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Incidence of infection in 39-month-old ewes with *TMEM154* diplotypes "1 1," "1 3," and "3 3" after natural exposure to ovine progressive pneumonia virus[1,2]

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ABSTRACT: Production and well-being of sheep and goats in many countries are harmfully impacted by small ruminant lentiviruses (SRLV) that cause incurable, progressive diseases. Susceptibility to ovine progressive pneumonia virus (OPPV), the North American form of SRLV, is influenced by variants of the ovine *transmembrane protein 154* gene (*TMEM154*). The experimental objective was to estimate additive and dominance effects of *TMEM154* haplotypes 1 and 3 on susceptibility of breeding ewes to infection after natural exposure to OPPV from birth to 39 mo of age. Sires and dams were heterozygous for *TMEM154* haplotypes 1 and 3, producing ewe lambs with diplotypes "1 1," "1 3," and "3 3." These lambs were raised by mature, infected dams to ensure natural, maternal exposure to OPPV. Ewe lambs (n = 108) were kept for breeding and joined an infected flock of ewes to guarantee natural, nonmaternal exposure to OPPV. Ewes were bred to lamb at 1, 2, and 3 yr of age. Serum samples were collected at breeding, 1 mo before lambing and shortly after weaning each year to monitor infection status to 39 mo of age. During the experiment, 9 of the 108 ewes died while uninfected and data collected on these ewes were not analyzed. Infection status of the remaining 99 ewes at 39 mo of age was analyzed using logistic regression procedures. Effects of ewe type of birth, ewe type of rearing, and breed type of dam were not detected ($P > 0.10$), and the estimated sire variance component was nil. Ewe diplotype affected infection status ($P < 0.0001$), as did additive ($P < 0.0001$) and dominance ($P < 0.0022$) effects. Predicted probabilities of infection for ewes with diplotypes "1 1," "1 3," and "3 3" were 0.10, 0.88, and 0.89, respectively, and confidence intervals for diplotypes "1 3" and "3 3" were distinct from "1 1." Haplotype 3 was completely dominant to haplotype 1 at 39 mo of age. The probability of infection for ewes with either diplotype "1 3" or "3 3" averaged 8.5 times that of ewes with diplotype "1 1." Diplotype "1 3" and "3 3" ewes were highly susceptible to nonmaternal transmission of OPPV, in contrast to diplotype "1 1" ewes. Therefore, the distribution of ewes with diplotypes "1 1," "1 3," and "3 3" within a flock will influence the number of infections caused by each route of transmission. Selection and mating strategies can be implemented to produce sheep that are genetically less susceptible to OPPV infection.

Key words: genetic resistance to disease, ovine progressive pneumonia, sheep, small ruminant lentivirus, *transmembrane protein 154* gene

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INTRODUCTION

Visna-maedi virus (**VMV**) and ovine progressive pneumonia virus (**OPPV**), the North American form of VMV, are small ruminant lentiviruses (**SRLV**) that adversely affect sheep production throughout much of the world. Visna-maedi (**VM**) and ovine progressive pneumonia (**OPP**) diseases caused by the SRLV may result in clinical symptoms such as loss of body condition, labored breathing, indurative mastitis, arthritis, and encephalitis (Blacklaws, 2012; Peterhans et al., 2004). A decrease in ewe productivity, increased labor requirements, and premature culling or death of breeding stock make OPP one of the most costly sheep diseases in the United States (Keen et al., 1997; Peterhans et al., 2004; Alvarez et al., 2005). Effective vaccines have not been developed, and there are no treatments. Depopulation, artificial rearing, and testing and culling of seropositive ewes are 3 managerial approaches to eradicate or reduce prevalence of VM and OPP in individual flocks but are expensive and labor intensive to implement.

