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Claudia Reckzeh, Christine Hoffmann, Anne Buschmann, Silke Buda, Klaus-Dieter Budras, Karl-Friedrich Reckling, Steffi Bellmann, Hartmut Knobloch, Georg Erhardt, Reinhard Fries, et al.

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1 **Rapid testing leads to the underestimation of the scrapie prevalence in an affected sheep**  
2 **and goat flock**

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5

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22

## 22 **Abstract**

23 To obtain a more detailed understanding of the prevalence of classical scrapie  
24 infections in a heavily affected German sheep flock (composed of 603 sheep and 6 goats), we  
25 analysed 169 sheep and 6 goats that carried the genotypes susceptible to the disease and that  
26 were therefore culled following discovery of the index case. The initial tests were performed  
27 using the Biorad TeSeE ELISA and reactive results were verified by official confirmatory  
28 methods (OIE-immunoblot and/or immunohistochemistry (IHC)) to demonstrate the  
29 deposition of scrapie-associated PrP<sup>Sc</sup> in the brain stem (obex). This approach led to the  
30 discovery of 40 additional subclinically scrapie-infected sheep. Furthermore, peripheral  
31 lymphatic tissue samples of the 129 sheep and six goats with a negative CNS result were  
32 examined by IHC in order to identify any preclinical infections which had not already spread  
33 to the central nervous system (CNS). Using this approach we found 13 additional sheep with  
34 PrP<sup>Sc</sup> depositions in the gut-associated lymph nodes (GALT) as well as in the enteric nervous  
35 system. Moreover, in most of these cases PrP<sup>Sc</sup> was also deposited in the spleen and in the  
36 retropharyngeal and superficial cervical lymph nodes. Taken together, these results show a  
37 30.3% infection prevalence in this scrapie-affected flock. Almost 7.4% of the infected animals  
38 harboured PrP<sup>Sc</sup> exclusively in the peripheral lymphatic tissue and were therefore missed by  
39 the currently used testing strategy.

40

41 *Keywords:* preclinical scrapie, immunohistochemistry, lymphoid tissue

42

## 43 **1. Introduction**

44 Scrapie belongs to the transmissible spongiform encephalopathies (TSE) of sheep and  
45 goats and is characterised by the accumulation of an abnormally folded isoform (PrP<sup>Sc</sup>) of the  
46 cellular prion protein (PrP<sup>C</sup>). Incubation times vary between a few months and several years  
47 depending on the infectious dose and route, the particular scrapie strain and the genotype and

48 age of the recipient animal (summarised in Detwiler and Baylis, 2003) According to the prion  
49 hypothesis (Prusiner et al., 1982), abnormally folded PrP<sup>Sc</sup> represents the causative infectious  
50 agent itself, the 'prion'. However, the existence of different strains remains as yet  
51 unexplained. PrP<sup>Sc</sup> is partially protease resistant, insoluble in detergents (as a consequence of  
52 an increased  $\beta$ -sheet content which increases its hydrophobicity) and forms scrapie-associated  
53 fibrils (Diringer et al., 1983; Hope et al., 1986; Oesch et al., 1985). These criteria form the  
54 basis for the demonstration of PrP<sup>Sc</sup> as a diagnostic marker.

