence bands result primarily for two reasons: great variation  
282 in data or low number of observations. For example, all confidence bands in Figures 2 and 3  
283 were wide in the right side of the graphs due to the generally low number of observations at  
284 the later time points, an intrinsic aspect in the analysis where the cows were sampling  
285 occurred over a 3 year time frame and first positive results could occur at any point within  
286 that time frame. However, a wide confidence band for the High shedding group around Time  
287 = -4 years (Fig. 2) was seen due to great variation in test-responses in combination with low

288 number of samples. The interpretation of the graphs was best at points with narrow  
289 confidence intervals.

290 The results of this study are the first observational longitudinal study in a large and  
291 naturally infected population of dairy cattle demonstrating the relation between test-  
292 responses of ELISA and FC, and occurrence of antibodies and bacterial shedding. The  
293 results are reasonably consistent with the theory described regarding the pathogenesis  
294 (Stabel, 2000), and with an experimental study (Lepper et al., 1989). However, Lepper et al.  
295 (1989) also detected shedding of MAP at early ages (<1 year), shedding that waned to  
296 subsequently reappear at older ages. The current study did not include young animals and  
297 could therefore not confirm this finding from the animals experimentally infected. More  
298 studies with longitudinal study designs should be carried out to verify the results presented  
299 here.

300 Implementation of the results from this study in control, surveillance and eradication  
301 programmes would require studies on technical and economical effects, to determine which  
302 test-strategy should be preferred. Sergeant et al. (2008) demonstrated that whole-herd  
303 ELISA would be the most cost-effective test-strategy for surveillance with the prevalences,  
304 herd-sizes and test-costs seen in Denmark, but this may be different in other scenarios. In  
305 control programmes, it would also be important to determine if farmers would respond to the  
306 recommendations given, while testing is of no use if appropriate actions are not made to a  
307 test-result.

308

### 309 **Conclusions**

310 The results in this study indicate that antibodies to MAP generally occur prior to  
311 shedding of MAP, although transient or intermittent shedding can occur prior to the  
312 occurrence of antibodies. Progression of MAP infection is generally accompanied by  
313 occurrence of antibodies. Positive ELISA-results can only be confirmed with FC in a fraction  
314 of animals, because time from occurrence of antibodies to shedding of MAP varies greatly.

315

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319

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**Table 1.** Description of 8 study herds: herd size, housing system, breed distribution and milk production level

<b>Herd</b>	<b>Housing system</b>	<b>Cow years<sup>2</sup></b>	<b>Kg ECM<sup>1</sup>/cow year<sup>2</sup></b>	<b>Cow years<sup>3</sup></b>	<b>Kg ECM/cow year<sup>3</sup></b>	<b>Breed distribution<sup>4</sup></b>
1	Tie stall	66.0	8152	64.1	8500	97% DH & 3% Cross
2	Bed stall	105.6	10002	112.5	10390	100% DH
3	Tie stall	119.9	7833	155.6	7282	71%DH % & 29% Cross
4	Tie stall	71.2	7368	69.6	7413	52%DH & 45%DJ
5	Bed stall	82.7	7284	122.5	7855	100%DJ
6	Bed stall	260.2	8270	258.9	8222	95%DH & 5%Cross
7	Tie stall	68.7	5910	70.5	5601	97%DJ & 3%Cross
8	Bed stall	81.5	8050	91.8	8211	100%DH

<sup>1</sup>Kg energy corrected milk yield (kg ECM) was estimated from the milk yield control scheme carried out in the herds 11 times per year; <sup>2</sup>For the period Oct. 1, 1999 to Sept. 30, 2000; <sup>3</sup>For the period Oct. 1, 2001 to Sept. 30, 2002; <sup>4</sup>Breed distribution at the beginning of the study. DH=Danish Holstein; DJ=Danish Jersey; Cross=Crossbred

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**Table 2.** Distribution of number of faecal samples per cow, and proportions of faecal culture positive samples per cow in total and after first positive sample, stratified by shedding group

Shedding group <sup>§</sup>	Total no. of samples					% positive samples among all samples					% positive samples after 1 <sup>st</sup> positive sample				
	Min	Q1	Q2	Q3	Max	0%	1-	26-	51-	>75%	0%	1-	26-	51-	>75%
							25%	50%	75%			25%	50%	75%	
Never	1	5	10	14	18	1512	0	0	0	0	1512	0	0	0	0
Transient	5	8	10	12	16	0	36	0	0	0	0	36	0	0	0
Intermittent	2	5	9	12	16	0	61	60	15	1	0	9	79	45	4
Low	1	5	9	13	16	0	54	42	14	33	0	0	0	2	141
High	1	4	7	10	14	0	5	20	16	37	0	0	1	3	74

Abbreviations: Min=Minimum; Q1, Q2 and Q3=1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> quartile, respectively; Max=maximum