Protein variants encoded by the ovine *transmembrane protein 154* gene (*TMEM154*) affect susceptibility to OPPV infection, providing selection and mating opportunities to produce sheep that are genetically less susceptible to infection (Heaton et al., 2012; Leymaster et al., 2013). Haplotypes 1, 2, and 3 are most common worldwide (Heaton et al., 2013). In retrospective case-control and cohort studies, mature sheep with 1 or 2 copies of either haplotype 2 or 3 had a greater risk of OPPV infection than sheep with diplotype "1 1" (Heaton et al., 2012). Haplotype 3 was completely dominant to haplotype 1 in an experiment measuring susceptibility of lambs to OPPV infection at 9 mo of age (Leymaster et al., 2013). The latter experiment was continued to estimate additive and dominance effects of haplotypes 1 and 3 on susceptibility of breeding ewes to infection after natural exposure to OPPV from birth to 39 mo of age.

MATERIALS AND METHODS

The Animal Care and Use Committee of the U.S. Meat Animal Research Center (**USMARC**) approved procedures used in this experiment. General design concepts, sheep populations, management, *TMEM154* genotyping, and diagnosis of infection were previously described in detail (Leymaster et al., 2013). The experiment was designed to account for 3 risk factors for OPPV transmission: OPPV-infection status of dams, ages of dams, and *TMEM154* diplotypes of sires and dams. A total of 154 infected ewes that were heterozygous for *TMEM154* haplotypes 1 and 3 (diplotype "1 3") was available from a USMARC flock of Rambouillet × Romanov reciprocal crosses. These seropositive ewes were 4 and 5 yr of age in 2010 when mated to 11 crossbred rams (50% Romanov, 25% White Dorper, and 25% Katahdin) that were also diplotype "1 3." The resulting lambs had diplotypes "1 1," "1 3," and "3 3" and allowed estimation of additive and dominance effects. The use of mature, OPPV-infected dams ensured natural exposure of lambs to the virus via infected colostrum and aerosolized respiratory secretions.

Table 1. Number of ewes at lambing by lambing year, ewe age, and ovine progressive pneumonia virus infection status[1]

Ewe	2012		2013		2014	
age, yr	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
1	27	81	–	70	28	82
2	–	–	51	44	34	26
3	–	–	–	–	49	35
6	83	8	–	–	–	–
7	58	24	41	6	–	–
8	–	–	19	10	15	–
9	–	–	–	–	7	–
Total	168	113	111	130	133	143

[1]Numbers of experimental ewes are underlined.

Lambs were born in March and April of 2011 and naturally reared in an open-fronted pole shed that was used for both drylot lambing and feedlot purposes. Male lambs were castrated at 2 to 3 wk of age, and all lambs were weaned at an average age of 60 d with a SD of 2.2 d. A total of 187 wether and ewe lambs, naturally reared by 5- and 6-yr-old infected dams, was used to provide genotypic and phenotypic data to estimate additive and dominance effects of haplotypes 1 and 3 on susceptibility to infection at 9 mo of age (Leymaster et al., 2013).

Wethers were sold after 9 mo of age, whereas the remaining 108 ewes were retained in the experiment to monitor OPPV-infection status over time. These experimental ewes permanently joined a flock that included mature, infected ewes to continue natural exposure to OPPV throughout the production cycle. Additional ewe lambs were brought into the flock in 2013 and 2014 to investigate effects of haplotypes 2 and 4. The number of infected and uninfected ewes in the combined flock at lambing in 2012, 2013, and 2014 is shown by ewe age in Table 1 to reflect the level of exposure to OPPV by experimental ewes. Thirteen of the 108 experimental ewes present at lambing in 2012 were absent (culled or dead) by lambing in 2013, while another 11 ewes left the flock before lambing in 2014.

Experimental ewes were multisire mated for 35 d to crossbred rams (50% Romanov, 25% White Dorper, and 25% Katahdin) to lamb in February and March of 2012 and to Dorset rams for March and April lambing

in both 2013 and 2014. About 30 d before lambing each year, ewes were shorn and moved into an open-fronted pole shed under semiconfinement conditions. The pole shed is designed to accommodate 450 ewes and their lambs, providing about 1.7 m^2 per lactating ewe. Lambs were weaned each year at 8 wk of age and ewes were then turned out to pasture.