55 Amino acid polymorphisms at positions 136, 154 and 171 of the prion protein  
56 determine the susceptibility of sheep to classical scrapie. Alleles on the PrP gene encoding  
57 alanine/arginine/arginine (PrP<sup>ARR</sup>) are associated with the lowest level of susceptibility,  
58 alleles encoding valine/arginine/glutamine (PrP<sup>VRQ</sup>) with the highest susceptibility,  
59 particularly if present in homozygous animals (Goldmann et al., 1990; Hunter, 1996; Hunter  
60 et al., 1997). The PrP genotype of sheep has a strong effect on the scrapie pathogenesis in  
61 sheep. While no, or only minor, traces of PrP<sup>Sc</sup> depositions can be found in the peripheral  
62 lymphatic tissues (e.g., spleen, tonsils, retropharyngeal lymph nodes, gut-associated lymph  
63 nodes) of sheep carrying at least one PrP<sup>ARR</sup> allele, PrP<sup>VRQ/VRQ</sup> and PrP<sup>ARQ/ARQ</sup> carriers can  
64 show PrP<sup>Sc</sup> accumulations in these lymphatic tissues as early as a few months after the  
65 infection (van Keulen et al., 1996 & 2002; Andreoletti et al., 2000; Jeffrey et al., 2002; Ersdal  
66 et al., 2005). Preclinical accumulation of PrP<sup>Sc</sup> in lymphoreticular tissues of sheep therefore  
67 provides an opportunity for an *in-vivo* diagnosis based on biopsy samples of the tonsils  
68 (Schreuder et al., 1998), the third eyelid (O'Rourke et al., 2002) or the rectum (Gonzalez et  
69 al., 2006; Espenes et al., 2006).

70 The gastrointestinal tract, in particular the gut-associated lymphoid tissues (GALT), is  
71 regarded as the major route of entry for natural scrapie (Hadlow et al., 1982; Heggebo et al.,  
72 2000). The earliest accumulation of PrP<sup>Sc</sup> can be found in the ileal Peyer's patches (IPP) and  
73 in the draining mesenteric lymph nodes (Andreoletti et al., 2000). From there, prions may

74 spread to other lymphatic tissues, which are not part of the GALT, resulting in a widespread  
75 distribution of disease-specific PrP deposition in the lymphoreticular system at relatively  
76 early stages of infection (Andreoletti et al., 2000; Heggebo et al., 2002; van Keulen et al.,  
77 2002). Neuroinvasion appears to start at the enteric nervous system (ENS) followed by a  
78 centripetal and retrograde spread via sympathetic and parasympathetic efferent fibres of the  
79 autonomic nervous system to the spinal cord and to the medulla oblongata (van Keulen et al.,  
80 2000).

81 The aim of the study was to analyse the prevalence of preclinical scrapie in a cohort of  
82 susceptible sheep that were compulsorily culled after the detection of scrapie in the flock. For  
83 this purpose LRS and the peripheral nervous system samples were examined with particular  
84 attention paid to animals which were tested negative in the initial rapid test on the brain stem.

85

## 86 **2. Materials and Methods**

### 87 *2.1. Sheep and goats*

88 All 169 German Blackheaded Mutton-Merino crossbreed sheep and 6 fawn goats  
89 belonged to a single flock (n=603) in Saxony-Anhalt in which an outbreak of classical scrapie  
90 occurred in December 2003.

91

### 92 *2.2. TSE rapid testing*

93 Brain stem samples from the obex region were examined using the Biorad TeSeE  
94 ELISA rapid test (Biorad, Munich, Germany) following the manufacturer's instructions.

95

### 96 *2.3. Genotyping*

97 Sheep were genotyped as described previously (Lühken et al., 2004).

98

### 99 *2.4. Histopathology and immunohistochemistry*

100 With some modifications, tissue samples were processed as described previously  
101 (Hardt et al., 2000). In short, all tissue samples were fixed in 4% buffered formalin, treated  
102 for one hour with 98% formic acid, rinsed for 40 minutes in tap water, embedded in paraffin,  
103 sectioned at 3-4  $\mu\text{m}$  and stained with haematoxylin and eosin.

