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1 **EU-approved rapid tests might underestimate bovine spongiform encephalopathy**
2 **infection in goats**

3

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24

25

26 **Abstract**

27 We report on the diagnostic sensitivity of 3 EU-approved rapid tests (1 from IDEXX and 2
28 from BIO-RAD) for the detection of transmissible spongiform encephalopathy (TSE) diseases
29 in goats. Ninety-eight goat brain stem samples were tested. All of rapid tests had 100%
30 specificity and $\geq 80\%$ sensitivity with the IDEXX test significantly more sensitive than the 2
31 Bio-Rad tests. All tests detected 100% of samples from goats with clinical scrapie, but missed
32 between 7% (IDEXX) and 24% (BIORAD-SG) of samples from pre-clinical goats.
33 Importantly, only IDEXX picked up all samples from clinical BSE-infected goats, whereas
34 the other 2 rapid tests missed between 15% (BIORAD-SG) and 25% (BIORAD-SAP). These
35 results show that a fraction of pre-clinical scrapie infections are likely missed by the EU
36 surveillance, with sensitivity of detection strongly depending on the choice of the rapid test.
37 Moreover, a significant proportion of clinical BSE infections are underestimated by using
38 either BIO-RAD test. Assuming that the same sensitivity on pre-clinical goats would also
39 occur in BSE-infected goats, our data suggest that IDEXX is likely the most sensitive test for
40 detecting preclinical field cases of BSE infection in goats, though with a 7% failure rate.
41 These results raise some concerns about the reliability of current EU surveillance figures on
42 BSE infection in goats.

43

44 **Key words:** BSE; diagnosis; goats; rapid tests; scrapie; sensitivity; surveillance.

45 Prion infection induces progressive and untreatable neurodegenerative diseases in humans,
46 wild and farmed ruminants, and occasionally in other mammalian species. Prion or
47 transmissible spongiform encephalopathy (TSE) diseases are characterized by the formation
48 and accumulation of an abnormal isoform of the natural prion protein (PrP^c) in the central
49 nervous system (CNS) and, occasionally, in peripheral tissues. The pathological prion protein
50 (PrP^{Sc}) differs from PrP^c because it appears refolded, aggregated and partially protease
51 resistant. These unique features of PrP^{Sc} have been used for the development of most
52 diagnostic methods currently used for the detection of TSE diseases.

53 Scrapie disease of sheep and goats has been endemic in Europe for ≥ 200 years, but has never
54 been convincingly associated with any form of human TSE disease, although recent data
55 based on experimental transmission of scrapie to humanized mice⁴ or non-human primates⁷
56 have re-opened this issue. On the other hand, the epidemic of bovine spongiform
57 encephalopathy (BSE) in the UK and other European cattle populations has been
58 unequivocally linked to the appearance of variant Creutzfeldt-Jakob disease in humans^{2,23,5}.
59 Because BSE is experimentally transmissible to sheep and goats¹⁰ and these small ruminants
60 were likely exposed to BSE-contaminated feed in the early 1980s, there is concern that the
61 BSE agent may circulate in the sheep and goat population representing a possible secondary
62 risk to humans^{8,11}.

63 In 2006 the Commission Regulation (EC) 253/2006⁶ approved 9 rapid postmortem tests to
64 monitor the prevalence of scrapie and BSE in small ruminant populations. Sensitivity, based
65 on the lowest detectable concentration of PrP^{Sc} above background noise, and specificity were
66 assessed in classical scrapie cases. In addition, the performance of these tests for the
67 identification of atypical scrapie and BSE in sheep was also evaluated^{20,21,18,17,19}. In the frame
68 of such evaluations, only IDEXX^a, BIORAD-SAP^b and BIORAD-SG^c tests were able to
69 detect atypical scrapie, a result also confirmed by routine screening for scrapie in sheep and

70 goats^{3, 22}. In 2012, EFSA also recommended PrioSTRIP SR^d test (visual reading protocol) for
71 the detection of TSE disease in small ruminants. However, a specific study on the suitability
72 of rapid methods for the detection of TSE diseases in goats was never performed.

73 The goat population in Europe is considerably smaller than that of sheep one, but these
74 ruminants were likely highly exposed to the BSE agent because of feeding of concentrate for
75 dairy farming purposes. Thus, evaluation of surveillance system in place for the goat
76 population is crucial.

77 We compared the performance of 3 EU-approved rapid postmortem tests for active
78 surveillance of TSE diseases on brain samples from goats with ‘natural’ scrapie or goats with
79 experimental scrapie or BSE. These three rapid tests resulted 100% specific and sensitive for
80 detecting TSE diseases in sheep.

