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Effect of *TMEM154* E35K variant (haplotypes 1 and 3) on the incidence of ovine lentivirus infection and ewe productivity during lifetime exposure

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Abstract

Ovine progressive pneumonia virus (OPPV) is a small ruminant lentivirus that is widespread throughout U.S. sheep flocks. Infections with OPPV are lifelong and effects are multi-systemic with significant implications for animal well-being and productivity. A protein isoform with lysine at position 35 (K35, haplotype "1") encoded by the ovine transmembrane protein 154 (*TMEM154*) gene has been associated with reduced susceptibility to infection when two copies are present (i.e., diplotype "1,1"). Conversely, the ancestral protein isoform with glutamate at position 35 (E35, haplotype "3") is associated with high susceptibility to infection when at least one copy is present. The beneficial effect of *TMEM154* K35 alleles on ewe productivity has not been previously measured in controlled challenge experiments and was a major objective of this study. Ewes with *TMEM154* diplotypes "1,1"; "1,3"; and "3,3" (n = 31, 47, and 30, respectively) were born and reared by OPPV-infected dams and managed under continual natural exposure to OPPV. Ewes were tested for serological status at 4-mo intervals for up to 5.5 yr. The incidence of infection in ewes with diplotype "1,1" was 6.5% to 9.7% and significantly lower ($P < 0.001$) than ewes with diplotype "1,3" (60.5 to 97.3%) or "3,3" (64.0 to 91.4%). Furthermore, the incidence among ewes with diplotype "1,1" did not increase from 10 to 67 mo of age ($P > 0.99$), whereas the incidence among diplotype "1,3" and "3,3" ewes increased steadily until reaching an asymptote at approximately 52 mo of age. Total number and weight of lamb weaned per ewe exposed through 5.5 yr from ewes with diplotype "1,1" far exceeded ($P \leq 0.05$) those with diplotypes "1,3" and "3,3" by, on average, 2.1 lambs and 40 kg, respectively. The present study confirmed that *TMEM154* diplotype "1,1" animals have reduced incidence of OPPV infection and, correspondingly, improved productivity. In flocks with a high frequency of *TMEM154* haplotype "3," selection for haplotype "1" appears to be a cost-effective approach to mitigate the impact of this economically important disease.

Key words: ewe productivity, longevity, ovine progressive pneumonia, small ruminant lentivirus, transmembrane protein 154 gene, sheep

Abbreviations

BW	body weight
LWW	total weight of lamb weaned per ewe exposed annually
LWWT	total weight of lamb weaned per ewe exposed over 5 yr
NLB	number of lambs born per ewe exposed annually
NLW	number of lambs weaned per ewe exposed annually
NLWT	number of lambs weaned per ewe exposed over 5 yr
OPP	ovine progressive pneumonia
OPPV	ovine progressive pneumonia virus
SRLV	small ruminant lentivirus
USMARC	U.S. Meat Animal Research Center
VM	visna/maedi.

Introduction

Ovine progressive pneumonia (OPP), the North American equivalent of visna/maedi (VM), is a multi-systemic disease caused by infection of the OPP virus (OPPV). Small ruminant lentiviruses (SRLV) such as OPPV are found throughout the world and clinical signs of infection include labored breathing, indurative mastitis, arthritis, encephalitis, and cachexia from chronic pulmonary inflammation (Cutlip et al., 1988; Brodie et al., 1998). The clinical state of OPP has major animal welfare and productivity implications but, because of the long latent period of SRLV, signs of disease are not usually observed for years after infection is established. The infection is persistent, incurable, and there are no treatments or vaccines.

All previous attempts to estimate production effects of SRLV infection have been conducted in sheep of varying genetic and management backgrounds and with limited repeated performance records and sampling points to establish seropositivity. These limitations have resulted in inconsistent results being reported for associations between SRLV infection status and ewe productivity (Snowder et al., 1990a; Arsenault et al., 2003). However, Keen et al. (1997) estimated that OPPV-infected ewes were less likely to lamb and weaned lighter litters than uninfected ewes. Moreover, the most recent U.S. surveys reported that 36% of flocks were OPPV infected (USDA APHIS, 2003) and clinical signs of OPP such as hard-bag (i.e., indurative mastitis) and chronic weight loss accounted for 9% of ewes culled (USDA APHIS, 2012). Therefore, strategies to reduce and then eliminate OPP in U.S. sheep flocks are needed.