104 The avidin-biotin-complex (ABC) method was used for PrP-IHC. This involved the  
105 paraffin wax tissue sections being mounted on superfrost plus slides (Menzel-Gläser,  
106 Braunschweig, Germany) and rehydrated. The subsequent pretreatment included incubation  
107 of the slides in 98% formic acid for 15 minutes, a 5-minute rinse in tap water, inhibition of the  
108 endogenous peroxidase activity with 3%  $\text{H}_2\text{O}_2$  (Merck, Darmstadt, Germany) in methanol for  
109 30 minutes, followed by 15 minutes digestion with proteinase K (4 $\mu\text{g}/\text{ml}$ , Boehringer,  
110 Mannheim, Germany) at 37 °C. The primary monoclonal antibody (mab) L 42, which binds to  
111 an epitope of amino acid 145-163 of ovine PrP, was applied at a dilution of 1:250 (Harmeyer  
112 et al., 1998) in Tris-buffered saline (TBS) containing 10 % goat serum and incubated  
113 overnight at 4°C. Negative control sections were treated with a monoclonal antibody against  
114 GP<sub>5</sub> of the Porcine Respiratory and Reproductive Syndrome virus (Weiland et al., 1999). As a  
115 secondary (link) antibody, biotinylated goat anti-mouse antiserum (Vector Laboratories,  
116 Burlingame, CA, USA) was incubated on the sections in a 1:200 dilution for 30 minutes at  
117 room temperature. Immunodetection was amplified using Vector ABC-elite avidin-  
118 horseradish peroxidase/biotin complex (Vector laboratories; Peterborough, UK). The slides  
119 were finally developed in DAB (diaminobenzidintetrahydrochloride) (Fluka Feinchemikalien,  
120 Neu Ulm, Germany) and counterstained with Mayer's haematoxylin. All sections were  
121 examined using light microscopy.

122 All positive results were verified using mab 2G11 binding to the epitope 146-182 of  
123 ovine PrP (Institut Pourquier, Montpellier, France) diluted 1:250 in TBS with 10 % goat  
124 serum.

125

126 **3. Results**

127 All 175 animals examined in this study came from a flock of 603 German Blackhead  
128 Mutton-Merino crossbreed sheep and six fawn goats which was kept in Saxony-Anhalt in  
129 Germany. The outbreak of classical scrapie was first recognised through TSE rapid testing in  
130 two fallen stock sheep. After the flock was quarantined and monitored, 10 more clinical cases  
131 of scrapie were identified. According to the EU 999/2001 regulation, all animals in the flock  
132 were genotyped with regard to codons 136, 154 and 171 of the sheep PrP gene and all  
133 genetically highly TSE-susceptible animals (169 sheep and six goats) were killed. The  
134 necropsy was carried out immediately after the death of the animals and a wide range of  
135 tissues of the central and peripheral nervous system as well as of the lymphoreticular system  
136 (LRS) was taken. Obex samples from these animals were subsequently TSE rapid tested using  
137 the Biorad TeSeE assay. Reactive results were obtained in another 40 of the 169 sheep and  
138 the TSE diagnosis in these cases was confirmed by using the OIE- approved methods, SAF-  
139 immunoblot and/or IHC. Table 1 shows an overview of relevant data of all sheep investigated.

140 All 135 animals with a negative rapid test result on the obex region were more closely  
141 examined by IHC to detect PrP<sup>Sc</sup> accumulations in the peripheral nervous and lymphoreticular  
142 system using a hierarchical approach, as illustrated in Figure 1. In a first step the five most  
143 likely sites of entry for the agent were analysed. In cases where there was a total absence of  
144 PrP<sup>Sc</sup> depositions in the samples, the animals were considered to be completely negative and  
145 no further studies were undertaken. However, if there was a positive result in one of the five  
146 tissues, the investigation was extended to several parts of the peripheral nervous system as  
147 well as to the lymphoreticular system.

148 PrP<sup>Sc</sup> was not found to be present in any of the initially investigated tissues of the six  
149 goats. However, PrP<sup>Sc</sup> was found to be present in the lymphoreticular system of 13 of the 129  
150 investigated sheep. Table 2 summarises the results for all animals with a clear PrP<sup>Sc</sup>-  
151 immunolabelling.