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**Table 3.** Distribution of ELISA-results (corrected optical density (OD<sub>C</sub>) for each shedding group

MAP Shedding group	No. of cows	No. of samples	OD <sub>C</sub>				
			Min.	Q1	Q2	Q3	Max.
Never	1,512	18,838	0.00	0.00	0.00	0.06	2.58
Transient	36	785	0.00	0.00	0.00	0.13	2.25
Intermittent	137	2,092	0.00	0.00	0.03	0.41	2.58
Low	143	1,597	0.00	0.00	0.09	0.55	2.81
High	78	764	0.00	0.00	0.20	0.81	2.68
Total	1,906	24,076	0.00	0.00	0.00	0.10	2.81

Abbreviations: Min=Minimum; Q1, Q2 and Q3=1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> quartile, respectively; Max=maximum

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**Table 4.** Proportion of faecal samples and positive faecal cultures (FC) overall and after first positive ELISA within each antibody category

Antibody category	No. of cows	No. of FC	Overall positive FC (%)	Positive FC after 1 <sup>st</sup> positive ELISA (%)
A0: Always ELISA <sup>-</sup>	932	4134	114 (2.8%)	NA
A1: ELISA <sup>-</sup> , but only 1 sample tested	53	71	4 (5.6%)	NA
A2: Last 3 samples ELISA <sup>-</sup> , but previously min. 1 sample ELISA <sup>+</sup>	100	517	8 (1.5%)	4 (1.9%)
A3: Fluctuating ELISA, last sample ELISA <sup>-</sup>	263	1664	92 (5.5%)	55 (9.7%)
A4: Last sample ELISA <sup>+</sup> , previous samples ELISA <sup>-</sup>	136	674	35 (5.2%)	7 (21.2%)
A5: Last 2 or more samples ELISA <sup>+</sup>	422	2873	459 (16.0%)	360 (27.7%)
NA: Not applicable				

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369 **Figure legends**

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371 **Fig. 1.** Schematic presentation of shedding groups. Each mark represent a test-date. +~1 to  
372 9 cfu; ++~10 to 49 cfu; +++~≥50 cfu.

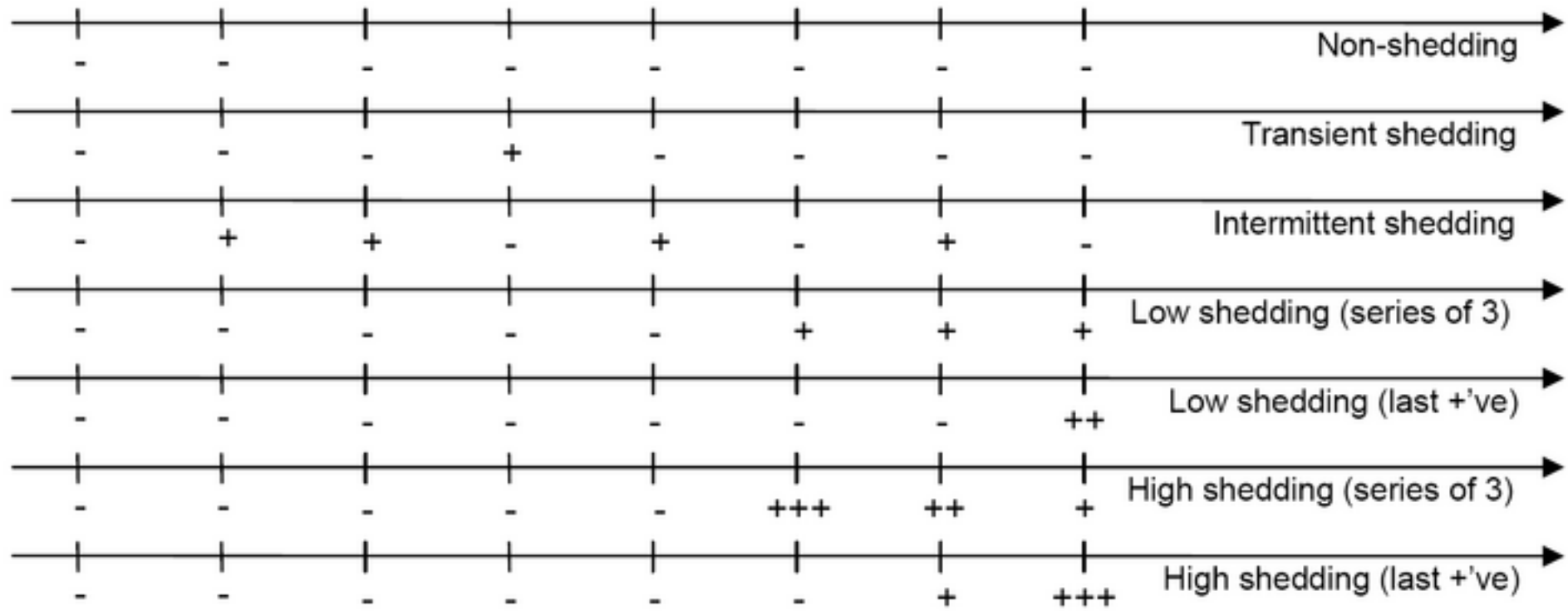
373

374 **Fig. 2.** Probability of testing positive by ELISA relative to time, where “Time” was the date of  
375 entering a shedding group. [§]For cows never shedding MAP, Time=0 was defined as the  
376 median ELISA test-date. 95% confidence bands are shown for each graph. Graphs only  
377 extent to the time for which data was available.

