

RESEARCH ARTICLE

Genes involved in immune, gene translation and chromatin organization pathways associated with *Mycoplasma ovipneumoniae* presence in nasal secretions of domestic sheep

Michelle R. Mousel^{1,2*}, Stephen N. White^{1,3,4}, Maria K. Herndon³, David R. Herndon¹, J. Bret Taylor⁵, Gabrielle M. Becker⁶, Brenda M. Murdoch^{4,6}

1 U.S. Department of Agriculture, Animal Disease Research Unit, Agricultural Research Service, Pullman, WA, United States of America, **2** Paul G. Allen School of Global Animal Health, Washington State University, Pullman, WA, United States of America, **3** Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, United States of America, **4** Center for Reproductive Biology, Washington State University, Pullman, WA, United States of America, **5** U.S. Department of Agriculture, Range Sheep Production Efficiency Research, Agricultural Research Service, Dubois, ID, United States of America, **6** Animal, Veterinary, and Food Sciences, University of Idaho, Moscow, ID, United States of America

* michelle.mousel@usda.ars



OPEN ACCESS

Citation: Mousel MR, White SN, Herndon MK, Herndon DR, Taylor JB, Becker GM, et al. (2021) Genes involved in immune, gene translation and chromatin organization pathways associated with *Mycoplasma ovipneumoniae* presence in nasal secretions of domestic sheep. PLoS ONE 16(7): e0247209. <https://doi.org/10.1371/journal.pone.0247209>

Editor: Hoh Boon-Peng, UCSI University, MALAYSIA

Received: January 31, 2021

Accepted: June 26, 2021

Published: July 12, 2021

Copyright: This is an open access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the [Creative Commons CC0](https://creativecommons.org/licenses/by/4.0/) public domain dedication.

Data Availability Statement: Genotypes and phenotypes by ewe for the significant SNPs may be found at <https://data.nal.usda.gov/dataset/data-genomic-regions-associated-mycoplasma-ovipneumoniae-presence-nasal-secretions-domestic-sheep>.

Funding: As USDA employees, MRM, SNW, and DRH received research funds by USDA-ARS CWA No. 2090-32000-036-00D (<http://www.ars.usda>).

Abstract

Mycoplasma ovipneumoniae contributes to polymicrobial pneumonia in domestic sheep. Elucidation of host genetic influences of *M. ovipneumoniae* nasal detection has the potential to reduce the incidence of polymicrobial pneumonia in sheep through implementation of selective breeding strategies. Nasal mucosal secretions were collected from 647 sheep from a large US sheep flock. Ewes of three breeds (Polypay $n = 222$, Rambouillet $n = 321$, and Suffolk $n = 104$) ranging in age from one to seven years, were sampled at three different times in the production cycle (February, April, and September/October) over four years (2015 to 2018). The presence and DNA copy number of *M. ovipneumoniae* was determined using a newly developed species-specific qPCR. Breed ($P < 0.001$), age ($P < 0.024$), sampling time ($P < 0.001$), and year ($P < 0.001$) of collection affected \log_{10} transformed *M. ovipneumoniae* DNA copy number, where Rambouillet had the lowest ($P < 0.0001$) compared with both Polypay and Suffolk demonstrating a possible genetic component to detection. Samples from yearlings, April, and 2018 had the highest ($P < 0.046$) detected DNA copy number mean. Sheep genomic DNA was genotyped with the Illumina OvineHD BeadChip. Principal component analysis identified most of the variation in the dataset was associated with breed. Therefore, genome wide association analysis was conducted with a mixed model (EMMAX), with principal components 1 to 6 as fixed and a kinship matrix as random effects. Genome-wide significant ($P < 9 \times 10^{-8}$) SNPs were identified on chromosomes 6 and 7 in the all-breed analysis. Individual breed analysis had genome-wide significant ($P < 9 \times 10^{-8}$) SNPs on chromosomes 3, 4, 7, 9, 10, 15, 17, and 22. Annotated genes near these SNPs are part of immune (*ANAPC7*, *CUL5*, *TMEM229B*, *PTPN13*), gene translation (*PIWIL4*), and

[gov/main/main.htm](https://doi.org/10.1371/journal.pone.0247209.g001)). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

chromatin organization (*KDM2B*) pathways. Immune genes are expected to have increased expression when leukocytes encounter *M. ovipneumoniae* which would lead to chromatin reorganization. Work is underway to narrow the range of these associated regions to identify the underlying causal mutations.

Introduction

Mycoplasma ovipneumoniae is considered a commensal bacterium [1] found in domesticated sheep and goats, but it can at times play a role in the aetiology of chronic, non-progressive pneumonia [2,3]. The bacteria adhere to and infect airway epithelial cells and can cause oxidative damage and apoptosis through an *extracellular-signal-regulated kinase* signaling-mediated mitochondrial pathway [4]. In addition, *M. ovipneumoniae* can moderate the host immune system by eliciting alterations in macrophage activity [5], suppress lymphocytes [6], and induce production of autoantibodies to host ciliary antigen [7].

Little is known about the lifecycle of *M. ovipneumoniae* in domestic sheep. At slaughter, 83 to 90% of lambs' lungs with chronic bronchopneumonia were positive for *M. ovipneumoniae* [8,9]. Nasal and lung isolates may not be independent populations, as a longitudinal study of New Zealand lambs found the same isolate of *M. ovipneumoniae* in lungs at slaughter as well as in the nasal cavity about three weeks before slaughter using older DNA technology [10]. In the U.S., 88.5% of flocks had individual sheep with *M. ovipneumoniae* detected in their nasal secretions (APHIS Info Sheet #708.0615). Using National Animal Health Monitoring System data, comparison of positive with negative *M. ovipneumoniae* flocks revealed a slight decrease in productivity but known effects that could contribute to this small difference (~4% [11]), such as litter size and ewe age, were not considered in the comparison. Seroprevalence of *M. ovipneumoniae* was found to be highly variable (9.76 to 30.61%) among Kazak, Hu, Merino, and Duolong sheep breeds [12]. This breed variability suggested there may be a host genetic component that is impacting bacterial shedding [12,13]. Genome-wide association studies (GWAS) can identify associated genomic regions of interest with underlying causal mutations that affect infectious disease susceptibility.

Technologies have been developed to improve the probability of identifying genomic regions associated with phenotypic traits of interest in sheep. The Ovine SNP50 beadchip [14] and the OvineHD BeadChip (600K) were collaboratively, internationally developed and have been used to identify new markers associated with inherited diseases [15–17], erythrocyte traits [18], parasite infection [19–21], and other infectious disease traits [22–25].

Previous exposure to *M. ovipneumoniae* can be assessed by the presence of antibodies [12], though this does not enable quantification of bacteria present. There are several molecular amplification assays used to detect this bacterium [26–28] with varying specificity depending on the genomic region of the bacterium targeted. In order to reduce false positives and ensure bacterium-specific quantification of *M. ovipneumoniae*, a species-specific quantitative PCR was developed for use with DNA from nasal secretions. Previous work utilizing a modified PCR [29] for screening of domestic animals and wildlife resulted in unintended or non-specific amplification of other bacteria [30]. The assay used in this study permitted a more precise measurement of *M. ovipneumoniae*.

The first objective of this study was to quantify longitudinal detection of *M. ovipneumoniae* DNA copy number from nasal secretions in a large, extensively managed flock composed of three prevalent US sheep breeds to determine patterns in detection per ewe and/or per breed. Our second objective was to identify genomic regions associated with detection of *M.*

ovipneumoniae DNA copy number given patterns of detection and/or breed differences identified in objective one. The breeds evaluated were developed and continue to be selected for high quality wool, fast growing, muscular lambs, or larger litters with good mothering ability to ensure wide applicability of the findings for the sheep industry. This study elucidated *M. ovipneumoniae* DNA copy number detection patterns over time and identified genomic regions associated with detected *M. ovipneumoniae* DNA copy number. Validation studies and fine mapping of markers from these genomic regions will allow for the possibility of applying selective breeding methods to reduce susceptibility to nasal shedding of *M. ovipneumoniae* in domestic sheep.

Materials and methods

Ethics statement

All animal care and handling procedures were reviewed and approved by the Washington State University Institutional Animal Care and Use Committee (Permit Number: 4885 and 4594) and/or by the U.S. Sheep Experiment Station Animal Care and Use Committee (Permit Numbers:15-04, 15-05).

Sheep

Whole blood (jugular venipuncture) and nasal mucosal secretions were collected from ewes of Rambouillet (N = 321), Polypay (N = 222), and Suffolk (N = 104) breeds, ranging in age from 1 to 7 years. Repeated sampling occurred three times per year (February, April, and September/October) for 4 years (2015–2018). Ewes were managed as a single flock in an extensive rangeland production system. Sampling corresponded with mid-pregnancy (February), early-lactation (~35 days post lambing, April), and post-weaning (~30 days post-weaning, September/October) production stages. At mid-pregnancy and early-lactation sampling events, ewes had been managed in a penned, close-contact environment (e.g., dry-lot) and fed harvested feeds for at least 45 and 120 days, respectively. At the post-weaning sampling event, ewes had been managed in an extensive rangeland grazing environment. Preliminary (unpublished) data of a very small number of sheep housed under the same intensive management conditions year-round, suggested that although some variation in the DNA copy number of *M. ovipneumoniae* existed, three sample time points were sufficient to detect a pattern. Therefore, a minimum of three time points was collected from each ewe but we were unable to sample every ewe at each timepoint. The number of sheep available over the four-year period was variable, as some animals were removed from the flock for other health or production reasons, each ewe was sampled 3 to 10 times, for a total of 2,876 nasal swabs collected for this study.

M. ovipneumoniae detection

Secretions were collected with sterile cotton swabs that were inserted approximately 10 cm into each nostril, consecutively, and rotated briefly. Swabs were stored without media at -20°C until DNA was extracted with QiaAmp DNA Mini kit (Qiagen, Germantown, MD). Nasal shedding status of *M. ovipneumoniae* was determined by qPCR with newly designed species-specific primers 439F: TGATGGAACATTATTGCGCT and 439R: TGCCATTATTTGAAACRAGA. Reactions consisted of 1uL each (10uM) primer, 10uL of SsoFast EvaGreen Super mix (BioRad), 6uL of distilled water and 2uL of DNA template diluted 1:10. Each sample was run in triplicate on a CFX96 Touch system (BioRad, Hercules, CA) and quantified using a 10-fold dilution series standard curve ranging from 0 to 10⁶ copies (S1 Fig). The standard curve was

generated with DNA from the *M. ovipneumoniae* Y98 type-strain cultured in SP4 medium, pelleted by centrifugation and extracted with the QiaAmp DNA Mini kit.