152 PrP<sup>Sc</sup> was discovered in the lymphoreticular system in most animals and in particular  
153 in the head-associated lymph nodes (i.e., tonsils, retropharyngeal or mandibular lymph  
154 nodes). Interestingly, two sheep showed a strong staining reaction in these non-GALT  
155 lymphoid tissues (Figure 2C), but not in the GALT. Additionally, a PrP<sup>Sc</sup> accumulation in the  
156 lymphoid follicles of the third eyelid was only apparent in the two sheep with the most  
157 widespread PrP<sup>Sc</sup> distribution in the lymphoreticular system. As the rectum samples examined  
158 did not totally comply with the proposed diagnostic requirements (i.e. too low a number of  
159 follicles), the absence of PrP<sup>Sc</sup> staining in any of the preclinically affected sheep at this site  
160 should be interpreted cautiously.

161 Only a sparse immunolabelling was found in the peripheral nervous system. While 11  
162 sheep showed a strong PrP<sup>Sc</sup> immunolabelling in the enteric nervous system of the distal  
163 ileum (Fig. 2A), only six of these sheep showed a detectable amount of PrP<sup>Sc</sup> in the coeliac  
164 and mesenteric ganglion complex (Fig. 2B). Three of these sheep showed an additional PrP-  
165 immunostaining in the ENS of the rectum. However, no PrP<sup>Sc</sup> accumulation was  
166 demonstrated in the Ganglion cervicale craniale, Ganglion stellatum and the Vagus nerve.

167 The preclinical scrapie-infected sheep carried either the PrP<sup>ARQ/ARQ</sup> (9 cases) or the  
168 PrP<sup>ARQ/AHQ</sup> (4 cases) genotype. There was no obvious correlation between the number of  
169 positive tissues and the age of the animal or between the quantity of accumulated PrP<sup>Sc</sup> and  
170 the genotype of the sheep. Although a widespread distribution of PrP<sup>Sc</sup> was detected in most  
171 animals, none of the 13 sheep was positive in every single sample.

172

#### 173 4. Discussion

174 In this study 169 sheep carrying the susceptible genotypes and six goats out of a flock,  
175 which underwent a selective culling to eradicate scrapie, were analysed. This investigation  
176 revealed that 30% of these animals were preclinically scrapie-infected and that 7.4% of them  
177 were PrP<sup>Sc</sup>-positive only at the peripheral sites but not in the brain stem, which is routinely

178 tested. This prevalence is within the range of 5% to 30.3% previously reported in similar  
179 studies (Jeffrey et al., 2002; Thorgeirsdottir et al. 2002; Ersdal et al. 2003; Vascellari et al.  
180 2005; Gonzalez et al., 2006). The classical scrapie prevalence in a flock depends on different  
181 factors: a) length of time period after the introduction of the infection into the flock; b)  
182 husbandry measures (e.g. postpartal removal of the placentas as the most infectious tissues,  
183 separation of lambing ewes, frequency of pasture changes, general hygiene measures); c)  
184 genetic status (in terms of scrapie susceptibility) of the animals in the flock; d) differences in  
185 the scrapie strains (Andreoletti et al., 2001; Jeffrey et al., 2001).

186         The origin of the scrapie infection in this flock, in which the oldest scrapie-infected  
187 sheep was born in 1998, is unknown. However, the large number of scrapie-infected sheep  
188 may reflect the fact that this flock has had a long scrapie history which was possibly started  
189 by the introduction of an infected animal from the outside. In this regard it is of particular  
190 interest that most of the sheep with a positive rapid test result are two years or younger, which  
191 corresponds to the reported changes in the age-genotype profile in flocks which have been  
192 infected for several years (Baylis et al., 2000). The last acquisition of ewes took place in  
193 1994, while rams were restocked from other flocks for many years. Interestingly, scrapie was  
194 diagnosed in a neighbourhood flock in 1979/1980, but the eradication measures taken at this  
195 time were not fully recorded. Little is known about the husbandry measures which were  
196 applied in this particular scrapie-affected flock.

