Extended scrapie incubation time in goats singly heterozygous for PRNP S146 or K222

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ABSTRACT

Scrapie is the transmissible spongiform encephalopathy (TSE) of sheep and goats, and scrapie eradication in sheep is based in part on strong genetic resistance to classical scrapie. Goats may serve as a scrapie reservoir, and to date there has been no experimental inoculation confirming strong genetic resistance in goats. Two prion protein variants (amino acid substitutions S146 and K222) in goats have been significantly underrepresented in scrapie cases though present in scrapie-exposed flocks, and have demonstrated low cell-free protein conversion efficiency to the disease form (PrPSc). To test degree of genetic resistance conferred in live animals with consistent exposure, we performed the first oral scrapie challenge of goats singly heterozygous for either PRNP S146 or K222. All N146-K222 homozygotes became clinically scrapie positive by an average of 24 months, but all S146 and K222 heterozygotes remain scrapie negative by both rectal biopsy and clinical signs at significantly longer incubation times (P<0.0001 for both comparisons). Recent reports indicate small numbers of S146 and K222 heterozygous goats have become naturally infected with scrapie, suggesting these alleles do not confer complete resistance in the scrapie-positive state but rather extend incubation. The oral challenge results presented here confirm extended incubation observed in a recent intracerebral challenge of K222 heterozygotes, and to our knowledge provide the first demonstration of extended incubation in S146 heterozygotes. These results suggest longer relevant trace-back histories in scrapie-eradication programs for animals bearing these alleles and strengthen the case for additional challenge experiments in both homozygotes to assess potential scrapie resistance.

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1. Introduction

Scrapie is a transmissible spongiform encephalopathy (TSE) of sheep and goats with varying symptoms appearing long after infection; these can include weight loss, lip smacking, and ataxia (Dickinson, 1976). In sheep, strong resistance to classical scrapie is conferred by the R171 substitution in PRNP which occurs on the “ARK” haplotype representing codons 136, 154, and 171 (Westaway et al., 1994). This resistance is a key component of scrapie eradication programs in many parts of the world, along with trace-backs of infected animals to eliminate as many infectious sources as possible (Lynn et al., 2007).

In goats, there have been no resistance alleles to classical scrapie confirmed following direct oral challenge, and only two alleles (M142 and K222) shown to have extended incubation period following any experimental challenge (Acutis et al., 2012; Goldmann et al., 1996). However, two amino acid substitutions (S146 and K222) have been present in flocks exposed to scrapie, but absent or strongly underrepresented among scrapie cases from those flocks (Acutis et al., 2006; Bouzalas et al., 2010; Papasavva-Stylianou et al., 2007, 2011; Vaccari et al., 2006). Further, both alleles have recently been shown to have low cell-free prion protein conversion efficiency to the disease form, PrPSc (Eiden et al., 2011). Therefore we hypothesized that goats singly heterozygous for S146 or K222 would have significantly longer scrapie incubation times than N146-K222 homozygotes, and we performed an experimental oral challenge experiment to test this hypothesis.

2. Material and methods

To study the degree of scrapie resistance conferred by S146 and K222, kids from three genetic backgrounds (Toggenburg, Alpine-Boer,
and Spanish-Boer) were removed from their dams at birth, genotyped using allele-specific PCR (Table 1), and orally inoculated within 24 h of birth. Genotypes were later confirmed by sequencing using previous protocols (White et al., 2008). Experimental genotypes were defined as one copy of the most common PRNP haplotype and one copy of either S146 (haplotype 1,7) or K222 (haplotype 1,10) as described in (White et al., 2008). Control genotypes were defined as two copies of the most common haplotype (1,1) that included N146 and Q222 (White et al., 2008). Eight of each experimental genotype and six controls were inoculated. One control goat was disqualified when it was subsequently determined to have an R101 allele previously reported only in Moroccan goats (Serrano et al., 2009). Even so, the control group still included breed-matched experimental goats singly heterozygous in PRNP for either S146 (P<0.0001) or K222 (P<0.0001). For the S146 group the average extension of incubation has been almost 10 months. The K222 group incubation has been extended by 20 months; this conservative estimate was made including the scrapie-free incubation of the animal euthanized for other reasons. Excluding this animal would result in average scrapie-free incubation of 46 months, with average extended incubation of 22 months for the K222 group.