We previously reported that differences in OPPV susceptibility were associated with variants in the ovine transmembrane protein 154 (*TMEM154*) gene, providing an opportunity to reduce the genetic susceptibility to OPP and VM (Heaton et al., 2012; Yaman et al. 2019). The ancestral, full-length polypeptide encoded by *TMEM154* has glutamate at position 35 (E35, haplotype "3") and is associated with high-susceptibility, whereas the single substitution of lysine at position 35 (K35, haplotype "1") is associated with greatly reduced susceptibility. In addition to retrospective field studies, Leymaster et al. (2013) designed experiments for testing the effects of specific *TMEM154* haplotypes in controlled environments. The experiment generated contemporary *TMEM154* diplotype "1,1"; "1,3"; and "3,3" lambs and monitored their infection status through 9 mo of age. Retained ewe lambs then joined a breeding flock in which OPPV was endemic and the incidence of infection through 39 mo of age was reported in Leymaster et al. (2015). Both studies showed that there was reduced OPPV incidence in diplotype "1,1" sheep. However, performance for economically relevant traits was not evaluated. A portion of these ewes were retained for up to 67 mo (i.e., 5.5 yr) of age and monitored for infection. The objectives of the present report were to evaluate OPPV infection status and productivity among *TMEM154* diplotype "1,1"; "1,3"; and "3,3" ewes through five production years.

Materials and Methods

Experimental design

The Institutional Animal Care and Use Committee of the USDA, ARS U.S. Meat Animal Research Center (USMARC; Clay Center, NE, USA) approved all procedures used in this experiment and its design was detailed in Leymaster et al. (2013 and 2015). Lambs were produced from a USMARC flock of Rambouillet x Romanov reciprocal cross ewes (n = 154; 100% diplotype "1,3") that were seropositive for OPPV. In the fall of 2010, these ewes were mated to USMARC Composite-IV rams (50% Romanov, 25% Kathadin, 25% White Dorper; 100% *TMEM154* diplotype "1,3") for lambing at 5 or 6 yr of age. The resulting lamb crop had a genetic background consisting of 50% Romanov, 25% Rambouillet, 12.5% Katahdin, and 12.5% White Dorper while segregating for all three *TMEM154* diplotypes ("1,1"; "1,3"; or "3,3"). Furthermore, lambs were naturally and continually exposed to OPPV via their dam's infected colostrum and milk, as well as interaction with infected ewes throughout their entire life.

A total of 187 lambs were serially tested for the presence of OPPV antibodies to estimate additive and dominance effects of *TMEM154* haplotypes "1" and "3" on susceptibility to OPPV infection through 9 mo of age (Leymaster et al., 2013). Wether lambs were then sold and 108 ewe lambs were retained for further evaluation. Leymaster et al. (2015) reported the incidence of OPPV infection of these experimental ewes at approximately 4-mo intervals through 39 mo of age. A portion of these ewes remained in the flock and continued to be serially tested through a maximum of 5 parities and 67 mo of age and were the focus of the present study.

Flock husbandry

The 108 experimental ewe lambs permanently joined a flock of mature ewes (≥ 6 yr) in which OPPV was endemic to ensure continued exposure. Additional ewe lambs were brought into this combined flock in 2013, 2014, and 2015 to evaluate the effects of *TMEM154* haplotypes "2" and "4," which were objectives of parallel study but contributed to the level of OPPV exposure experienced by the experimental ewes of the present study. The age structure and OPPV infection rate near lambing each year in the combined flock are displayed in Table 1. Although there were multiple, concurrent experimental objectives of this combined flock, it was managed as one contiguous group with an OPPV infection rate > 30% and direct or fence line contact throughout the production year.