197         In our study, all affected animals were either PrP<sup>ARQ/ARQ</sup> or PrP<sup>AHQ/ARQ</sup> carriers and no  
198 differences in the distribution of PrP<sup>Sc</sup> were found between these groups. These results are in  
199 contrast to previous studies carried out in Scotland (Jeffrey et al., 2002) and Iceland  
200 (Thorgeirsdottir et al., 2002) in which these genotypes seemed to be more resistant to a  
201 scrapie infection. However, the results reported here are in accordance with the results from  
202 an outbreak in Italy (Vascellari et al., 2005). The most probable explanation for this difference  
203 is that scrapie strains with a bias towards different genotypes had been going round the flocks

204 in question. All seven PrP<sup>ARR</sup> heterozygous sheep were negative in our study. Although most  
205 of the sheep were at least four years or older (5/6), the low number of animals with this semi-  
206 resistant genotype does not allow us to exclude that the scrapie agent targets genotypes with  
207 an age-dependent lower prevalence as shown in a previous study (Gonzalez et al., 2006).

208 All preclinically scrapie-infected sheep showed a marked and widespread distribution  
209 of PrP<sup>Sc</sup> throughout the lymphoreticular system and in some parts of the peripheral nervous  
210 system. However, none of the animals was positive in every tissue sampled. In accordance  
211 with several studies most animals (n=11) showed an accumulation of PrP<sup>Sc</sup> in the Peyer's  
212 patches of the distal ileum and/or the draining lymph nodes, suggesting the intestine as the  
213 port of entry for the agent (Heggebo et al., 2000; van Keulen et al., 2002). Additionally, six of  
214 the sheep showed a clear staining reaction in the enteric nervous system as well as in the  
215 CMGC, indicating the subsequent spread to the CNS (van Keulen et al., 2000). In contrast, in  
216 two sheep (5, 11) no detectable amount of PrP<sup>Sc</sup> was found in the intestine and the mesenteric  
217 lymph nodes. One possible explanation could be a direct neuroinvasion (Jeffrey et al., 2002).  
218 However, none of the neuronal tissues examined contained PrP<sup>Sc</sup> depositions, whereas a  
219 strong immunostaining was seen in the tonsil and in the Ln. retropharyngealis lateralis.  
220 Therefore the oronasal cavity may have served as a port of entry in these two sheep.

221 The current EU strategy for the active and passive surveillance of sheep TSEs is based  
222 on the testing of CNS samples. The results of the present study reveal that a better estimation  
223 of the "real" prevalence of scrapie-infected sheep can be obtained when LRS samples are  
224 examined as well.

225 However, it must be noted that a diagnostic examination of the LRS alone (as  
226 suggested by Gonzalez et al., 2006) would lead to studies missing the large number of  
227 atypical scrapie cases which lack a PrP<sup>Sc</sup> dissemination in the LRS (Gavier-Widen et al.,  
228 2005). The same applies for sheep carrying the PrP<sup>ARR</sup> haplotype. Therefore a combination of

229 CNS and LRS testing is necessary to detect all clinical and preclinically scrapie-infected  
230 animals in affected flocks.

231

## 232 **5. Conclusion**

233 In conclusion, this study shows that a negative result of the rapid test on brain stem  
234 samples does not exclude the presence of PrP<sup>Sc</sup> in peripheral tissues. In consequence, the  
235 actual prevalence of preclinical scrapie is significantly underestimated when it is only based  
236 on studies using brain stem samples.

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239 **References**

240

241 Andreoletti, O., Berthon, P., Marc, D., Sarradin, P., Grosclaude, J., van Keulen, L., Schelcher,  
242 F., Elsen, J.M., Lantier, F., 2000. Early accumulation of PrP(Sc) in gut-associated  
243 lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural  
244 scrapie. *J. Gen. Virol.* 81, 3115-3126.

245 Baylis, M., Houston, F., Goldmann, N., Hunter, N., McLean, A.R., 2000. The signature of  
246 scrapie: differences in the PrP genotype profile of scrapie affected and scrapie-free  
247 UK sheep flocks. *Proc. R. Soc. Lond. B* 267, 2029-2035.