The *TMEM154* diplotypes of experimental ewes were determined by use of Sanger sequencing as described in detail by Heaton et al. (2012). Sequences from each sheep were manually scored and recorded to assign haplotypes defined by distinct AA sequences of 6 missense SNP (L14H, T25I, D33N, E35K, T44M, and N70I) and 2 frame-shift deletion polymorphisms ($R4A^{\Delta}$ and $E82Y^{\Delta}$). Haplotypes 1 and 3 differ only at position 35: haplotype 1 encodes lysine whereas haplotype 3 encodes glutamate (Heaton et al., 2012). All diplotypes were confirmed by a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assay as previously described (Heaton et al., 2013).

Serum samples were collected from ewes at approximately 4-mo intervals during each production cycle: at breeding, 1 mo before lambing, and shortly after weaning lambs at 8 wk of age. Samples were drawn from ewes via jugular venipuncture using 9-mL S-Monovette serum Z syringes (Sarstedt, Newton, NC) on 8 collection dates (January 4, 2012; May 9, 2012; October 22, 2012; February 13, 2013; June 20, 2013; October 22, 2013; February 18, 2014; and June 18, 2014) to monitor serological trends in anti-OPPV antibodies over time. These collection dates correspond to average ages of experimental ewes of 9.8, 13.9, 19.4, 23.1, 27.3, 31.4, 35.3, and 39.2 mo, respectively.

Serological testing was performed at GeneSeek (Lincoln, NE), a Neogen company, using competitive ELISA kits manufactured by VMRD Inc. (Pullman, WA). In this study, experimental ewes with percentage inhibition values greater than 22.8 were classified as infected, according to previous estimates of cutoff values (Herrmann et al., 2003; Leymaster et al., 2013).

Statistical Analysis

Nine of the 108 experimental ewes died while still uninfected. Two ewes were diplotype "1 1," 5 were diplotype "1 3," and 2 were diplotype "3 3." Four ewes died before the second collection date and all but 1 ewe died before the fifth collection date. Serological data recorded on these ewes provided little information to predict infection status on the final collection date and, therefore, data collected on these 9 ewes were excluded from analysis.

Infection status (infected or uninfected) of 99 experimental ewes (the 84 ewes listed in Table 1 for 2014 plus 15 infected ewes that left the flock before lambing in 2014) at 39 mo of age was analyzed using logistic regression procedures (logit link function; GLIMMIX; SAS Inst. Inc., Cary, NC). Of these 99 ewes, 28, 43, and 28 had diplotypes of "1 1," "1 3," and "3 3," respectively. Inspection of the data revealed that all diplotype "1 1" ewes born to 6-yr-old dams were uninfected. Also, effects of age of dam were not detected ($P = 0.663$) at 9 mo of age by Leymaster et al. (2013). Therefore, age-of-dam effects were not fitted in the present statistical model to avoid potential spurious estimates due to the lack of variance for infection status within the specific diplotype × age of dam classification. Effects of type of birth (single, twin, triplet, and quadruplet; $P = 0.14$) and type of rearing (single, twin, and triplet; $P = 0.17$) were fitted in exploratory models but subsequently deleted due to lack of statistical significance, consistent with results reported by Leymaster et al. (2013) for infection status at 9 mo of age. The final model included fixed effects of reciprocal cross of dam (Rambouillet rams × Romanov ewes and Romanov rams × Rambouillet ewes) and diplotype of ewe ("1 1," "1 3," and "3 3") and random effects of sires of ewes. These effects were incorporated into the experimental design and therefore included in the statistical model regardless of levels of significance. Linear contrasts of effects of diplotypes "1 1," "1 3," and "3 3" were used to estimate levels of significance for additive (–1, 0, and 1) and dominance (–0.5, 1, and –0.5) effects. Least-squares means of diplotypes on the logit scale were transformed to the raw scale (inverse logit scale) to give predicted probabilities of OPPV infection.