81 Ninety-eight goat brain stem samples were included in the study. All samples were prepared
82 as 50% tissue macerates in water as below. Thirty-one of these samples were sourced from
83 goats with ‘natural’ scrapie from seven different EU countries (Table 1), 7 from clinically
84 affected goats and 24 from clinically healthy animals. Other samples (n=32) from goats with
85 experimentally induced scrapie or BSE were provided by the CVI, FLI, Roslin, INRA and
86 CEA (full names in Table 1). All samples from TSE positive animals resulted also PrP^{Sc}
87 positive at western blot or immunohistochemical analyses as required by the EU Regulation
88 (EC) N. 999/2001⁹. PRNP analyses revealed that 60% of goats carried the wild genotype,
89 while in a few animals polymorphisms I142M (11%), H143R (9%), R154H (2%), R211Q
90 (23%) or repeats deletion (4%) were found in a few animals. Negative control samples were
91 from clinically healthy goats slaughtered in Italy and they were, as expected, negative by
92 Western blot analysis¹⁴. The whole brain stem sample tissue was trimmed, pooled, mildly
93 minced with a scalpel blade, until the tissue appeared homogeneous. Sterile nuclease-free
94 water was added in an equal amount (50% water/volume) to create a 1:1 dilution. The

95 suspension was subjected to cycles of homogenization using a low-speed hand-held
96 homogenizing unit until achievement of a homogeneous paste. The resulting homogenate was
97 immediately stored at -80°C and kept frozen until tested. Samples were tested by the IDEXX,
98 the BIORAD-SAP, and the BIORAD-SG ELISAs tests according to the manufacturer's test
99 instructions. The PrioSTRIP SR test was not included in this analysis. The 3 tests are based
100 on semi-quantitative ELISA methods that produce a qualitative result relative to a cut-off
101 value. The two BIORAD tests include a PK digestion step to unmask cryptic epitopes,
102 whereas the IDEXX test relies on conformational detection technology using a specific
103 proteinase resistant binding dextran polymer¹².

104 The manufacturers specifically provided a unique batch of each rapid test well before the
105 expiry dates to avoid false results produced by old, though still unexpired batches. Samples
106 were coded and then tested in duplicate except for 3 samples from Greece and 1 from the UK
107 because of insufficiently available material. The 3 rapid tests use semi-quantitative ELISA
108 methods that produce qualitative results based on cut-off values. Samples with optical density
109 lower than the cut-off value on both replicates were considered negative. Samples showing an
110 optical density greater than or equal to the cut-off value at least on one replicate were
111 considered positive. However, because the *Bio-Rad* specifications suggest a cautious
112 interpretation of samples situated just below the cut-off value (cut-off value - 10%), we
113 arbitrarily chose to consider these samples as positive. Environmental conditions that might
114 influence testing, such as temperature and humidity, were strictly controlled and monitored
115 during analytical sessions.

116 The efficiency of each rapid test was assessed by the receiver operating characteristic (ROC)
117 curve analyses (STATA 11, StataCorp LP). Nonparametric ROC curves analyzed TSE-
118 infected goats vs healthy and unaffected goats. The area under the ROC curve (AUC) and its
119 95% confidence interval (95% CI) indicate diagnostic efficiency.

120 Overall, the 3 EU-approved rapid tests analyzed showed 100% specificity and >80%
121 sensitivity (Table 2). However, ROC curves showed that the IDEXX test was significantly
122 more sensitive (97%) than the 2 BIORAD rapid tests (Table 3, 4; Figure 1A), which showed
123 sensitivity just >80%.

124 A more detailed analysis showed that all three rapid tests recognized 100% of samples from
125 goats with experimental scrapie regardless of the route of infection, but only IDEXX showed
126 100% sensitivity in detecting BSE-infected goats (Table 2, 4). The other 2 rapid tests missed
127 3 (BIORAD-SG) to 5 (BIORAD-SAP) of the 20 BSE samples (Table 2) with differences that
128 reached significance only between IDEXX and BIORAD-SAP tests (Table 4, Figure 1C).

129 In goats with natural ‘classical’ scrapie, the IDEXX test missed 2 of 29 samples and none of
130 the ‘atypical’ scrapie-infected samples; BIORAD-SAP missed 4 samples and BIORAD-SG 7
131 (a further sample gave an uncertain result, but was considered positive in the ROC curve
132 analyses) (Table 2). It is of note that the only 2 samples from asymptomatic goats, which
133 were not recognized by the IDEXX test, were also not detected by 2 two Bio-Rad tests. ROC
134 curves showed that the sensitivity of the IDEXX was significantly higher only compared to
135 the BIORAD-SG test (Table 4). Other comparisons did not show any significant differences
136 (Table 4).