Genotyping

Blood was collected into EDTA-coated vacutainer tubes. Genomic DNA was isolated using the Invitrogen GeneCatcher gDNA 3–10 ml Blood Kit (Life Technologies, Carlsbad, CA) or GenTra PureGene (Qiagen, Germantown, MD), with minor modifications of manufacturers' instructions. Quality and quantity of DNA were quantified using an ND-1000 spectrophotometer (Nanodrop, Wilmington, DE) and 300ng per ewe was sent for genotyping. Genotyping services were provided by Geneseek Inc. (Lincoln, NE) using the OvineHD BeadChip (Illumina Inc., San Diego, CA) with a set of greater than 600,000 SNP designed by the International Sheep Genome Consortium.

Phenotype analysis

Univariate analysis was conducted (SAS v9.4; SAS Inst. Inc., Cary, NC) to identify outliers and test for normality. *Mycoplasma ovipneumoniae* DNA copy number data were not normal, and therefore, values greater than zero were transformed to \log_{10} to reduce heterogenous variances. To test if there were shedding differences a reduced mixed model in SAS that accounted for fixed effects of breed, age at sample collection, year of sample collection, and approximate month of sample collection, ewe as a repeated effect, and sire as a random effect was conducted. Tukey-Kramer adjustment was implemented to identify pair-wise differences.

Association analysis

A preliminary screen for high genotype call rates (>95%) was performed to select individuals for further analysis. Single nucleotide polymorphism inclusion screening criteria was conducted with PLINK1.9 [31] as previously described [23]: missingness by individual (0.1), missingness by marker (0.05), minor allele frequency (0.01), and Hardy-Weinberg equilibrium (0.0000001, which corresponds to $P = 0.05$ after Bonferroni correction for 600,000 marker tests).

Quality control was performed on 647 individual samples and 606,006 SNP sites using Plink1.90 software. The following SNPs were excluded: 1) 203 for duplicate base pair location on the same chromosome; (2) 14,953 not in accordance with the Hardy-Weinberg equilibrium; 3) 31,770 with a minor allele frequency less than 1%; 4) 26,412 with genotyping rate less than 95%. In addition, multidimensional scaling was conducted to identify breed outliers. Three individuals were excluded because of failure to cluster with breed in multidimensional scaling (Fig 1). Genotyping rate was 99.8% for 644 sheep and 532,668 SNPs were used in the analyses.

Data were reformatted in PLINK1.9 for use with Efficient Mixed-Model Association eXpedited (EMMAX) [32]. The full model evaluated included fixed effects of breed or principal components 1 to 6 (eigenvalues greater than 4.9; generated with PLINK) and random effect of relationship matrix (Balding-Nichols estimate). For individual breed analysis, principal components with eigenvalues greater than 4.9 were Polypay PC1-2, Rambouillet PC1-4, and Suffolk PC1 were considered fixed effects and random effect of relationship matrix (Balding-Nichols estimate). Phenotypes considered were 1) binomial where all samples from an ewe were either greater or were less than or equal to 100 DNA copy numbers, 2) categorical where a mixed detection among sampling times was added to the binomial data, and 3) untransformed mean DNA copy number. Genome-wide suggestive significance was set at a nominal $P < 9 \times 10^{-8}$ consistent with other sheep GWAS using the OvineHD Beadchip [33]. In R, qqman [34] was used

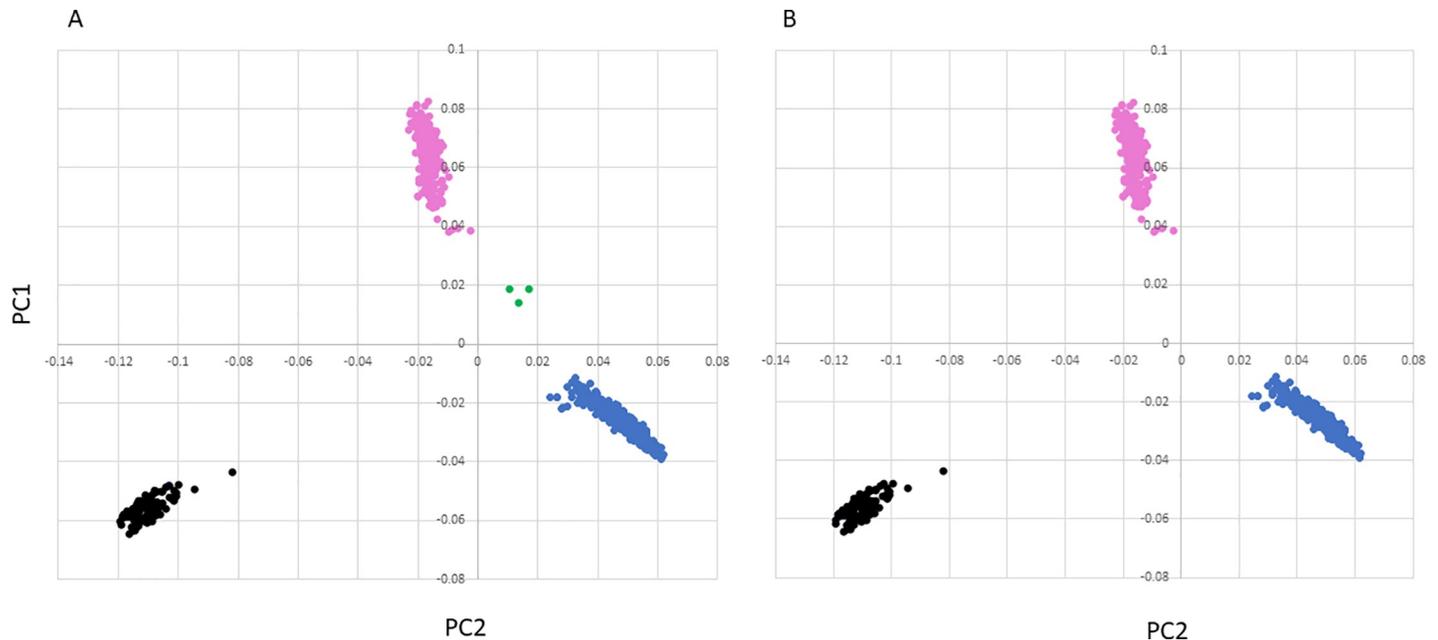


Fig 1. Plots of the first 2 principal components with 647 sheep showing breed outliers (A) and without the 3 outliers (B). Blue represents Rambouillet, pink represents Polypay, black represents Suffolk and green represents the 3 outliers.

<https://doi.org/10.1371/journal.pone.0247209.g001>

for visualization of results in Manhattan and quantile-quantile plots. Further, the significant SNPs were interrogated using a mixed model similar to those described above, accounting for \log_{10} transformed *M. ovipneumoniae* DNA copy number, breed, age, year, sample time, and genotype with SAS.

Ensembl [35] and NCBI [36] were used to determine the location of the SNP within genome assembly OAR_Rambouillet_v1.0 as well as identify annotated genes within 100 kb of the SNP. The Illumina designated name of the SNP with the dbSNP rs# cluster id, the dataset where the significant SNP was identified, minor allele, and *M. ovipneumoniae* mean DNA copy trend associated with the minor allele can be found in [S1 Table](#). Genotypes and phenotypes by ewe for the significant SNPs may be found at <https://data.nal.usda.gov/dataset/data-genomic-regions-associated-mycoplasma-ovipneumoniae-presence-nasal-secretions-domestic-sheep>. GeneCards [37] and a search of Pubmed [36] was used to provide known information about genes within 100kb of the associated SNPs. Reactome [38] was used to determine the pathway(s) associated with the annotated genes.

Results

Mycoplasma ovipneumoniae DNA copy number

Nasal mucosal secretions were collected from three to 10 times from each ewe, median number of collections was four. Untransformed *M. ovipneumoniae* DNA copy number ranged from 0 to 109,000 with a mean of 1,461 and median of 22. Transformed (\log_{10}) *M. ovipneumoniae* DNA copy number ranged from 0 to 5.03 with mean of 1.52 and median of 1.34. There were 224 ewes with DNA copy number categorized as ≤ 100 (zero to low detection, considered not detected) for all samplings; specifically, these ewes were sampled three to eight times with a mode of four samplings. Ninety-four ewes consistently had DNA copy number greater than 100 (considered positive for each sample time) for all sampling times; these ewes were sampled

Table 1. Mean *M. ovipneumoniae* log₁₀ transformed DNA copy number in breed and age of ewes; year and month of sample collection.

	N	Mean	StdErr	P-value
Breed				<0.0001
Polypay	220	1.73 ^a	0.083	
Rambouillet	320	1.12 ^b	0.071	
Suffolk	104	1.97 ^a	0.12	
Age (years)				0.0236
1	519	1.91 ^a	0.096	
2	599	1.68 ^b	0.079	
3	453	1.58 ^b	0.081	
4	464	1.52 ^b	0.082	
5	407	1.52 ^b	0.084	
6	293	1.51 ^b	0.01	
7	141	1.54 ^{a,b}	0.13	
Year				<0.0001
2015	370	1.22 ^a	0.086	
2016	904	1.65 ^b	0.064	
2017	974	1.6 ^b	0.066	
2018	628	1.96 ^c	0.085	
Month				<0.0001
February	917	1.2 ^a	0.064	
April	1137	1.91 ^b	0.062	
September/October	822	1.71 ^c	0.068	

a–c Means within Mean column for each effect with different superscripts differ ($P < 0.05$). Mean separation tests were adjusted for multiple comparisons using the Tukey-Kramer option in PROC Mixed (SAS Inst. Inc., Cary, NC).