248 Detwiler, L.A., Baylis, M., 2003. The epidemiology of scrapie. *Rev. Sci. Tech.* 22, 121-143.

249 Diringer, H., Hilmert, H., Simon, D., Werner, E., Ehlers, B., 1983. Towards purification of  
250 the scrapie agent. *Eur. J. Biochem.* 134, 555-560.

251 Ersdal, C., Ulvund, M.J., Benestad, S.L., Tranulis, M.A., 2003. Accumulation of pathogenic  
252 prion protein (PrP<sup>Sc</sup>) in nervous and lymphoid tissues of sheep with subclinical  
253 scrapie. *Vet. Pathol.* 40, 164-174.

254 Ersdal, C., Ulvund, M.J., Espenes, A., Benestad, S.L., Sarradin, P., Landsverk, T., 2005.  
255 Mapping PrP<sup>Sc</sup> propagation in experimental and natural scrapie in sheep with  
256 different PrP genotypes. *Vet. Pathol.* 42, 258-274.

257 Espenes, A., Press, C.M., Landsverk, T., Tranulis, M.A., Aleksandersen, M., Gunnes, G.,  
258 Benestad, S.L., Fuglesteit, R., Ulvund, M.J., 2006. Detection of PrP<sup>Sc</sup> in Rectal  
259 Biopsy and Necropsy Samples from Sheep with Experimental Scrapie. *J. Comp.*  
260 *Pathol.* 134, 115-125.

261 Gavier-Widen, D., Stack, M.J., Baron, T., Balachandran, A., Simmons, M., 2005. Diagnosis  
262 of transmissible spongiform encephalopathies in animals: a review. *J. Vet. Diagn.*  
263 *Invest.* 17, 509-527.

- 264 Goldmann, W., Hunter, N., Foster, J.D., Salbaum, J.M., Beyreuther, K., Hope, J., 1990. Two  
265 alleles of a neural protein gene linked to scrapie in sheep. *Proc. Natl. Acad. Sci.*  
266 *U.S.A.* 87, 2476-2480.
- 267 Gonzalez, L., Dagleish, M.P., Bellworthy, S.J., Siso, S., Stack, M.J., Chaplin, M.J., Davis,  
268 L.A., Hawkins, S.A., Hughes, J., Jeffrey, M., 2006. Postmortem diagnosis of  
269 preclinical and clinical scrapie in sheep by the detection of disease-associated PrP in  
270 their rectal mucosa. *Vet. Rec.* 158, 325.
- 271 Hadlow, W.J., Kennedy, R.C., Race, R.E., 1982. Natural infection of Suffolk sheep with  
272 scrapie virus. *J. Infect. Dis.* 146, 657-664.
- 273 Hardt, M., Baron, T., Groschup, M.H., 2000. A comparative study of immunohistochemical  
274 methods for detecting abnormal prion protein with monoclonal and polyclonal  
275 antibodies. *J. Comp. Path.* 122, 43-53.
- 276 Harmeyer, S., Pfaff, E., Groschup, M.H., 1998. Synthetic peptide vaccines yield monoclonal  
277 antibodies to cellular and pathological prion proteins of ruminants. *J. Gen. Virol.* 79,  
278 937-945.
- 279 Heggebo, R., Press, C.M., Gunnes, G., Lie, K.I., Tranulis, M.A., Ulvund, M., Groschup,  
280 M.H., Landsverk, T., 2000. Distribution of prion protein in the ileal Peyer's patch of  
281 scrapie-free lambs and lambs naturally and experimentally exposed to the scrapie  
282 agent. *J. Gen. Virol.* 81, 2327-2337.
- 283 Heggebo, R., Press, C., Gunnes, G., Gonzalez, L., Jeffrey, M., 2002. Distribution and  
284 accumulation of PrP in gut-associated and peripheral lymphoid tissue of scrapie-  
285 affected Suffolk sheep. *J. Gen. Virol.* 83, 479-489.
- 286 Hope, J., Morton, L.J., Farquhar, C.F., Multhaup, G., Beyreuther, K., Kimberlin, R.H., 1986.  
287 The major polypeptide of scrapie-associated fibrils (SAF) has the same size, charge  
288 distribution and N-terminal protein sequence as predicted for the normal brain protein  
289 (PrP). *EMBO J.* 5, 2591-2597.