Flock health was monitored according to USMARC standard operating procedures and included annual vaccinations (*Campylobacter jejuni/fetus*, *Chlamydia psittaci*, and *Clostridia*) and, when necessary, chemical treatment for internal and external parasites. Experimental ewes were multi-sire mated for 35 d each fall for lambing in 2012–2016. Ewes were mated to Composite-IV rams to lamb in February and March of 2012 and mated to Dorset rams to lamb in March and April of 2013–2015 and February and

Table 1. Number of ewes in the combined flock and OPPV infection rate during January/February of each year

	Production year, number of ewes (OPPV infection rate)				
Age, yr	2012	2013	2014	2015	2016
1	108 (19%)[a]	70 (0%)	110 (25%)	137 (4%)	59 (2%)
2	–	105 (47%)[a]	60 (57%)	101 (36%)	121 (18%)
3	–	–	92 (54%)[a]	53 (62%)	93 (59%)
4	–	–	–	75 (57%)[a]	45 (87%)
5	–	–	–	–	56 (57%)[a]
≥ 6	173 (82%)	76 (79%)	22 (100%)	–	–
Total	281 (58%)	251 (44%)	284 (39%)	366 (32%)	374 (40%)

[a]Experimental ewes of the present study.

March of 2016. Approximately 1 mo before expected lambing date each year, ewes were shorn and managed in drylot with access to an open-fronted pole barn until lamb weaning. Ewes were fed a corn-silage based ration while in semi-confinement (breeding and late gestation through lactation) and rotated through actively growing or stockpiled forage for the remainder of the production year.

After lambing, ewes and newborn lambs were placed into individual bonding pens for approximately 24 h before joining larger contemporary groups. First parity ewes were permitted to rear a maximum of two lambs, whereas second parity and older ewes were permitted to rear up to three lambs. No grafting took place and excess lambs or those born to dams that had died or had insufficient milk production were transferred to the nursery for artificial rearing until 4 wk of age. Dam-reared lambs had access to supplemental feed while still nursing and were weaned at 8 wk of age.

During their first three parities, nonpregnant ewes were retained and ewes were only removed from the flock if they died or were functionally unsound (e.g., prolapse, mastitis, and insufficient milk production). By approximately 3.5 yr of age (October 2014), there were 84 experimental ewes remaining. The feeding and housing facilities for lamb rearing could accommodate approximately 400 ewes and, as the combined flock grew to integrate additional objectives, otherwise functional experimental ewes were removed as "surplus" in 2014 (3.5 yr of age, n = 8) and 2015 (4.5 yr of age, n = 13). Frequencies of *TMEM154* diplotypes "1,1"; "1,3"; and "3,3" among the 21 surplus ewes were 3, 12, and 6, respectively. All other experimental ewes had the opportunity to remain in the flock and 50 ewes were present at the end of the experiment (September 2016) at approximately 5.5 yr of age. The most common reasons for ewe death/removal were surplus (n = 21), unknown/other (n = 17), insufficient milk production (n = 10), mastitis (n = 6), and pneumonia (n = 4).

TMEM154 genotyping and OPPV diagnosis

Methods for scoring *TMEM154* genotypes and testing OPPV serological status were previously described (Heaton et al., 2012). Briefly, genomic DNA was extracted from whole blood and *TMEM154* diplotypes were determined by both Sanger sequencing and matrix-assisted laser desorption and ionization time-of-flight mass spectrometry. Phased diplotypes for *TMEM154* were unambiguously inferred in ewes by genotyping SNPs encoded at amino acid positions: R4A(delta), L14H, D33N, K35E, T44M, N70I, and E82Y(delta). Specifically, this resolved ambiguous haplotypes "1" from "10"; and "3" from "2", "4," and "6." Of the 108 ewe lambs that entered the present experiment, 31, 47, and 30 were diplotypes "1,1"; "1,3"; and "3,3," respectively.