<https://doi.org/10.1371/journal.pone.0247209.t001>

three to ten times with a mode of four samplings. Finally, there were 326 ewes with mixed levels of detection (above and below 100) when sampled over time where each ewe was sampled three to six times with a mode of four samplings. There were only 28 ewes with zero DNA copy numbers detected at all time points tested and these ewes were sampled three to six times with a mode of four samplings.

There were significant ($P < 0.0001$) differences among breeds of log₁₀ transformed *M. ovipneumoniae* DNA copy number (Table 1). Rambouillet ewes had lower ($P < 0.0001$) detected DNA copy numbers than Polypay and Suffolk ewes which were not different ($P > 0.21$). Detected DNA copy number was greatest for yearlings and declined with increasing age until a slight increase at 7 years old (Table 1). Yearling ewes had significantly ($P < 0.046$) higher detected DNA copy numbers than ewes 2 through 6 years of age. No other pair-wise age comparisons were different ($P > 0.08$). Time during the year samples were collected significantly ($P < 0.0001$) impacted DNA copy number (Table 1). All three sample periods were different ($P < 0.003$) from one another where April had the highest and February had the lowest mean DNA copy number. Year of sample collection significantly ($P < 0.0001$) impacted detected DNA copy number, with means increasing with time (Table 1). All years were different ($P < 0.004$) in pair-wise comparisons except for 2016 and 2017 ($P > 0.87$).

Association analysis

No significant genomic associations were identified when *M. ovipneumoniae* DNA copy number detection was considered binomial, where all samples from an ewe were either positive or

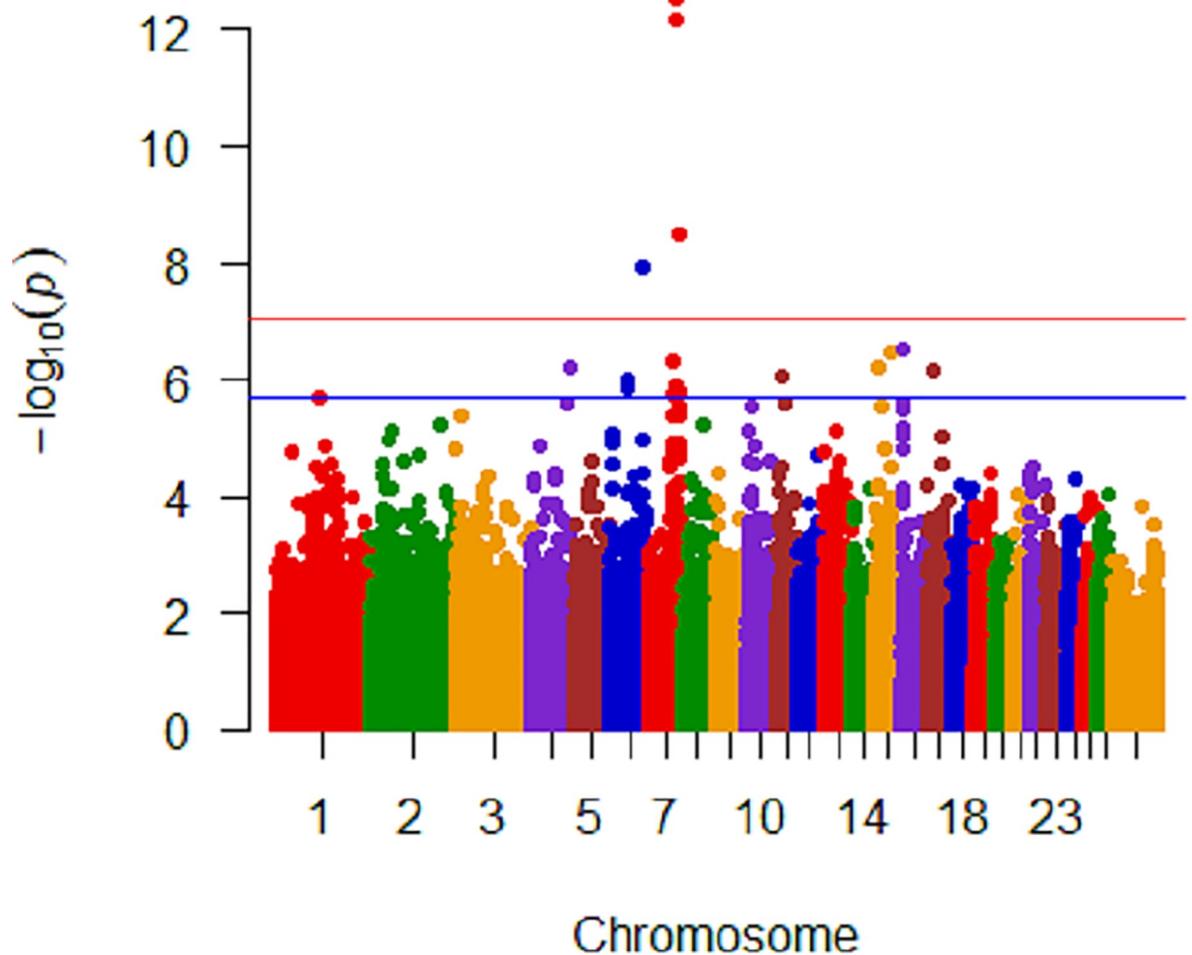


Fig 2. Manhattan plot of all breed analysis with untransformed *M. ovipneumoniae* mean DNA copy number showing significant SNPs on chromosomes 6 and 7. Genome significant line is red and genome suggestive line is blue.

<https://doi.org/10.1371/journal.pone.0247209.g002>

undetected or when categorization included mixed detection. Seven SNPs were identified as significantly ($P < 10^{-8}$) associated with untransformed mean *M. ovipneumoniae* nasal shedding (Fig 2). These SNPs represented 3 regions on chromosomes 6 and 7 (Table 2) where the least frequent genotype for all seven SNPs had the highest \log_{10} DNA copy number and the minor allele was present in all breeds. There were 4 closely spaced SNPs on chromosome 7, approximately 21Kb apart, that were highly significant (2.72 to 6.60×10^{-13}) within 50Kb of *Transmembrane protein 229B* (*TMEM229B*). An additional SNP, at approximately 91Kb, on chromosome 7 was significant at 3.15×10^{-9} and was within 50Kb of a postulated gene *ENSOARG00000026723*. The two SNPs on chromosome 6 were in introns of *Protein Tyrosine Phosphatase Non-Receptor Type 13* (*PTPN13*; 1.21×10^{-8}). Quantile-Quantile plots are shown in Fig 3, where A includes all SNPs in the dataset analyzed, showing extreme deviation with the most significant SNPs. Panel B in Fig 3 shows a Q-Q plot that had the top seven SNPs included in the model when reanalyzed, demonstrating their impact.

Analysis of untransformed *M. ovipneumoniae* DNA copy number was conducted within each breed with 320, 220, and 104 ewes classified as Rambouillet, Polypay, and Suffolk, respectively (Table 3). Within Rambouillet, there were nine SNPs on five chromosomes (3, 4, 9, 10,

Table 2. Significant SNPs associated with untransformed *M. ovipneumoniae* mean DNA copy number in the all-breed analysis.

SNP	CHR	Position	MAF (%)	Nominal P-value	Closest gene
rs159990985	6	111960643	3.261	1.21E-08	PTPN13
rs159991022	6	111973304	3.261	1.21E-08	PTPN13
rs426816169	7	82832532	3.571	2.72E-13	TMEM229B
rs411153659	7	82840000	3.571	2.72E-13	TMEM229B
rs400632990	7	82852837	3.339	6.60E-13	TMEM229B
rs427671730	7	82854206	3.339	6.60E-13	TMEM229B
rs424565925	7	99167837	4.51	3.15E-09	ENSOARG00000026723

Listed chromosome position is from Oar_Rambouillet v1.0.

<https://doi.org/10.1371/journal.pone.0247209.t002>

and 22) that were significantly ($<10^{-8}$) associated with *M. ovipneumoniae* mean DNA copy number (Fig 4). Three significant SNPs on chromosome 4 were within *Thyrotropin Releasing Hormone Degrading Enzyme (TRHDE)* and one significant SNP was within 25Kb of *TRHDE*. The two significant SNPs on chromosome 22 were within *Solute Carrier Family 16 Member 12 (SLC16A12)*. Analysis of only Polypay identified three SNPs on two chromosomes (7 and 17) that were significantly ($P < 4.63 \times 10^{-8}$ and 9.34×10^{-8} , respectively) associated with *M. ovipneumoniae* mean DNA copy number (Fig 5). The significant SNPs on chromosome 17 were within *Lysine-specific demethylase 2b (KDM2B)* and *Anaphase Promoting Complex Subunit 7 (ANAPC7)*. Nine SNPs on two chromosomes (7 and 15) were significantly associated with *M. ovipneumoniae* mean detection in the Suffolk-only analysis (Fig 6). Eight of these nine significant SNPs were on chromosome 15, with three of the SNPs within 31Kb of one another and two SNPs within *piwi (P element-induced Wimpy)-like RNA-mediated gene silencing 4 (PIWIL4)*. In addition, one SNP was within *Unc-13 Homolog C (UNC13C)* on chromosome 15 and one on chromosome 7 was within 10Kb of *Cullin 5 (CUL5)*.