137 Finally, we compared the sensitivity of rapid tests in recognizing goats with scrapie in the
138 pre-clinical or clinical phase of disease. While all rapid tests were systematically able to pick
139 up both natural and experimental scrapie samples from symptomatic goats (Table 3), IDEXX
140 missed 2 of 24 samples with ‘natural’ scrapie in the pre-clinical phase of disease, BIORAD-
141 SAP missed 4 samples, and BIORAD-SG 7 (Table 3). ROC curves analysis showed that
142 IDEXX and BIORAD-SAP were significantly more sensitive than BIORAD-SG (Table 4) in
143 detecting positive samples from pre-clinical animals.

144 Several important features of our study should be considered for the surveillance of TSE
145 diseases in goats. All tests detected 100% of samples from goats with clinical scrapie,
146 regardless of whether they were experimentally or naturally infected. In contrast, sensitivity
147 was lower in goats with pre-clinical scrapie and rapid tests missed between 7% (IDEXX) and
148 24% (BIORAD-SG) of these samples. A second important consideration is that only IDEXX
149 detected all samples from clinical BSE-infected goats, whereas the other 2 rapid tests missed
150 between 15% (BIORAD-SG) and 25% (BIORAD-SAP) of samples. These results suggest
151 that a consistent fraction of pre-clinical scrapie infections are likely missed by the EU
152 surveillance, mostly in areas where BIORAD tests are in use, and that BSE infection in goats
153 may also be underreported in areas using the BIORAD rapid tests (Table 2, 4). Assuming that
154 the same sensitivity on pre-clinical goats would also occur in BSE-infected goats, our data
155 show that the IDEXX test may detect eventual preclinical field case of BSE infection in goats,
156 though with a disappointing 7% failure rate. Although the analytical sensitivity of some TSE
157 rapid tests might be reduced by the method used to prepare our samples^{16,1}, the results raise
158 some concerns in relation to the current figures on BSE infections in goats deriving from EU
159 surveillance.

160 In goats, the difference in performance of rapid tests between scrapie and BSE infection
161 might depend on the use of proteinase K (PK) digestion, the choice of the primary anti-PrP
162 antibodies, or both. Interestingly, PK digestion is used by both BIORAD tests but not by
163 IDEXX and is likely that antibodies used in each kit recognize different PrP epitopes. This
164 last hypothesis, however, is purely speculative because the details on anti-PrP antibodies are
165 covered by patents and are therefore not publicly unavailable.

166 The other interesting result, though based solely on 2 samples, is that only IDEXX and
167 BIORAD-SAP were able to fully recognize samples from goats with the atypical Nor98
168 scrapie infection suggesting that the in place surveillance system in countries using the

169 BIORAD-SG test would miss a proportion of atypical scrapie infections in the goat
170 population. The small number of samples, however, is too low to allow a firm conclusion.
171 All rapid tests in this study failed to recognize the same 2 samples of ‘natural’ preclinical
172 scrapie. This finding is somewhat of concern because it might indicate that there is a small
173 subpopulation of ‘naturally’ scrapie-infected goats (e.g. early pre-clinical animals) that would
174 be missed by all available rapid tests, and thus by the surveillance system. *PRNP*
175 polymorphisms might reduce the sensitivity of the assays in goats carrying specific genotypes
176 by reducing antibody binding epitopes^{15,4}. In our samples, however, statistical analysis did not
177 show any association between failure of each test and goat genotypes (data not shown). The
178 reason for this finding remains therefore unknown and might simply depend on low levels of
179 PrP^{Sc}.
180 Ultimately, none of the three rapid tests picked up any false positives showing a reassuring
181 100% specificity.

182

183 **Sources and manufactures**

- 184 a. *IDEXX HerdChek*® *BSE-scrapie* Antigen Test Kit, EIA. IDEXX Laboratories,
185 Westbrook, ME, USA.
- 186 b. *Bio-Rad*® *TeSeETM SAP* Purification-Detection Test Kit, Bio-Rad Laboratories, Marnes-
187 La-Coquette, France.
- 188 c. *Bio-Rad*® *TeSeETM Sheep/Goat* Purification-Detection Test Kit, Bio-Rad Laboratories,
189 Marnes-La-Coquette, France.
- 190 d. *Prionics*® - *Check PrioSTRIP SR* Prionics AG, Wagistrasse 27A Schlieren-Zürich, CH
191 8952 Switzerland.