Serum samples were collected from ewes at approximately 4-mo intervals coinciding with breeding (September/October), late pregnancy (January/February), and lamb weaning (May/June). Ewes were bled by jugular venipuncture using 9-mL S-Monovette serum Z syringes (Sarstedt, Newton, NC, USA) on up to 8 dates through 39 mo of age (Leymaster et al., 2015) and up to 7 additional dates through 67 mo of age in the present study. Competitive ELISA tests (VMRD Inc., Pullman, WA) were used for serological testing of serum samples at either USMARC or GeneSeek Neogen (Lincoln, NE, USA). The threshold value for percentage inhibition used to signify positivity of a single test was 35% based on the manufacturer's recommendation. Three sequential positive tests over 12 mo were required to confirm infection but were retrospectively considered infected at the first date of three repeated positive tests.

Ewe performance

Annually recorded ewe traits included body weight (BW) at mating and number of lambs born (NLB) and weaned (NLW) per ewe exposed. Additionally, lamb BW at birth was used to adjust BW at weaning to 65 d of age which was summed within dam to calculate total litter weaning weight per ewe exposed (LWW). Ewes were not credited with nursery-reared lamb performance in NLW or LWW calculations. Longevity was expressed as absence or presence in the flock at the end of the experiment (0 or 1, respectively) at 67 mo of age. Lifetime productivity was assessed as total number (NLW_T) and weight (LWW_T) of lamb weaned through the five production years.

Statistical analysis

All statistical analyses were conducted in the GLIMMIX procedure of SAS (v 9.4; SAS Institute Inc., Cary, NC, USA). Annually recorded performance traits (BW, NLB, NLW, and LWW) were analyzed as repeated measures with fixed effects of birth type of the ewe (≤ 2 or ≥ 3), age (1–5 yr), and *TMEM154* diplotype ("1,1"; "1,3"; or "3,3"). Additionally, a random sire of the ewe effect (n = 11) was fit and a compound symmetric (co)variance structure with heterogenous variance across age was chosen to model the random ewe effect. Lifetime performance traits (longevity, NLW_T, and LWW_T) were analyzed with fixed effects of birth type of the ewe and *TMEM154* diplotype and a random effect of sire of the ewe. Ewe OPPV infection status was analyzed as repeated measures with fixed effects of ewe age in nearest month (up to 15 timepoints) and *TMEM154* diplotype and the random ewe effect was modeled with a compound symmetric (co)variance structure with heterogenous variance across age. All two-way interactions among fixed effects were included in these analyses.

Ewe longevity and OPPV infection status were modeled as binary traits (logit link function) and means were

back-transformed to the original scale. All other traits were considered normally distributed. Records from ewes culled from the flock as "surplus" were not included in the analysis of lifetime performance but were for annual performance and OPPV infection status. Therefore, frequencies of *TMEM154* diplotypes "1,1"; "1,3"; and "3,3" were, respectively, 31, 47, and 30 for annual performance traits and 28, 35, and 24 for lifetime performance traits. For consistency with Leymaster et al. (2015), OPPV infection status was not included for 9 ewes that left the flock before 39 mo of age and frequencies of *TMEM154* diplotypes "1,1"; "1,3"; and "3,3" in this analysis were 31, 43, and 25, respectively. Summary statistics for all traits are presented in Table 2.

Results

OPPV infection status

The ewe *TMEM154* diplotype x age at sampling interaction effect was significant in the longitudinal analysis of ewe OPPV infection status ($P < 0.01$). This interaction was mostly due to a difference in relative magnitude of OPPV seropositivity between diplotypes within sampling date, rather than a significant re-ranking among diplotypes across sampling dates. Since animals that become infected with OPPV are infected for life, any decreases in proportion of infected individuals over time in Figure 1 are due to ewes leaving the flock.

Within the first and second sampling dates (10 and 14 mo, respectively), differences in seropositivity between diplotypes were not significant ($P \geq 0.08$). However, by the third sampling date (20 mo and later), seropositivity of diplotype "1,1" ewes was lower ($P < 0.001$) than diplotype "1,3" and "3,3" ewes. Diplotype "1,3" and "3,3" ewe seropositivity was not different at any sampling point ($P \geq 0.51$). These results are consistent with complete dominance of haplotype "3" relative to haplotype "1". Within diplotype "1,1" ewes, OPPV infection status was not statistically different between any sampling point ($P > 0.99$). However, diplotype "1,3" and "3,3" ewe seropositivity increased over time until reaching an asymptote at approximately 52 mo of age.