- 290 Hunter, N. 1996. Genotyping and susceptibility of sheep to scrapie. In: Baker, H.F., Ridley,  
291 R.M. (Eds.), Prion diseases, Humana Press Inc., Totowa, New Jersey, pp. 211-222.
- 292 Hunter, N., Goldmann, W., Foster, J.D., Cairns, D., Smith, G., 1997. Natural scrapie and PrP  
293 genotype: case-control studies in British sheep. *Vet. Rec.* 141, 137-140.
- 294 Jeffrey, M., Martin, S., Thomson, J.R., Dingwall, W.S., Begara-McGorum, I., Gonzalez, L.,  
295 2001. Onset and distribution of tissue PrP accumulation in scrapie-affected Suffolk  
296 sheep as demonstrated by sequential necropsies and tonsillar biopsies. *J. Comp.*  
297 *Pathol.* 125, 48-57.
- 298 Jeffrey, M., Begara-McGorum, I., Clark, S., Martin, S., Clark, J., Chaplin, M., Gonzalez, L.,  
299 2002. Occurrence and distribution of infection-specific PrP in tissues of clinical  
300 scrapie cases and cull sheep from scrapie-affected farms in Shetland. *J. Comp. Pathol.*  
301 127, 264-273.
- 302 Lühken, G., Buschmann, A., Groschup, M.H., Erhardt, G., 2004. Prion protein allele  
303 A136H154Q171 is associated with high susceptibility to scrapie in purebred and  
304 crossbred German Merionland sheep. *Arch. Virol.* 149, 1571-1580.
- 305 Oesch, B., Westaway, D., Walchli, M., McKinley, M.P., Kent, S.B., Aebersold, R., Barry,  
306 R.A., Tempst, P., Teplow, D.B., Hood, L.E., 1985. A cellular gene encodes scrapie  
307 PrP 27-30 protein. *Cell* 40, 735-746.
- 308 Prusiner, S.B., Bolton, D.C., Groth, D.F., Bowman, K.A., Cochran, S.P., McKinley, M.P.,  
309 1982. Further purification and characterization of scrapie prions. *Biochemistry* 21,  
310 6942-6950.
- 311 Schreuder, B.E., van Keulen, L.J., Vromans, M.E., Langeveld, J.P., Smits, M.A., 1998.  
312 Tonsillar biopsy and PrPSc detection in the preclinical diagnosis of scrapie. *Vet. Rec.*  
313 142, 564-568.
- 314 Thorgeirsdottir, S., Georgsson, G., Reynisson, E., Sigurdarson, S., Palsdottir, A., 2002.  
315 Search for healthy carriers of scrapie: an assessment of subclinical infection of sheep