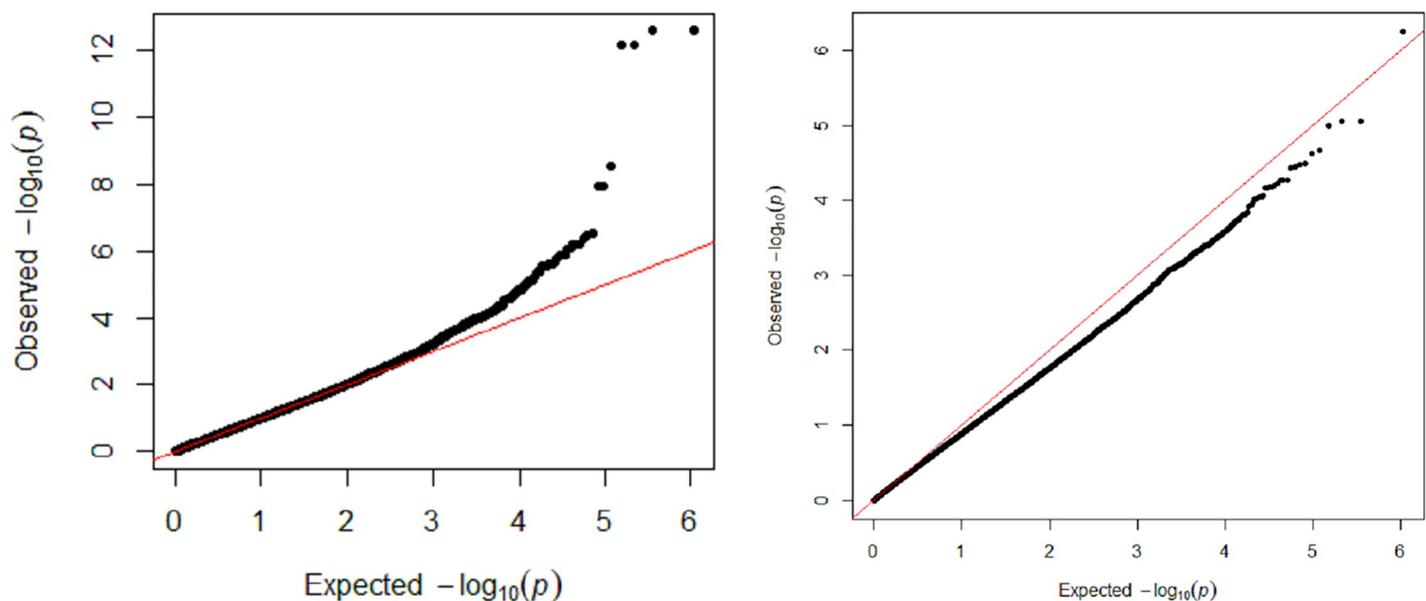


Fig 3. Quantile-Quantile plots for the all-breed analysis with untransformed *M. ovipneumoniae* mean DNA copy number, showing deviation due to significant SNPs (A) and less deviation when top 7 SNPs are included as covariates in the analysis (B).

<https://doi.org/10.1371/journal.pone.0247209.g003>

Table 3. Significant SNPs associated with untransformed *M. ovipneumoniae* mean DNA copy number in the individual breed analysis.

SNP	CHR	Position	MAF(%)	Breed	Nominal P-value	Closest Gene
rs403152750	3	115055558	1.56	Rambouillet	5.30E-08	TRHDE
rs404662185	3	115060178	1.57	Rambouillet	5.24E-08	TRHDE
rs409357561	3	115062407	1.56	Rambouillet	5.30E-08	TRHDE
rs407012017	3	115135930	1.56	Rambouillet	5.30E-08	TRHDE
rs398278706	4	10400045	1.25	Rambouillet	8.31E-13	ENSOARG00000025219
rs420722286	7	57732050	5.34	Suffolk	7.23E-09	UNC13C
rs402243563	7	99714676	1.406	Polypay	4.63E-08	N/A
rs160633679	9	15144452	1.122	Rambouillet	1.43E-10	ENSOARG00000016023
rs429475222	10	75915173	1.406	Rambouillet	6.81E-09	N/A
rs410915115	15	17401773	1.923	Suffolk	3.53E-10	PIWIL4
rs406526355	15	17412929	1.923	Suffolk	3.53E-10	PIWIL4
rs413355105	15	17431988	3.365	Suffolk	4.79E-08	PIWIL4
rs406613692	15	17480590	2.885	Suffolk	4.82E-09	ENSOAG0000009780
rs418601541	15	19401648	2.885	Suffolk	2.02E-08	CUL5
rs590224797	15	21333112	4.327	Suffolk	2.92E-08	C11of87
rs406524055	15	21333678	4.327	Suffolk	2.92E-08	C11of87
rs409884491	15	21442130	2.404	Suffolk	1.27E-10	C11of87
rs402253048	17	62654668	1.591	Polypay	9.34E-08	KDM2B
rs160856157	17	63273726	1.591	Polypay	9.34E-08	ANAPC7
rs429858348	22	12903174	1.406	Rambouillet	1.31E-08	SLC16A12
rs413436283	22	12912440	1.406	Rambouillet	1.31E-08	SLC16A12

Listed chromosome position is from Oar_Rambouillet v1.0.

<https://doi.org/10.1371/journal.pone.0247209.t003>

Discussion

This is the first known publication of repeated, sequential *M. ovipneumoniae* nasal secretion testing in domestic sheep, with testing spanning 4 contiguous years. Detection and quantification of *M. ovipneumoniae* was influenced by sheep breed and age, and sampling time and year of sample collection. In concordance with Cheng et al. [12], we identified differences in the detection of *M. ovipneumoniae* in three different breeds of sheep. Rambouillet had the lowest detected DNA copy numbers for all sampling times measured. Similar studies that found Rambouillet had lower levels of ovine progressive pneumonia virus compared with Polypay and Columbia [23,39]. While not significantly different from Polypay, Suffolk had numerically the highest mean detection of *M. ovipneumoniae*. A Japanese survey study found variable detection (0 to 53%) in 13 different flocks from three prefectures and the positive flocks had at least one serologically positive animal representing 9 different breeds and crossbreeds, including Suffolk [40]. Flocks with greater than 150 sheep or Suffolk tended to have a higher percentage of positive sheep [40] which this study agrees with and supports.

We identified a shedding pattern among the sheep, where a subset consistently did not have detectable *M. ovipneumoniae* DNA, was always detected, or had mixed detection. Understanding this variability is important for conducting genomic studies and improves the possibility for selection of non-shedding sheep. Unfortunately, there were too few animals in the always detected category to identify genomic associations with the categorical analysis in this study. In other livestock, approximately 4.8% of cattle were positive (nasal secretions) for *Mycoplasma bovis* testing [41]. A longitudinal study of dairy cattle also found a low positivity rate of about 4% but identified one heifer that was shedding *M. bovis* for each time tested over 2 years

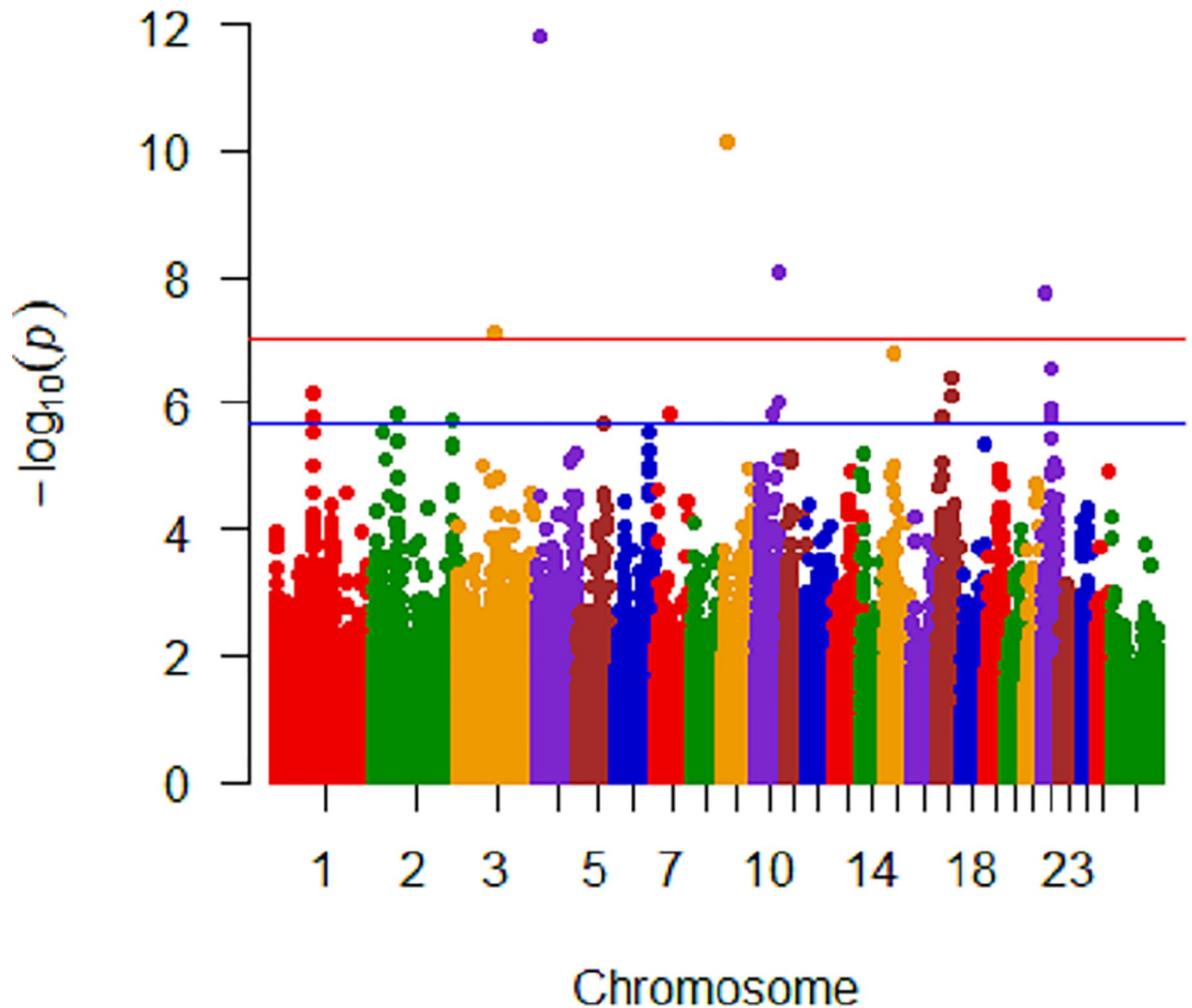


Fig 4. Manhattan plot of Rambouillet analysis with untransformed *M. ovipneumoniae* mean DNA copy number. Significant SNPs are on chromosomes 3, 4, 9, 10, and 22. Genome significant line is red and genome suggestive line is blue.