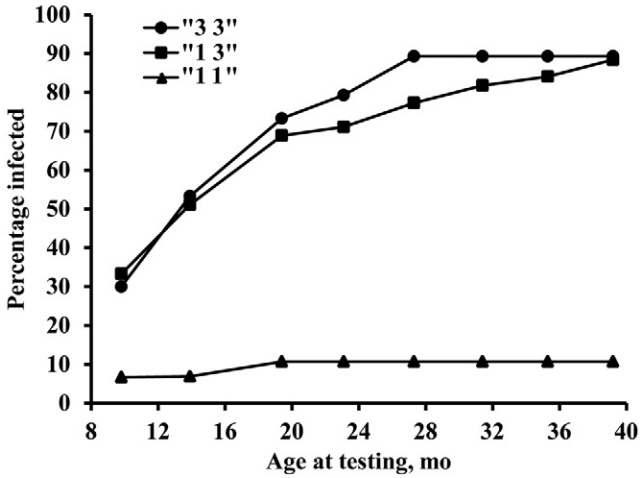

Figure 1. Cumulative percentage of ewes infected with ovine progressive pneumonia virus due to natural exposure from birth to 39 mo of age. Diplotypes "1 1," "1 3," and "3 3" for ovine *transmembrane protein 154* gene are illustrated.

RESULTS

Distinct effects of *TMEM154* diplotypes on OPPV infection from 10 to 39 mo of age are shown in Fig. 1.

Table 2. Probability values for fixed sources of variation and additive and dominance effects on infection status at 39 mo of age

Item	*P*-value
Reciprocal cross of dam	0.7189
TMEM154[1] diplotype of ewe	0.0001
Additive	0.0001
Dominance	0.0022

[1]*Transmembrane protein 154* gene.

Table 3. Predicted probability of infection at 39 mo of age and 95% confidence interval by *TMEM154*[1] diplotype of ewe

TMEM154 diplotype	Probability of infection	Confidence interval
"1 1"	0.10	0.03, 0.28
"1 3"	0.88	0.75, 0.95
"3 3"	0.89	0.71, 0.96

[1]*Transmembrane protein 154* gene.

Effects of reciprocal cross of dam were not detected ($P = 0.7189$; Table 2) and the estimate of the sire variance component was nil, indicating that loci other than *TMEM154* were not significantly affecting infection status in this population.

Infection status at 39 mo of age was affected by diplotype of ewe ($P < 0.0001$), and both additive ($P < 0.0001$) and dominance ($P = 0.0022$) effects were detected. The predicted probability of infection and confidence intervals for each *TMEM154* diplotype are given in Table 3. Confidence intervals for diplotypes "1 3" and "3 3" are similar and do not overlap with the confidence interval for diplotype "1 1." These results are consistent with complete dominance of haplotype 3 relative to haplotype 1. The probability of infection at 39 mo of age for ewes with either diplotype "1 3" or "3 3" averaged 8.5 times that of ewes with diplotype "1 1."

DISCUSSION

Two diplotype "1 1" ewes were infected with OPPV at 9 mo of age, most likely due to maternal transmission of OPPV (Leymaster et al., 2013). During the current phase of the experiment, a single "1 1" ewe seroconverted, resulting in 3 infected ewes out of 28 (10.7%). Therefore, under the experimental conditions at USMARC, diplotype "1 1" ewes were essentially resistant to OPPV infection due to exposure to infected flock mates, that is, nonmaternal or horizontal exposure.