- 316 in an Icelandic scrapie flock by three diagnostic methods and correlation with PrP  
317 genotypes. *Arch. Virol.* 147, 709-722.
- 318 van Keulen, L.J., Schreuder, B.E., Meloen, R.H., Mooij-Harkes, G., Vromans, M.E.,  
319 Langeveld, J.P., 1996. Immunohistochemical detection of prion protein in lymphoid  
320 tissues of sheep with natural scrapie. *J. Clin. Microbiol.* 34, 1228-1231.
- 321 van Keulen, L.J., Vromans, M.E., van Zijderveld, F.G., 2002. Early and late pathogenesis of  
322 natural scrapie infection in sheep. *APMIS* 110, 23-32.
- 323 van Keulen, L.J., Schreuder, B.E., Vromans, M.E., Langeveld, J.P., Smits, M.A., 2000.  
324 Pathogenesis of natural scrapie in sheep. *Arch. Virol. Suppl.* 16, 57-71.
- 325 Vascellari, M., Aufiero, G.M., Nonno, R., Agrimi, U., Vaccai, G., Basilicata, L., Falcaro, C.,  
326 Mancin, S., Marcon, S., Mulinelli, F., 2005. Diagnosis and PrP genotype target of  
327 scrapie in clinically healthy sheep of Massese breed in the framework of a scrapie  
328 eradication programme. *Arch. Virol.* 150, 1959-1976.
- 329 Weiland, E., Wiczorek-Krohmer, M., Kohl, D., Conzelmann, K.K., Weiland, F., 1999.  
330 Monoclonal antibodies to the GP5 of porcine reproductive and respiratory syndrome  
331 virus are more effective in virus neutralization than monoclonal antibodies to the GP4.  
332 *Vet. Microbiol.* 66, 171-186.
- 333



**Table 1:** Numbers, genotypes and age of all culled sheep and goats

	Total number	Number of sheep with genotypes						Age (in years)*				
		ARQ/ARR	ARQ/ARQ	ARQ/AHQ	AHQ/ARQ	VRQ/ARQ	VRQ/ARR	0-1	1-2	2-3	3-4	> 4
<b>Obex positive</b>	40	--	38	--	2	--	--	13	16	2	3	0
<b>Obex negative</b>												
<b>sheep</b>	129	4	105	13	--	4	3	23	24	39	11	9
<b>goats</b>	6	--	--	--	--	--	--	unknown				

\* 29 sheep of unknown age

**Table 2:** Immunohistochemical results relative to the distribution of PrP<sup>Sc</sup> in the different tissues of 13 sheep

Sheep	IPP	Lnn. ileo- colici	RPLN	Tonsil	3rd eyelid	Ln. Spleen cerv. superf.	ENS Ileum	ENS Rectum	CMGC	Ggl. cerv. cran.	Ggl. stella- tum	N. vagus	Tongue	PrP genotype	Age (in years)
1	+	-	+ <sup>1*</sup>	+	-	+	+	-	-	-	-	-	-	ARQ/ARQ	>4
2	-	+	+ <sup>1*</sup>	+	-	+	+	-	-	-	-	-	-	ARQ/ARQ	>4
3	+	+	+	+	+	+	+	+	-	+	-	-	-	ARQ/ARQ	2-3
4	+	-	-	-	-	-	-	-	-	-	-	-	-	ARQ/ARQ	2-3
5	-	-	+	+	-	+	+	-	-	-	-	-	-	ARQ/ARQ	2-3
6	+	+	-	-	-	+	-	+	+	+	-	-	-	ARQ/ARQ	1-2
7	+	+	+	+	+	+	+	+	+	+	-	-	-	ARQ/ARQ	1-2
8	-	+	+	+	-	-	+	-	-	-	-	-	-	ARQ/ARQ	0-1
9	-	+	+	+	-	+	+	-	-	-	-	-	-	ARQ/ARQ	0-1
10	+	+	+	+	-	+	+	+	-	+	-	-	-	AHQ/ARQ	1-2
11	-	-	+	+	-	-	-	-	-	-	-	-	-	AHQ/ARQ	1-2

<b>12</b>	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	AHQ/ARQ 0-1
<b>13</b>	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	AHQ/ARQ
<b>Total</b>	8	7	9	9	2	8	8	6	3	6	0	0	0	0		

\* Lnn. mandibulares

IPP Peyer's patches of the distal ileum

RPLN Retropharyngeal lymph node

ENS Enteric nervous system CMGC Coeliac and mesenteric ganglion complex

**Figure legends****Figure 1:**

Hierarchical approach in the sample selection from culled sheep and goats that underwent a further immunohistochemical examination

**Figure 2:**

Distinct PrP<sup>Sc</sup> immunolabelling in different tissues of sheep

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