<https://doi.org/10.1371/journal.pone.0247209.g004>

[42]. In this study, we found 14.6% of ewes were positive every time tested and an overall positivity rate of 38.3%. Detection of *M. ovipneumoniae* was greatest in yearlings and decreased as the sheep aged. Converse of DNA copy number data, serological data indicated the number of positive animals increased with age [40] which might suggest that some individuals seroconvert at extended intervals post-exposure, or that colonization is reduced in aging animals to below levels of detection.

April had the greatest mean DNA copy number. When sheep are in close confinement, such as for the longest period before testing in April, opportunity for direct-contact transmission increases. Experimental transmission of *M. hyopneumoniae* in pigs has been demonstrated to be due to direct contact and less effective with indirect exposure [43]. September/October mean DNA copy number was intermediate to February and April. This was somewhat unexpected as the sheep were on open range grazing for approximately 3 months before testing. Although, while on open range, sheep are herded into a small area with close contact every night to better protect them from predators. Temperature and humidity were found to impact *Mycoplasma* infectivity in a Spanish study of lambs, with higher temperature and lower humidity having greater rates of infection [44]. Specifically, they found February, March, and

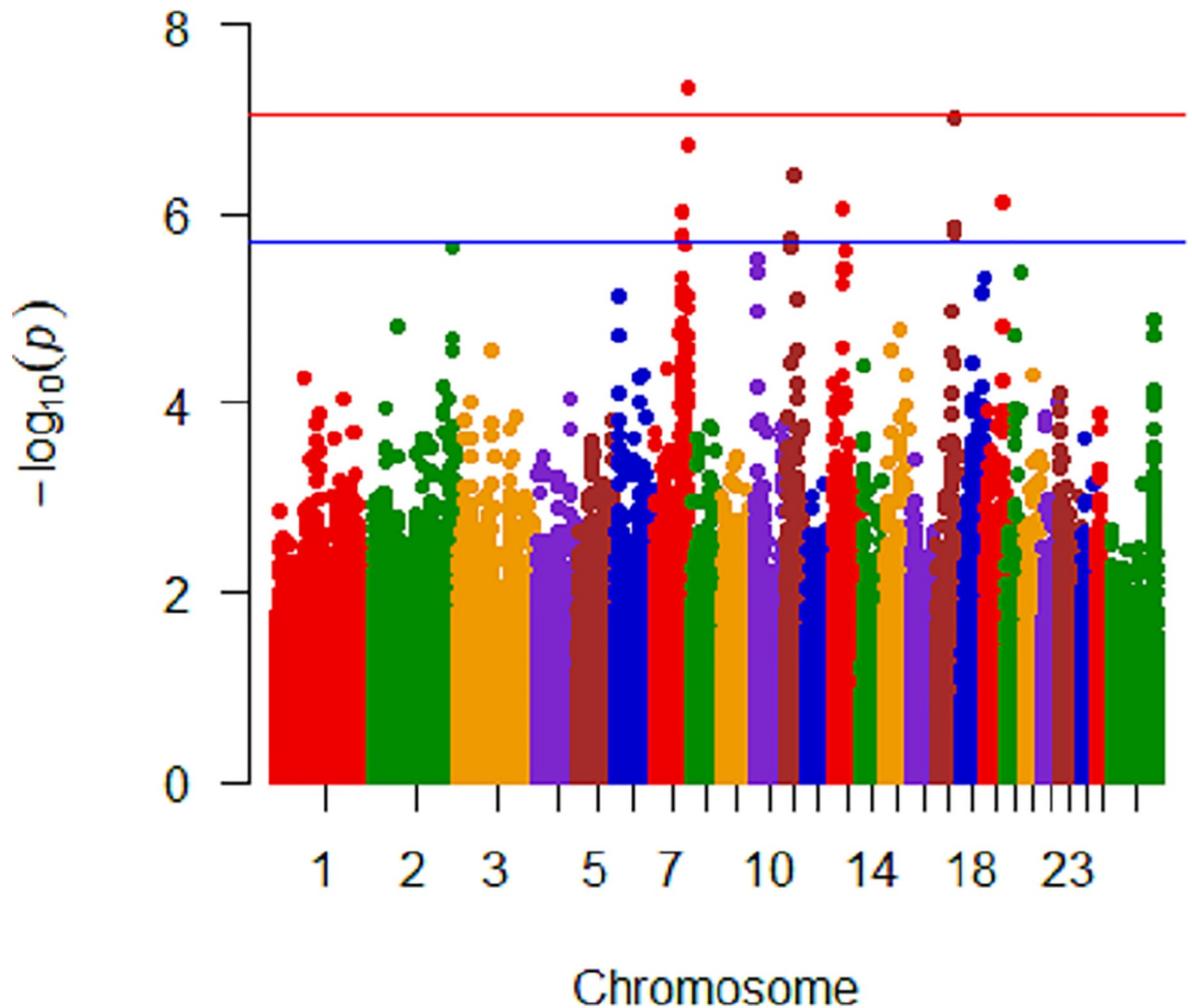


Fig 5. Manhattan plot of Polypay analysis with untransformed *M. ovipneumoniae* mean DNA copy number. Significant SNPs are on chromosomes 7 and 17. Genome significant line is red and genome suggestive line is blue.

<https://doi.org/10.1371/journal.pone.0247209.g005>

September to October had the lowest detection and April through August had the greatest. The Spanish and this study together demonstrate that transmission of *M. ovipneumoniae* among sheep is not necessarily just a function of proximity, but that climate may also be a factor.

Genomic associations with *M. ovipneumoniae* detection were identified in all-breed and individual-breed analyses. Within the all-breed analysis, significant SNPs were close to *TMEM229B* on chromosome 7 and *PTPN13* on chromosome 6. Both these genes are found in immune pathways, and impact T cells and innate lymphoid cell type 2. Specifically, *TMEM229B* was found to be down regulated in memory CCR6+ T cells in an HIV study [45]. The CCR6+ T cells direct Th17 cells to effector mucosal sites [46,47], where they secrete cytokines. Some of these cytokines stimulate neutrophils and other immune cells to promote clearance of extracellular bacteria, such as *Streptococcus pyogenes* (*Sp*), *Klebsiella pneumoniae*, and *Bordetella pertussis* [48–53]. Interestingly, *TMEM229B* was identified as under extreme selection in chickens from tropical and arid climates [54]. The immune system of these chickens would be hypothesized to respond differently to pathogens as the two environments in the signatures of selection study were vastly different. While *PTPN13* has been shown to play a role

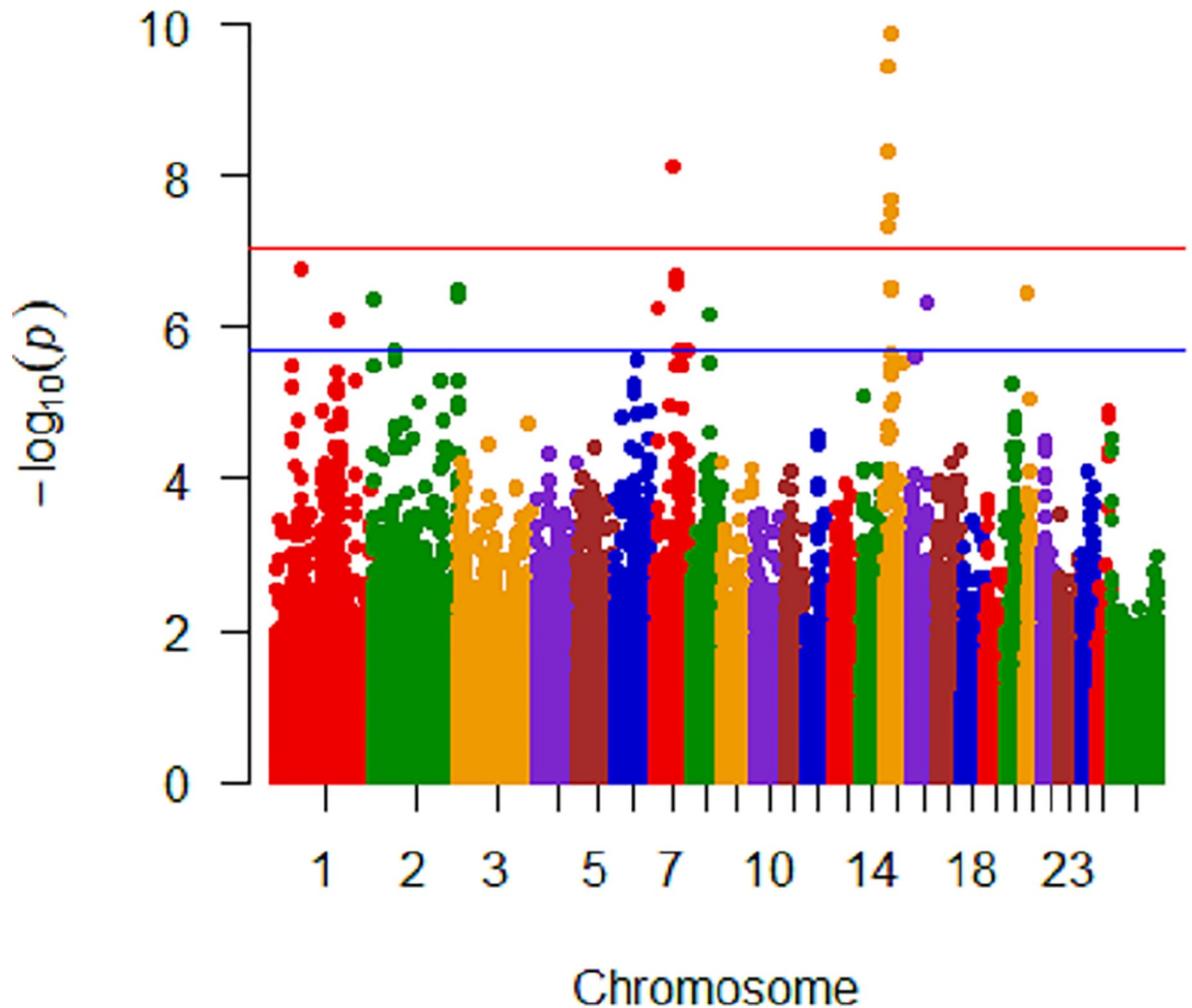


Fig 6. Manhattan plot of Suffolk analysis with untransformed *M. ovipneumoniae* mean DNA copy number. Significant SNPs are on chromosomes 7 and 15 Genome significant line is red and genome suggestive line is blue.