About one-third of diplotype "1 3" and "3 3" ewes were infected at 9 mo of age, presumably due to maternal transmission of OPPV (Leymaster et al., 2013). However, diplotype "1 3" and "3 3" ewes were highly susceptible to OPPV infection due to nonmaternal exposure to OPPV during the current experimental phase, in stark contrast to diplotype "1 1" ewes. The percentage of infected "1 3" and "3 3" ewes increased from about 32% at 10 mo of age to 89% at 39 mo of age. The period between the first and second collection dates, from January 4, 2012, to May 9, 2012, spanned the interval from shearing to weaning. During this intensive phase of production, ewes were managed at the pole shed in close proximity to one another and physiologically challenged due to parturition and lactation. The combined effects of greater exposure and physiological stress may explain the substantial increase from 32 to 52% infection during this phase of production as well as the increase to an average of 71% during the following period.

The complete dominance of haplotype 3 relative to haplotype1 for greater susceptibility to OPPV infection at 9 mo of age ($P = 0.052$; Leymaster et al., 2013) was substantiated at 39 mo of age ($P = 0.0022$). The magnitude of the additive effect was remarkable, with an absolute difference between homozygous diplotypes of 79% for percentage infected at 39 mo of age (10% for diplotype "1 1" ewes versus 89% for diplotype "3 3" ewes; Table 3). Selection and mating strategies to increase the proportion of diplotype "1 1" sheep in a flock should reduce genetic susceptibility to OPPV infection and decrease disease prevalence. Estimated frequencies of highly susceptible diplotypes for 74 breeds of sheep have been reported (Heaton et al., 2013).

Combined with results from the first phase of this experiment (Leymaster et al., 2013), this research has documented that mature ewes with diplotypes "1 3" and "3 3" were more likely to be infected by nonmaternal transmission of OPPV than by maternal transmission, whereas there was scant evidence that nonmaternal transmission caused infection in diplotype "1 1" ewes. Accordingly, the distribution of ewes with diplotypes "1 1," "1 3," and "3 3" within a flock will influence the number of infections caused by each route of transmission. The current understanding that nonmaternal transmission is the primary cause of lifetime infection in a flock of mature sheep (Berriatua et al., 2003; Alvarez et al., 2005; Leginagoikoa et al., 2006a,b; Herrmann-Hoesing et al., 2007; Broughton-Neiswanger et al., 2010; Leymaster et al., 2013) seems to apply to ewes with diplotypes "1 3" and "3 3" but may not apply to "1 1" ewes. Stated differently, *TMEM154* diplotype and route of transmission seem to interact to affect infection status.

Understanding OPPV transmission is further complicated by research of Sider et al. (2013), who documented an interaction between *TMEM154* diplotypes of infected sheep and 2 OPPV genetic subgroups present at USMARC. Subgroup 1 was significantly more likely

than subgroup 2 to have caused infection in sheep with diplotype "1 1," whereas the opposite situation existed for infected sheep with 1 or 2 copies of haplotype 3. These results imply that OPPV have evolved to infect sheep with specific *TMEM154* diplotypes.

Our current general recommendation to reduce or eradicate OPP in a flock is the following:

1. Serologically test a random sample of the oldest ewes to determine prevalence of OPP.
2. Keep all productive ewes, regardless of infection status, for breeding.
3. Mate to rams with 1 or 2 copies of haplotype 1.
4. Naturally rear the resulting lambs and serologically test replacement ewe lambs at 7 mo of age or older.
5. Permanently isolate seronegative ewe lambs from the infected flock.
6. Mate ewes in the seronegative flock to rams that will increase the frequency of haplotype 1.
7. Monitor infection status in the seronegative flock by serologically testing the oldest ewes.

This approach requires the ability to manage 2 flocks separately until the infected flock is completely replaced. Three advantages of this protocol are minimal testing of ewes in the inflected flock, the potential to produce seronegative replacement lambs from infected ewes, and natural rearing of lambs born in the infected flock. These benefits address impediments such as culling of genetically superior infected ewes and the expense of artificially rearing lambs that are often associated with traditional eradication programs.

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