Ewe performance

The birth type of the ewe x *TMEM154* diplotype interaction effect was significant ($P = 0.02$) in the analysis of BW near breeding. However, within ewe birth type, there were no significant differences in BW between *TMEM154* diplotypes ($P \geq 0.07$). No two-way interactions were significant in the analysis of annually recorded ewe reproductive performance ($P \geq 0.08$) and least-squares means for main effects are displayed in Table 3.

Birth type of the ewe did not affect BW or NLB ($P \geq 0.76$), but, interestingly, ewes born as singles or twins had greater NLW and LWW ($P < 0.01$) than those born in triplet or larger litters. Body weight at mating generally increased through 5 yr of age, whereas NLB, NLW, and LWW were greatest at 3 yr of age and decreased thereafter. Ewe *TMEM154* diplotype did not affect BW, NLB, or NLW ($P \geq 0.13$). However, LWW was greater for diplotype "1,1" than "3,3" ewes ($P = 0.01$) and similar between "1,3" ewes and either homozygote ($P \geq 0.25$).

The birth type of the ewe x *TMEM154* diplotype interaction effect was not significant in the analysis of NLW_T, LWW_T, or longevity ($P \geq 0.16$) and least-squares means for their main effects are displayed in Table 4. Ewes born as singles or twins had greater NLW_T, LWW_T, and longevity ($P \leq 0.05$) than those born in triplet or larger litters. Ewe NLW_T and LWW_T were greater ($P \leq 0.05$) for diplotype "1,1" ewes than diplotype "1,3" and "3,3" ewes which were not different ($P \geq 0.59$). At the end of the experiment, there were 21/31, 17/47, and 12/30 diplotype "1,1"; "1,3"; and "3,3" ewes remaining, respectively. Longevity was numerically greatest for diplotype "1,1" ewes but the main effect of *TMEM154* diplotype was not significant ($P = 0.08$). Overall, results suggest greater lifetime resiliency to OPPV infection for *TMEM154* diplotype ewes which was accompanied with greater lifetime productivity.

Table 2. Summary statistics for ewe performance traits and OPPV infection status

Trait	Records, n	Ewes, n	Min.	Max.	Mean	SD
BW, kg	440	108	30.4	82.6	55.4	10.7
NLB, n	426	108	0	5	2.09	0.85
NLW, n	426	108	0	3	1.45	0.81
LWW, kg	426	108	0	49.6	22.6	13.5
NLW_T, n	87	87	0	11	5.97	3.15
LWW_T, kg	87	87	0	186.8	93.1	53.1
Longevity, *p*	87	87	0	1	0.58	–
OPPV infection status, *p*[1]						
10 mo	99	99	0	1	0.19	–
23 mo	99	99	0	1	0.46	–
36 mo	92	92	0	1	0.54	–
48 mo	75	75	0	1	0.57	–
59 mo	56	56	0	1	0.57	–

[1]Approximate age (in nearest mo) of ewes at sampling during late gestation.

Discussion

Infection with SRLV, including OPPV, is lifelong and no effective treatment or vaccine is available. Eradication strategies such as testing and culling or artificial rearing of lambs born to infected dams (Houwers et al., 1983; Williams-Fulton and Simard, 1989) are effective but are costly, can hinder genetic improvement programs, and do not promote greater resilience should exposure occur again. Heaton et al. (2012) were the first to identify a large effect genomic region associated with OPPV susceptibility in a retrospective, matched case-control genome-wide association study in USMARC flocks. In those closely matched ewes, animals with one or more copy of *TMEM154* haplotype "2" or "3" were 28 times more likely to be infected with OPPV than those homozygous for haplotype "1." Results from the genome-wide association study of White et al. (2012) agreed that *TMEM154* was an important contributor to OPPV susceptibility in other U.S. sheep populations. More recently, *TMEM154* variants have been associated with VM virus susceptibility in Italian, Iranian, German, and Turkish sheep (Molaee et al., 2019; Yaman et al., 2019; Arcangenli et al., 2021). However, the design of previous studies did not permit conclusions regarding longitudinal differences in SRLV serology among diplotypes as was done in Leymaster et al. (2013) and (2015) and the present study. Furthermore, this is the first to report associated effects between ewe *TMEM154* diplotype and productivity for commercially relevant traits.