192

193 **Declaration of conflicting interests**

194 The author(s) declare no potential conflicts of interest with respect to the research, authorship,
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196

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201

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268 **Table 1.** Details and origin of goat samples used in the study.

Disease	Type	County of origin (Institute^o)	n
Natural scrapie	Classical	Cyprus	3
		France	5
		Greece	4
		Italy	9
		Netherlands	3
		Spain	2
	UK	3	
	Atypical (Nor98)	Italy	2
TOTAL			31
Experimental scrapie	Classical	Italy (CEA)	5
		France (INRA)	1
		France (INRA)	6
TOTAL			12
Experimental BSE	Classical	France (INRA)	1
		Netherlands (CVI)	6
		France (INRA)	4
		France (INRA)	1
		Netherlands (CVI)	4
		Germany (FLI)	3
		UK (Roslin)	1
TOTAL			20
TOTAL TSE diseases			63
Negative controls	Healthy	Italy	35

270 °INRA, Institut national de la recherche agronomique, France; CVI, Central Veterinary
271 Institute, The Netherlands; FLI, Friedrich-Loeffler-Institut, Germany; CEA, Centro di
272 referenza nazionale per lo studio e le ricerche sulle encefalopatie animali e neuropatologie
273 comparate, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin,
274 Italy; Roslin, The Roslin Institute, University of Edinburgh, UK.
275

276 **Table 2.** Number and percentage of positive samples in goats with different forms of TSE
 277 diseases by different rapid tests

Disease	Type	Inoculum	n	Positive test, n (%)		
				IDEXX	BIORAD SG	BIORAD SAP
Natural scrapie	Classical	-	29	27 (93.1)	22° (75.9)	25 (86.2)
	Atypical (Nor98)	-	2	2 (100)	1 (50.0)	2 (100)
Experimental scrapie	Classical	Scrapie	12	12 (100)	12 (100)	12 (100)
Experimental BSE	Classical	Bovine BSE	20	20 (100)	17 (85.0)	15 (75.0)
TOTAL TSE diseased			63	61 (96.8)	52° (82.5)	54 (85.7)
Negative controls	Healthy	-	35	0 (0.0)	0 (0.0)	0 (0.0)

278 °One sample gave uncertain result

279 **Table 3.** Number and percentage of positive samples by different tests on ‘natural scrapie’
 280 affected goats

Disease	Type	Clinical signs	n	Positive test, n (%)		
				IDEXX	BIORAD SG	BIORAD SAP
Natural scrapie	Classical	No	22	20 (90.9)	15° (68.2)	18 (81.8)
	Atypical (Nor98)	No	2	2 (100)	1 (50)	2 (100)
TOTAL			24	22 (91.7)	16 (66.6)	20 (83.3)
Natural scrapie	Classical	Yes	7	7 (100)	7 (100)	7 (100)
Negative controls	Healthy	No	35	0	0	0

281 °One sample gave uncertain result

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Table 4. ROC curve analyses

	Goats with natural and experimental TSEs (n= 63) vs. controls (n=35)	Goats with natural classical scrapie (n=29) vs. controls (n=35)	Goats with experimental BSE (n=20) vs. controls (n=35)	Goats with experimental scrapie (n=12) vs. controls (n=35)	Goats with TSE with no clinical signs (n=24) vs. controls (n=35)
Diagnostic tests	AUC (95% CI)	AUC (95% CI)	AUC (95% CI)	AUC (95% CI)	AUC (95% CI)
IDEXX	0.9841 (0.96231-1.0000)	0.9655 (0.91859-1.0000)	1.0000 (1.00000-1.00000)	1.0000 (1.00000-1.00000)	0.9583 (0.90186-1.0000)
BIORAD SG	0.9127 (0.86545-0.95995)	0.8793 (0.8006-0.95856)	0.9250 (0.84472- 1.00000)	1.0000 (1.00000-1.00000)	0.8333 (0.73701-0.92966)
BIORAD SAP	0.9286 (0.88502-0.97212)	0.9310 (0.86717- 0.99490)	0.8750 (0.77765-0.97235)	1.0000 (1.00000-1.00000)	0.9167 (0.84051-0.99282)
	p value	p value	p value	p value	p value
IDEXX vs. BIORAD SG	0.0013	0.0157	0.0671	=	0.0056
IDEXX vs. BIORAD SAP	0.0054	0.1498	0.0118	=	0.1482
BIORAD SG vs. BIORAD SAP	0.5291	0.0723	0.4183	=	0.0320