<https://doi.org/10.1371/journal.pone.0247209.g006>

in T cell activation [55], it is specifically expressed in Th17 T cells [56] and has variable expression in T cells of people with Rett Syndrome [57]. In addition, *PTPN13* is a conserved transcript in innate lymphoid cell type 2 (ILC2), which are considered helper-like and found in upper and lower airway and blood of humans [58] and mice [59]. In mouse lung, ILC2s were found to proliferate upon intranasal application of allergens, and memory-like lung ILC2s displayed upregulated expression of cytokines and their respective receptors, but also receptors for alarmins (*IL25R*, *ST2*), and genes associated with cell activation and proliferation (*BCL2A1B*, *IER3*) [60]. Also, *PTPN13* (PTP-BL) interacts with the Rho effector kinase protein kinase C-related kinase 2 (*PRK2*) [61] and *PRK2* has been demonstrated to have a role in cilia signaling [62] as well as regulation of the formation of apical junctions in bronchial epithelial cells [63]. Infection of a murine macrophage cell line with *M. ovipneumoniae* increases levels of nucleotide-binding oligomerization domain-containing 2 (*NOD2*) and *NOD2* along with activation of *c-Jun NH2-terminal protein kinase* (*JNK*) induces autophagy [64]. *NOD2* interacts with *RAC family small GTPase 1* (*Rac1*) in membrane ruffles [65] and it has been suggested that Rho GTPases such as *Rac1* regulate *PRK2* activity during control of the cell cycle [66].

In individual-breed analyses, 21 SNPs on 8 chromosomes were identified as associated with mean *M. ovipneumoniae* detection. One of these SNPs was in a similar chromosomal region as the all-breed analysis, but the majority were on different chromosomes. Specifically, eight SNPs were identified on chromosome 15 within three regions in Suffolk. *PIWIL4*, on chromosome 15, has been identified in the chromatin pathway [67,68], where human *PIWIL4* has a role in regulating gene expression in immune cells with gene silencing [69]. *In vitro* experiments showed that *PIWIL4* knockdown in primary human cells from people with HIV-1 on suppressive combined antiretroviral therapy had increased HIV-1 transcription and reversed HIV-1 latency in HIV-1 latently infected Jurkat T cells and primary CD4+ T lymphocytes, as well as resting CD4+ T lymphocytes [70]. Mutations in *CUL5*, also located on chromosome 15, have been associated with more rapid loss of CD4+ T cell in HIV infected patients [71].

Two SNPs on chromosome 17, near two different genes, were significantly associated in the Polypay analysis. The expression of *KDM2B* has been shown to help maintain ILC3 cells, while reduction in ILC3s leads to susceptibility to bacterial infection [72]. Interestingly, optimal Th17 cell formation in mice after *Streptococcus pyogenes* infection requires polycomb repressive complex 1.1, which includes *KDM2B* [73]. The other significant gene, *ANAPC7*, is a part of the anaphase promoting complex/cyclosome (APC/C), which is a substantial E3 ubiquitin ligase that controls mitosis by catalyzing ubiquitination of key cell cycle regulatory proteins [74]. In human lungs, an overexpression of *ANAPC7* protein has been identified and differential expression was found between smokers and non-smokers [75].

In the Rambouillet analysis, four SNPs were within or very near *TRHDE* on chromosome 3. This is an important regulator of thyroid hormones [76]. Although there does not appear to be a direct involvement of this gene with the immune system, a study found T- and B-cells from hyperthyroidic mice had higher mitogen-induced proliferation compared with the same cells from hypothyroidic mice [77]. Two SNPs were near an annotated gene, *SLC16A12*, a transmembrane transporter on chromosome 22. This gene was found to be induced by budesonide (a corticosteroid to treat asthma by reducing inflammation) in A549, BEAS-2B and primary human bronchial epithelial cells [78]. Interestingly, another GWAS examining susceptibility of mice to *Streptococcal pneumoniae* infection found an association with *Slc16a12* as well and the authors made the correlation with *Slc11a1*, another known solute carrier transporter, that has been found to affect susceptibility to bacterial infections [79].

As indicated above there are other bacteria and viruses that are associated with changes in immune function genes and future studies may further support findings here. It is not unexpected that a host's immune system reacts similarly to certain organisms. Additional studies will require the qualification and quantification of the repertoire of viruses and bacteria present in nasal mucosal secretions to improve our understanding of which organisms contribute to polymicrobial pneumonia and potentially confirm the findings of this study. Further studies are also needed to quantify the strains of *M. ovipneumoniae* in domestic sheep and if different strains contribute to polymicrobial pneumonia.

Conclusion

Domestic sheep nasal mucosal secretions had variable mean *M. ovipneumoniae* DNA copy number as detected with a new species-specific qPCR. Rambouillet had the lowest mean while Suffolk and yearlings had the greatest. Time of year samples were collected affected detected DNA copy number. Twenty-eight SNPs on 9 different chromosomes were associated with mean DNA copy number. Annotated genes near these SNPs have been identified in immune function pathways (*TMEM229B*, *PTPN13*, *CUL5*, and *ANAPC7*), chromatin organization (*KDM2B*), and gene silencing (*PIWIL4*). Nasal shedding of *M. ovipneumoniae* may affect

mucosal immunity, specifically Th17 cells. These data will be used to identify causal mutations, validate an association with improved control of *M. ovipneumoniae*, and causal mutations then may be developed into commercial markers for the domestic sheep industry.

Supporting information

S1 Fig. Representative standard curve for serial dilution of Y98 type-strain *M. ovipneumoniae* DNA.

(PPTX)

S1 Table. Name of significant SNPs on OvineHD Beadchip, rs number, the animal set in which the SNPs were identified, the minor allele, and trend of *M. ovipneumoniae* transformed \log_{10} mean DNA copy number associated with minor allele.

(DOCX)

Acknowledgments

The authors thank Dr. Maggie Highland for participating in concept development, sample coordination and collection, qPCR results analysis, and technical consultation. We very much appreciate the exceptional efforts of Nic Durfee in sample handling and processing and generation of qPCR data which were crucial to the success of the project, as well as Caylee Birge and Codie Durfee for sample handling and Ralph Horn, James Allison, Lori Fuller, and ADRU staff for technical assistance. We also acknowledge current and former USDA, Range Sheep Production Efficiency Research Unit (Dubois, ID, USA) employees Tom Kellom and Natalie Pierce for data collection, curation, and management. We gratefully appreciate Shiquan Wang, Mark Williams, Boyd Leonard, Ella Ybarlucea, Harley Carpenter, Joel Billman, Brad Eddins, Lyn Mortensen, Jack Hensley, Nicole Strong, and Jennifer Barnett for animal management, husbandry, and sample-collection assistance.

Author Contributions

Conceptualization: Michelle R. Mousel, Stephen N. White.

Data curation: Michelle R. Mousel, Stephen N. White, Maria K. Herndon.

Formal analysis: Michelle R. Mousel, Brenda M. Murdoch.

Funding acquisition: Michelle R. Mousel, Stephen N. White.

Investigation: Michelle R. Mousel, Maria K. Herndon, David R. Herndon.

Methodology: Michelle R. Mousel, Stephen N. White, David R. Herndon.

Project administration: Michelle R. Mousel, Stephen N. White.

Resources: J. Bret Taylor.

Supervision: Michelle R. Mousel, Stephen N. White, Brenda M. Murdoch.

Validation: Michelle R. Mousel.

Visualization: Michelle R. Mousel.

Writing – original draft: Michelle R. Mousel.

Writing – review & editing: Michelle R. Mousel, Stephen N. White, Maria K. Herndon, David R. Herndon, J. Bret Taylor, Gabrielle M. Becker, Brenda M. Murdoch.