At 9 mo of age, predicted probabilities of infection for diplotype "1,1"; "1,3"; and "3,3" ewe and wether lambs were 0.09, 0.32, and 0.35, respectively, in Leymaster et al. (2013). Ewe lambs were retained in Leymaster et al. (2015) and predicted probabilities of infection at 39 mo of age for diplotypes "1,1"; "1,3"; and "3,3" were 0.10, 0.88, and 0.89, respectively. By 67 mo of

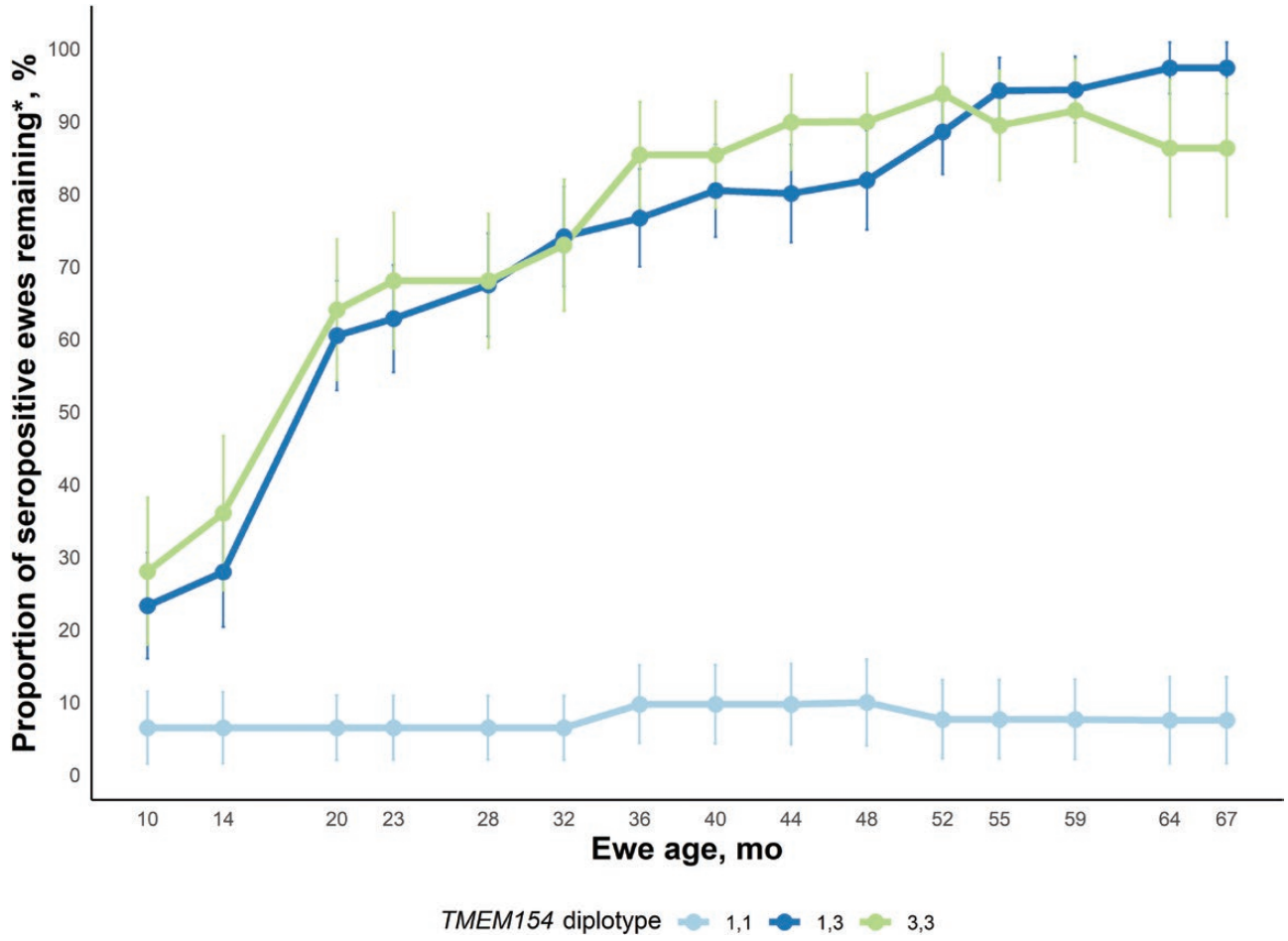

Figure 1. Least-squares means (± SE) for the ewe age at sampling x *TMEM154* diplotype interaction effect on OPPV infection status. (*Since ewes were culled from the flock for welfare reasons, the apparent seroprevalence can decrease within a diplotype group.).

age in the current study, predicted probabilities of infection for remaining diplotype "1,1"; "1,3"; and "3,3" ewes were 0.08, 0.97, and 0.86, respectively. In addition to a longer OPPV exposure and fewer remaining animals, slight numerical differences between the present study and Leymaster et al. (2015) may also be attributed to use of a more conservative competitive ELISA inhibition value to classify seropositivity (35% vs. 22%).

At the final sampling of the present study and at 5.5 yr of age, 2/21, 16/17, and 11/12 of the remaining *TMEM154* diplotype "1,1"; "1,3"; and "3,3" ewes were seropositive for OPPV, respectively. While this provides strong evidence for lifetime resiliency to SRLV infection for diplotype "1,1" sheep, it confounds the ability to jointly estimate the effects of both *TMEM154* diplotype and OPPV infection status on ewe productivity. Past studies evaluating production effects of SRLV have generally been survey designs whereby ewe infection status based on a single serology test is associated with concurrent performance measures. As summarized below, results from these studies vary for multiple reasons including the type and accuracy of serological test used, heterogeneity of breeds and management