### 3. Results

One K222 goat was euthanized at 30 months when it became lame with swollen joints. This clinical presentation coupled with positive serology for caprine arthritis encephalitis virus (CAEV) (Herrmann et al., 2003) suggest lameness was a result of arthritis associated with CAEV. PrP<sup>Sc</sup> was not detected in any tissues from this goat, including obex and retropharyngeal lymph node.

All control animals became clinical with scrapie at an average of 23.8 months post-inoculation (Table 2). PrP<sup>Sc</sup> was detected in the rectal biopsy of all but one control goat by 18 months post-inoculation. In that one control goat, PrP<sup>Sc</sup> was not detected in rectal mucosal tissue despite progressing to clinical symptoms and having positive IHC confirmation of PrP<sup>Sc</sup> in obex.

None of the experimental animals have been positive by either rectal biopsy or clinical signs (Table 2). Scrapie-free survival times have been significantly prolonged in these experimentally challenged goats singly heterozygous in PRNP for either S146 (P<0.0001) or K222 (P<0.0001). For the S146 group the average extension of incubation has been almost 10 months. The K222 group incubation has been extended by 20 months; this conservative estimate was made including the scrapie-free incubation of the animal euthanized for other reasons. Excluding this animal would result in average scrapie-free incubation of 46 months, with average extended incubation of 22 months for the K222 group.

### 4. Discussion

Existing data on the degree of scrapie resistance conferred by caprine PRNP S146 and K222 rely on potentially variable exposure in natural scrapie situations, in vitro correlates of resistance, or an unnatural intracerebral challenge. Experimental challenge can provide the advantages of consistent inoculum and known incubation time, and this study provided both with homogenized, goat-derived inoculum delivered orally for what may be a more natural exposure than other experimental routes. All the homozygous haplotype 1 control animals developed clinical disease and have been euthanized, demonstrating the infectivity of the inoculum was consistent. None of the animals from either the S146 or K222 heterozygote groups have developed clinical signs, demonstrating consistent extension of incubation for both alleles. Specifically, the current results (Table 2) suggest animals with one copy of S146 or K222 may not become rectal biopsy positive or clinically affected in the same timeframe post-exposure as common haplotype homozygous control genotypes. Extended incubation time can create difficulties in correctly diagnosing exposed animals. Furthermore, retrospective tracing of chains of exposure in scrapie eradication programs (Lynn et al., 2007) may become more difficult with longer incubation times such as those conferred by S146 or K222 because of the longer relevant exposure histories.

Recently, scrapie cases have been reported in small numbers of naturally-infected S146 and K222 heterozygotes (Barillet et al., 2009; Papasavva-Stylianou et al., 2011). In each of these cases, the S146 and K222 heterozygotes were significantly underrepresented in the scrapie cases, which is consistent with some degree of resistance to scrapie conferred by each allele. It is possible that differing scrapie strains and/or additional PRNP variants may alter the degree of resistance conferred by S146 and/or K222, but the very small number of such positives to date limits conclusions concerning those issues at present. However, the existence of any scrapie positives bearing these alleles, including at least one animal with the same apparent K222 haplotype combination as this study (Papasavva-Stylianou et al., 2011) and two animals with the same apparent S146 haplotype combinations as this study (Barillet et al., 2009), suggests the S146

### Table 1

Primer sets and conditions for allele-specific PCR genotyping.