References

1. Alley MR, Ionas G, Clarke JK. Chronic non-progressive pneumonia of sheep in New Zealand—a review of the role of *Mycoplasma ovipneumoniae*. *N Z Vet J*. 1999 Oct; 47(5):155–60. <https://doi.org/10.1080/00480169.1999.36135> PMID: 16032095
2. St George TD, Carmichael LE. *Mycoplasma ovipneumoniae* from sheep with chronic pneumonia. *Vet Rec*. 1975 Sep 13; 97(11):205–6. <https://doi.org/10.1136/vr.97.11.205> PMID: 1162872
3. Nicholas R, Ayling R, McAuliffe L. *Mycoplasma Diseases of Ruminants*. Wallingford: CABI. pp 171–179. 2008.
4. Li Y, Jiang Z, Xue D, Deng G, Li M, Liu X, et al. *Mycoplasma ovipneumoniae* induces sheep airway epithelial cell apoptosis through an ERK signaling-mediated mitochondria pathway. *BMC Microbiol*. 2016 Sep 23; 16(1):222. <https://doi.org/10.1186/s12866-016-0842-0> PMID: 27663303
5. Niang M, Rosenbusch RF, Lopez-Virella J, Kaeberle ML. Expression of functions by normal sheep alveolar macrophages and their alteration by interaction with *Mycoplasma ovipneumoniae*. *Vet Microbiol*. 1997 Oct 31; 58(1):31–43. [https://doi.org/10.1016/s0378-1135\(97\)00141-7](https://doi.org/10.1016/s0378-1135(97)00141-7) PMID: 9451459
6. Shahzad W, Ajuwape AT, Rosenbusch RF. Global suppression of mitogen-activated ovine peripheral blood mononuclear cells by surface protein activity from *Mycoplasma ovipneumoniae*. *Vet Immunol Immunopathol*. 2010 Jul; 136(1–2):116–21. <https://doi.org/10.1016/j.vetimm.2010.02.001> PMID: 20189658
7. Niang M, Rosenbusch RF, Andrews JJ, Lopez-Virella J, Kaeberle ML. Occurrence of autoantibodies to cilia in lambs with a ‘coughing syndrome’. *Vet Immunol Immunopathol*. 1998 Jul 31; 64(3):191–205. [https://doi.org/10.1016/s0165-2427\(98\)00133-0](https://doi.org/10.1016/s0165-2427(98)00133-0) PMID: 9730216
8. Lindstrom L, Tauni FA, Vargmar K. Bronchopneumonia in Swedish lambs: a study of pathological changes and bacteriological agents. *Acta Vet Scand*. 2018 Sep 17; 60(1):54. <https://doi.org/10.1186/s13028-018-0409-1> PMID: 30223865
9. Sheehan M, Cassidy JP, Brady J, Ball H, Doherty ML, Quinn PJ, et al. An aetiopathological study of chronic bronchopneumonia in lambs in Ireland. *Vet J*. 2007 May; 173(3):630–7. <https://doi.org/10.1016/j.tvjl.2006.01.013> PMID: 16632391
10. Ionas G, Mew AJ, Alley MR, Clarke JK, Robinson AJ, Marshall RB. Colonisation of the respiratory tract of lambs by strains of *Mycoplasma ovipneumoniae*. *Vet Microbiol*. 1985 Dec; 10(6):533–9. [https://doi.org/10.1016/0378-1135\(85\)90062-8](https://doi.org/10.1016/0378-1135(85)90062-8) PMID: 4095899
11. Manlove K, Branam M, Baker K, Bradway D, Cassirer EF, Marshall KL, et al. Risk factors and productivity losses associated with *Mycoplasma ovipneumoniae* infection in United States domestic sheep operations. *Prev Vet Med*. 2019 Jul 1; 168:30–38. <https://doi.org/10.1016/j.prevetmed.2019.04.006> PMID: 31097121
12. Cheng C, Jun Q, Qingling M, Zhengxiang H, Yu M, Xuepeng C, et al. Serological and molecular survey of sheep infected with *Mycoplasma ovipneumoniae* in Xinjiang, China. *Trop Anim Health Prod*. 2015 Dec; 47(8):1641–7. <https://doi.org/10.1007/s11250-015-0908-2> PMID: 26315151
13. Todorov D. [*Mycoplasma* carrier state in the respiratory apparatus of sheep and lambs]. *Vet Med Nauki*. 1985; 22(2):21–6. PMID: 3992923
14. Kijas JW, Lenstra JA, Hayes B, Boitard S, Porto Neto LR, San Cristobal M, et al. Geonome-wide analysis of the world’s sheep breeds reveals high levels of historic mixture and strong recent selection. *PLoS Biol*. 2012 Feb; 10(2):e1001258. <https://doi.org/10.1371/journal.pbio.1001258> PMID: 22346734
15. Becker D, Tetens J, Brunner A, Bürstel D, Ganter M, Kijas J, et al. Microphthalmia in Texel sheep is associated with a missense mutation in the paired-like homeobox domain 3 (PITX3) gene. *PLoS One*. 2010 Jan 13; 5(1):e8689. <https://doi.org/10.1371/journal.pone.0008689> PMID: 20084168
16. Zhao X, Dittmer KE, Blair HT, Thompson KG, Rothschild MF, Garrick DJ. A novel nonsense mutation in the DMP1 gene identified by a genome-wide association study is responsible for inherited rickets in Corriedale sheep. *PLoS One*. 2011; 6(7):e21739. <https://doi.org/10.1371/journal.pone.0021739> PMID: 21747952
17. Mousel MR, Reynolds JO, White SN. Genome-wide association identifies *SLC2A9* and *NLN* gene regions as associated with entropion in domestic sheep. *PLoS One*. 2015 Jun 22; 10(6):e0128909. <https://doi.org/10.1371/journal.pone.0128909> PMID: 26098909
18. Gonzalez MV, Mousel MR, Herndon DR, Jiang Y, Dalrymple BP, Reynolds JO, et al. A divergent artiodactyl *MYADAM*-like repeat is associated with erythrocyte traits and weight of lamb weaned in domestic sheep. *PLoS One*. 2013 Aug 30; 8(8):e74700. <https://doi.org/10.1371/journal.pone.0074700> PMID: 24023702
19. Silva MVB, Sonstegard TS, Hanotte O, Mugambi JM, Garcia JF, Nagda S, et al. Identification of quantitative trait loci affecting resistance to gastrointestinal parasites in a double backcross population of Red

- Maasai and Dorper sheep. *Anim Genet.* 2012 Feb; 43(1):63–71. <https://doi.org/10.1111/j.1365-2052.2011.02202.x> PMID: 22221026
20. Benavides MV, Sonstegard TS, Kemp S, Mugambi JM, Gibson JP, Baker RL, et al. Identification of novel loci associated with gastrointestinal parasite resistance in a Red Maasai x Dorper backcross population. *PLoS One.* 2015 Apr 13; 10(4):e0122797. <https://doi.org/10.1371/journal.pone.0122797> PMID: 25867089
 21. Atlija M, Arranz JJ, Martinez-Valladares M, Gutiérrez-Gil B. Detection and replication of QTL underlying resistance to gastrointestinal nematodes in adult sheep using the ovine 50K SNP array. *Genet Sel Evol.* 2016 Jan 20; 48:4. <https://doi.org/10.1186/s12711-016-0182-4> PMID: 26791855
 22. Heaton MP, Clawson ML, Chitko-Mckown CG, Leymaster KA, Smith TPL, Harhay GP, et al. Reduced lentivirus susceptibility in sheep with TME154 mutations. *PLoS Genet.* 2012 Jan; 8(1):e1002467. <https://doi.org/10.1371/journal.pgen.1002467> PMID: 22291605
 23. White SN, Mousel MR, Herrmann-Hoesing LM, Reynolds JO, Leymaster KA, Neibergs HL, et al. Genome-wide association identifies multiple genomic regions associated with susceptibility to and control of ovine lentivirus. *PLoS One.* 2012; 7(10):e47829. <https://doi.org/10.1371/journal.pone.0047829> PMID: 23082221
 24. White SN, Mousel MR, Reynolds JO, Herrmann-Hoesing LM, Knowles DP. Variant near *ZNF389* is associated with control of ovine lentivirus in multiple flocks of sheep. *Anim Genet.* 2014 Apr; 45(2):297–300. <https://doi.org/10.1111/age.12107> PMID: 24303974
 25. Niggeler A, Tetens J, Stäuble A, Steiner A, Drögemüller C. A genome-wide significant association on chromosome 2 for footrot resistance/susceptibility in Swiss White Alpine sheep. *Anim Genet.* 2017 Dec; 48(6):712–715. <https://doi.org/10.1111/age.12614> PMID: 28983925
 26. McAuliffe L, Hatchell FM, Ayling RD, King AIM, Nicholas RAJ. Detection of *Mycoplasma ovipneumoniae* in *Pasteurella*-vaccinated sheep flocks with respiratory disease in England. *Vet Rec.* 2003 Nov 29; 153(22):687–8. <https://doi.org/10.1136/vr.153.22.687> PMID: 14682543
 27. Zhang J, Cao J, Zhu M, Xu M, Shi F. Loop-mediated isothermal amplification-lateral-flow dipstick (LAMP-LFD) to detect *Mycoplasma ovipneumoniae*. *World J Microbiol Biotechnol* 2019 Jan 30; 35(2):31. <https://doi.org/10.1007/s11274-019-2601-5> PMID: 30701329
 28. Wang J, Li R, Sun X, Liu L, Hao X, Wang J, et al. Development and validation of the isothermal recombinase polymerase amplification assays for rapid detection of *Mycoplasma ovipneumoniae* in sheep. *BMC Vet Res.* 2020 Jun 1; 16(1):172. <https://doi.org/10.1186/s12917-020-02387-3> PMID: 32487081
 29. Highland MA, Herndon DR, Bender SC, Hansen L, Gerlach RF, Beckmen KB. *Mycoplasma ovipneumoniae* in Wildlife Species beyond Subfamily Caprinae. *Emerg Infect Dis.* 2018 Dec; 24(12):2384–2386. <https://doi.org/10.3201/eid2412.180632> PMID: 30457547
 30. Herndon DR, Beckmen KB, Highland MA. Draft Genome Sequence of a Novel *Mycoplasma* Species Identified from the Respiratory Tract of an Alaska Moose (*Alces alces gigas*). *Microbiol Resour Announc.* 2021 Feb 25; 10(8):e01371–20. <https://doi.org/10.1128/MRA.01371-20> PMID: 33632866
 31. Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, et al. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience.* 2015 Dec; 4(1):s13742–015–0047–8. <https://doi.org/10.1186/s13742-015-0047-8> PMID: 25722852
 32. Kang HM, Sul JH, Service SK, Zaitlen NA, Kong SY, Freimer NB, et al. Variance component model to account for sample structure in genome-wide association studies. *Nat Genet.* 2010 March; 42:348–54. <https://doi.org/10.1038/ng.548> PMID: 20208533
 33. McRae KM, Rowe SJ, Baird HJ, Bixley MJ, Clarke SM. Genome-wide association study of lung lesions and pleurisy in New Zealand lambs. *J Anim Sci.* 2018 Nov 21; 96(11):4512–4520. <https://doi.org/10.1093/jas/sky323> PMID: 30099550
 34. Turner SD. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. *JoSS.* 2018 May; 3:731.
 35. Howe KL, Achuthan P, Allen J, Allen J, Alvarez-Jarreta J, et al. Ensembl 2021. *Nucleic Acids Res.* 2021 Jan 8; 49(D1):D884–D891. <https://doi.org/10.1093/nar/gkaa942> PMID: 33137190
 36. NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* 2018 Jan 4; 46(D1):D8–D13. <https://doi.org/10.1093/nar/gkx1095> PMID: 29140470
 37. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. *Curr Protoc Bioinformatics.* 2016 Jun 20; 54:1.30. <https://doi.org/10.1002/cpbi.5> PMID: 27322403
 38. Jassal B, Matthews L, Viteri G, Gong C, Lorente P, Fabregat A, et al. The reactome pathway knowledgebase. *Nucleic Acids Res.* 2020 Jan 8; 48(D1):D498–D503. <https://doi.org/10.1093/nar/gkz1031> PMID: 31691815