378

379 **Fig. 3.** Probability of testing positive by FC for cows with different ELISA profiles. “Time” = 0  
380 was the date of 1<sup>st</sup> positive ELISA for a given cow. 95% point-wise confidence bands are  
381 shown with shading around the point estimates.

Figure 1



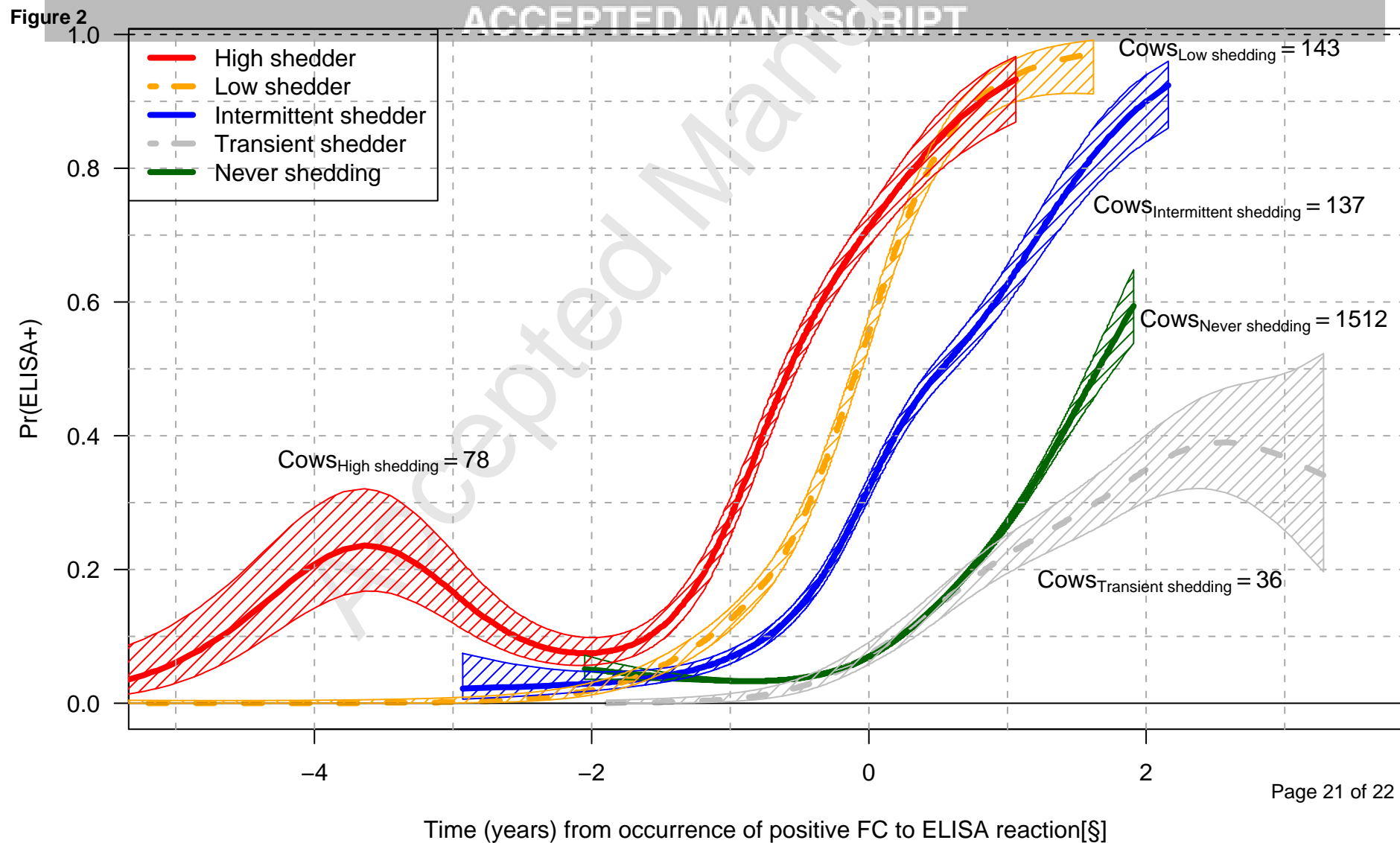


Figure 3