systems, and an inability to determine duration of infection and past exposure level. In contrast, ewes in the present study were of the same breed composition, had been born to infected dams, and were managed together with exposure to OPPV throughout their productive life. Additionally, multiple sampling timepoints to signify seropositivity and repeated reproductive performance records were used.

Keen et al. (1997) determined OPPV infection status in 9 USMARC flocks of varying breed types and estimated seronegative ewes had 8% greater NLB, 10% greater NLW, and 22% greater LWW than seropositive ewes. Arsenault et al. (2003) quantified VM status in ewes across 29 commercial flocks in Canada and reported that lambs reared by ≥ 4-yr-old seronegative dams weighed 0.9 kg more at weaning than those reared by seropositive dams, but infection status did not impact productivity of younger dams. In five flocks across Canada, Dohoo et al. (1987) found that, while the odds of conception for seropositive ewes were 0.69 that of seronegative ewes in one flock, there was little effect of infection status on other lamb production traits. Finally, Snowder et al. (1990a) found no significant effect of OPPV infection status on ewe reproductive performance traits in Idaho.

Hard-bag, or indurative mastitis, is a common sign of OPP and is associated with intramammary lesions (van der Molen and Houwers, 1987) which greatly reduce milk production. However, similar survey designs conducted in dairy flocks have not reported associations between ewe SRLV infection status and milk production (Legrottaglie et al., 1999), though Pazzola et al. (2020) did find greater milk bacterial count in seropositive ewes. The effect of OPPV infection status on milk production of

Table 3. Least-squares means (± SE) for the main effects of birth type of the ewe, age, and *TMEM154* diplotype on annual BW at breeding and reproductive performance