<table>
<thead>
<tr>
<th>Forward PCR primer</th>
<th>Reverse PCR primer</th>
<th>Annealing temp</th>
<th>Detects allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCTTATACATTGGCAC</td>
<td>GACAGCTAAAGAAATGCACA</td>
<td>53</td>
<td>S146</td>
</tr>
<tr>
<td>TCTTATACATTGGCAA</td>
<td>GACAGCTAAAGAAATGCACA</td>
<td>53</td>
<td>N146</td>
</tr>
<tr>
<td>TGTCG(A/C)/GCACC(G/T)/TACA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GACAGCTAAAGAAATGCACA</td>
<td>59</td>
<td>K222</td>
</tr>
<tr>
<td>TGTCG(A/C)/GCACC(G/T)/TACC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GACAGCTAAAGAAATGCACA</td>
<td>59</td>
<td>Q222</td>
</tr>
</tbody>
</table>

<sup>a</sup> Degenerate primers designed to accommodate nearby single nucleotide polymorphisms.

### Table 2

Summary of scrapie status by genotype.

<table>
<thead>
<tr>
<th>N</th>
<th>Genotype</th>
<th>Number biopsy positive</th>
<th>Number clinical scrapie</th>
<th>Ave time to first clinical signs in months (range)</th>
<th>Ave time without clinical signs in months (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Control</td>
<td>4</td>
<td>5</td>
<td>23.8 (20, 27)</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>S146</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>33.6&lt;sup&gt;b&lt;/sup&gt; (33, 38)</td>
</tr>
<tr>
<td>8</td>
<td>K222</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>44.0&lt;sup&gt;b&lt;/sup&gt; (30, 47)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly extended incubation compared to control (P<0.0001).

<sup>b</sup> One K222 goat was euthanized for swollen joints and lameness at 30 months but remained scrapie negative by IHC. That animal was included in the statistical test for extended incubation.
and K222 alleles may not convey as strong resistance to classical scrapie as does R171 in sheep in the heterozygous condition (Westaway et al., 1994), but rather may confer extended incubation time like M142 (Goldmann et al., 1996).

In their study on M142, Goldmann et al. used many different sources of inoculum and routes of inoculation, making direct comparisons with the current study challenging. However, an average of about 14 months extended incubation was observed for M142 heterozygotes (Goldmann et al., 1996) compared to homozygotes for the most common haplotype (I142). In the final stages of preparing this manuscript, a study was published demonstrating extended incubation of K222 heterozygotes for 4.5 years following intracerebral scrapie challenge (Acutis et al., 2012). In this oral challenge, we have already observed a 20 month average extended survival following oral challenge for K222 heterozygotes without clinical signs to date. This confirms the intracerebral challenge result that heterozygous K222 has the longest extended scrapie incubation time shown by experimental challenge in goats to date and may convey a longer extended scrapie incubation than heterozygous M142.

A separate question regards the scrapie resistance conveyed by either S146 or K222 in the homozygous condition. Since the current results demonstrate that one copy of either allele conveys some partial scrapie resistance in goats, it is possible that homozygotes would possess stronger resistance. Allele frequencies for both S146 and K222 are sufficient to enable such an approach to achieve a high level of such animals in a small number of generations (Vaccari et al., 2009), should they be shown to possess strong scrapie resistance. Additional experimental challenge data will be necessary to demonstrate sufficient resistance to pursue this approach.

5. Conclusions

In conclusion, goats singly heterozygous for S146 or K222 have extended scrapie incubation times following oral scrapie challenge. The K222 heterozygote result confirms a recent intracerebral challenge, and to our knowledge this is the first report demonstrating extended incubation time in S146 following any experimental scrapie challenge. These results suggest difficulty in correctly diagnosing exposed S146 and K222 animals, and longer relevant trace-back histories of exposed animals bearing these alleles. An open question is the length of extended incubation for each genotype, and this experiment will be ongoing to address that issue. Since the present results also demonstrate partial scrapie resistance conferred by S146 and K222, they further suggest additional investigation into the scrapie resistance of homozygotes for each allele.

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