		Trait			
Effect	Level	BW, kg	NLB, n	NLW, n	LWW, kg
Birth type, n[1]	≤ 2	56.9 ± 1.02	2.14 ± 0.07	1.58 ± 0.08[a]	24.3 ± 1.25[a]
	≥ 3	57.1 ± 0.96	2.11 ± 0.07	1.28 ± 0.08[b]	19.8 ± 1.20[b]
Age, yr	1	42.3 ± 0.64[d]	1.53 ± 0.07[c]	1.15 ± 0.07[c]	12.1 ± 0.76[c]
	2	53.5 ± 0.73[c]	2.08 ± 0.07[b]	1.56 ± 0.07[a,b]	27.4 ± 1.10[a]
	3	62.0 ± 0.84[b]	2.46 ± 0.09[a]	1.70 ± 0.10[a]	26.6 ± 1.41[a]
	4	61.6 ± 0.88[b]	2.30 ± 0.11[a,b]	1.50 ± 0.10[a,b]	25.3 ± 1.61[a,b]
	5	65.4 ± 1.02[a]	2.25 ± 0.13[a,b]	1.25 ± 0.14[b,c]	19.0 ± 2.11[b]
TMEM154 diplotype	"1,1"	56.2 ± 1.22	2.12 ± 0.08	1.56 ± 0.09	25.1 ± 1.46[a]
	"1,3"	57.8 ± 1.03	2.17 ± 0.07	1.43 ± 0.08	22.2 ± 1.32[a,b]
	"3,3"	56.7 ± 1.24	2.08 ± 0.09	1.30 ± 0.10	18.9 ± 1.64[b]

[1]Ewes born as singles and twins (≤ 2) or as triplets or greater (≥ 3).
[a–d]Means within an effect with no common superscript are different ($P \leq 0.03$).

Table 4. Least-squares means (± SE) for the main effects of birth type of the ewe and *TMEM154* diplotype on lifetime performance

		Trait		
Effect	Level	NLW_T, kg	LWW_T, kg	Longevity, *p*
Birth type, n[1]	≤ 2	6.82 ± 0.48[a]	105.6 ± 7.98[a]	0.70 ± 0.08[a]
	≥ 3	5.14 ± 0.44[b]	79.9 ± 7.31[b]	0.47 ± 0.08[b]
TMEM154 diplotype	"1,1"	7.39 ± 0.56[a]	119.4 ± 9.39[a]	0.77 ± 0.08
	"1,3"	5.56 ± 0.52[b]	86.0 ± 8.60[b]	0.50 ± 0.09
	"3,3"	5.00 ± 0.60[b]	72.9 ± 10.1[b]	0.48 ± 0.11

[1]Ewes born as singles and twins (≤ 2) or as triplets or greater (≥ 3).
[a,b]Means within an effect with no common superscript are different ($P \leq 0.05$)

meat and wool type ewes was dependent on breed and lactation stage in Snowder et al. (1990b), but seronegative ewes generally had greater milk production than seropositive ewes. While milk production or measures of udder health were not collected in the present study, 13/16 ewes culled for observed mastitis or insufficient milk production were diplotype "1,3" or "3,3."

Results from this study demonstrate that variants within *TMEM154* impact lifetime susceptibility to OPPV infection in naturally exposed ewes. Haplotype "3" had complete dominance over haplotype "1", and < 10% of diplotype "1,1" ewes became infected through 5.5 yr of age, whereas > 80% of diplotype "1,3" and "3,3" ewes were infected by 3.3 yr of age. Reduced productivity of diplotype "1,3" and "3,3" ewes is expected to be due to associated effects of clinical and subclinical OPP rather than direct effects of *TMEM154*. The length of the experiment was similar to the average cull age of ewes in the U.S. (6.3 yr; USDA APHIS, 2012) but shorter than the natural expected lifespan of sheep. Nevertheless, diplotype "1,1" ewes weaned, on average, 2.1 more lambs and 40 kg greater weight of lamb over 5.5 production year than diplotype "1,3" and "3,3" ewes. Based on an average price of $4.27/kg for 18- to 27-kg feeder lambs marketed in the states of CO, SD, and TX during the years of this study (2012–2016; USDA AMS, 2020), this equates to US$171 in additional lifetime revenue per *TMEM154* diplotype "1,1" ewe. Increasing the frequency of *TMEM154* haplotype "1" within a flock promotes genetic resilience to OPP and should be considered for incorporation into routine husbandry to mitigate the effects of this costly disease.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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