39. Herrmann-Hoesing LM, White SN, Mousel MR, Lewis GS, Knowles DP. Ovine progressive pneumonia provirus levels associate with breed and Ovar-DRB1. *Immunogenetics*. 2008 Dec; 60(12):749–58. <https://doi.org/10.1007/s00251-008-0328-9> PMID: 18797863
40. Giangaspero M, Nicholas RA, Hlusek M, Bonfini B, Osawa T, Orusa R, et al. Seroepidemiological survey of sheep flocks from Northern Japan for *Mycoplasma ovipneumoniae* and *Mycoplasma agalactiae*. *Trop Anim Health Prod*. 2012 Mar; 44(3):395–8. <https://doi.org/10.1007/s11250-011-9952-8> PMID: 21830171
41. Moore SJ, O'Dea MA, Perkins N, O'Hara AJ. Estimation of nasal shedding and seroprevalence of organisms known to be associated with bovine respiratory disease in Australian live export cattle. *J Vet Diagn Invest*. 2015 Jan; 27(1):6–17. <https://doi.org/10.1177/1040638714559741> PMID: 25525134
42. Hazelton MS, Morton JM, Parker AM, Bosward KL, Sheehy PA, Dwyer CJ, et al. *Mycoplasma bovis* and other Mollicutes in replacement dairy heifers from *Mycoplasma bovis*-infected and uninfected herds: A 2-year longitudinal study. *J Dairy Sci*. 2020 Dec; 103(12):11844–11856. <https://doi.org/10.3168/jds.2020-18921> PMID: 32981720
43. Fano E, Pijoan C, Dee S. Dynamics and persistence of *Mycoplasma hyopneumoniae* infection in pigs. *Can J Vet Res*. 2005 Jul; 69(3):223–8. PMID: 16187553
44. Fernández S, Galapero J, Rey J, Pérez CJ, Ramos A, Rosales R, et al. Investigations into the seasonal presence of *Mycoplasma* species in fattening lambs. *Vet J*. 2016 Jun; 212:80–2. <https://doi.org/10.1016/j.tvjl.2016.04.006> PMID: 27256030
45. Planas D, Fert A, Zhang Y, Goulet JP, Richard J, Finzi A, et al. Pharmacological Inhibition of PPAR γ Boosts HIV Reactivation and Th17 Effector Functions, While Preventing Progeny Virion Release and *de novo* Infection. *Pathog Immun*. 2020 Sep 30; 5(1):177–239. <https://doi.org/10.20411/pai.v5i1.348> PMID: 33089034
46. Sinclair E, Ronquillo R, Lollo N, Deeks SG, Hunt P, Yiannoutsos CT, et al. Antiretroviral treatment effect on immune activation reduces cerebrospinal fluid HIV-1 infection. *J Acquir Immune Defic Syndr*. 2008 Apr 15; 47(5):544–52. <https://doi.org/10.1097/QAI.0b013e318162754f> PMID: 18362693
47. Yamazaki T, Yang XO, Chung Y, Fukunaga A, Nurieva R, Pappu B, et al. CCR6 regulates the migration of inflammatory and regulatory T cells. *J Immunol*. 2008 Dec 15; 181(12):8391–401. <https://doi.org/10.4049/jimmunol.181.12.8391> PMID: 19050256
48. Higgins SC, Jarnicki AG, Lavelle EC, Mills KH. TLR4 mediates vaccine-induced protective cellular immunity to *Bordetella pertussis*: role of IL-17-producing T cells. *J Immunol*. 2006 Dec 1; 177(11):7980–9. <https://doi.org/10.4049/jimmunol.177.11.7980> PMID: 17114471
49. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med*. 2006 Oct 2; 203(10):2271–9. <https://doi.org/10.1084/jem.20061308> PMID: 16982811
50. Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, Collins M, et al. An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. *J Immunol*. 2007 Dec 1; 179(11):7791–9. <https://doi.org/10.4049/jimmunol.179.11.7791> PMID: 18025225
51. Auja SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med*. 2008 Mar; 14(3):275–81. <https://doi.org/10.1038/nm1710> PMID: 18264110
52. Dileepan T, Linehan JL, Moon JJ, Pepper M, Jenkins MK, Cleary PP. Robust antigen specific th17 T cell response to group A *Streptococcus* is dependent on IL-6 and intranasal route of infection. *PLoS Pathog*. 2011 Sep; 7(9):e1002252. <https://doi.org/10.1371/journal.ppat.1002252> PMID: 21966268
53. Fan X, Wang X, Li N, Cui H, Hou B, Gao B, et al. Sortase A induces Th17-mediated and antibody-independent immunity to heterologous serotypes of group A streptococci. *PLoS One*. 2014 Sep 18; 9(9):e107638. <https://doi.org/10.1371/journal.pone.0107638> PMID: 25232948
54. Walugembe M, Bertolini F, Dematawewa CMB, Reis MP, Elbeltagy AR, Schmidt CJ, et al. Detection of Selection Signatures Among Brazilian, Sri Lankan, and Egyptian Chicken Populations Under Different Environmental Conditions. *Front Genet*. 2019 Jan 14; 9:737. <https://doi.org/10.3389/fgene.2018.00737> PMID: 30693019
55. Freiss G, Chabos D. PTPN13/PTPL1: an important regulator of tumor aggressiveness. *Anticancer Agents Med Chem*. 2011 Jan; 11(1):78–88. <https://doi.org/10.2174/187152011794941262> PMID: 21235435
56. Ramesh R, Kozhaya L, McKeivitt K, Djuretic IM, Carlson TJ, Quintero MA, et al. Pro-inflammatory human Th17 cells selectively express P-glycoprotein and are refractory to glucocorticoids. *J Exp Med*. 2014 Jan 13; 211(1):89–104. <https://doi.org/10.1084/jem.20130301> PMID: 24395888

57. Delgado IJ, Kim DS, Thatcher KN, LaSalle JM, Van den Veyver IB. Expression profiling of clonal lymphocyte cell cultures from Rett syndrome patients. *BMC Med Genet*. 2006 Jul 21; 7:61. <https://doi.org/10.1186/1471-2350-7-61> PMID: 16859563
58. Mjösberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol*. 2011 Sep 11; 12(11):1055–62. <https://doi.org/10.1038/ni.2104> PMID: 21909091
59. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*. 2010 Apr 29; 464(7293):1367–70. <https://doi.org/10.1038/nature08900> PMID: 20200518
60. Martinez-Gonzalez I, Mathä L, Steer CA, Ghaedi M, Poon GF, Takei F. Allergen-Experienced Group 2 Innate Lymphoid Cells Acquire Memory-like Properties and Enhance Allergic Lung Inflammation. *Immunity*. 2016 Jul 19; 45(1):198–208. <https://doi.org/10.1016/j.immuni.2016.06.017> PMID: 27421705
61. Gross C, Heumann R, Erdmann KS. The protein kinase C-related kinase PRK2 interacts with the protein tyrosine phosphatase PTP-BL via a novel PDZ domain binding motif. *FEBS Lett*. 2001 May 11; 496(2–3):101–4. [https://doi.org/10.1016/s0014-5793\(01\)02401-2](https://doi.org/10.1016/s0014-5793(01)02401-2) PMID: 11356191
62. Patel H, Li J, Herrero A, Kroboth J, Byron A, Kriegsheim AV, et al. Novel roles of PRK1 and PRK2 in cilia and cancer biology. *Sci Rep*. 2020 Mar 3; 10(1):3902. <https://doi.org/10.1038/s41598-020-60604-3> PMID: 32127582
63. Wallace SW, Magalhaes A, Hall A. The Rho target PRK2 regulates apical junction formation in human bronchial epithelial cells. *Mol Cell Biol*. 2011 Jan; 31(1):81–91. <https://doi.org/10.1128/MCB.01001-10> PMID: 20974804
64. Luo H, Wu X, Xu Z, Hao X, Wang Y, Li M. NOD2/c-Jun NH₂-Terminal Kinase Triggers *Mycoplasma ovipneumoniae*-Induced Macrophage Autophagy. *J Bacteriol*. 2020 Sep 23; 202(20):e00689. <https://doi.org/10.1128/JB.00689-19> PMID: 32778560
65. Legrand-Poels S, Kustermans G, Bex F, Kremmer E, Kufer TA, Piette J. Modulation of Nod2-dependent NF-kappaB signaling by the actin cytoskeleton. *J Cell Sci*. 2007 Apr 1; 120(Pt 7):1299–310. <https://doi.org/10.1242/jcs.03424> PMID: 17356065
66. Schmidt A, Durgan J, Magalhaes A, Hall A. Rho GTPases regulate PRK2/PKN2 to control entry into mitosis and exit from cytokinesis. *EMBO J*. 2007 Mar 21; 26(6):1624–36. <https://doi.org/10.1038/sj.emboj.7601637> PMID: 17332740
67. Sugimoto K, Kage H, Aki N, Sano A, Kitagawa H, Nagase T, et al. The induction of H3K9 methylation by PIWIL4 at the p16Ink4a locus. *Biochem Biophys Res Commun*. 2007 Aug 3; 359(3):497–502. <https://doi.org/10.1016/j.bbrc.2007.05.136> PMID: 17544373
68. Ponnusamy M, Yan KW, Liu CY, Li PF, Wang K. PIWI family emerging as a decisive factor of cell fate: An overview. *Eur J Cell Biol*. 2017 Dec; 96(8):746–757. <https://doi.org/10.1016/j.ejcb.2017.09.004> PMID: 28993008
69. Zhang X, Cozen AE, Liu Y, Chen Q, Lowe TM. Small RNA Modifications: Integral to Function and Disease. *Trends Mol Med*. 2016 Dec; 22(12):1025–1034. <https://doi.org/10.1016/j.molmed.2016.10.009> PMID: 27840066
70. He Z, Jing S, Yang T, Chen J, Huang F, Zhang W, et al. PIWIL4 Maintains HIV-1 Latency by Enforcing Epigenetically Suppressive Modifications on the 5' Long Terminal Repeat. *J Virol*. 2020 May 4; 94(10):e01923–19. <https://doi.org/10.1128/JVI.01923-19> PMID: 32161174
71. An P, Duggal P, Wang LH, O'Brien SJ, Donfield S, Goedert JJ, et al. Polymorphisms of CUL5 are associated with CD4+ T cell loss in HIV-1 infected individuals. *PLoS Genet*. 2007 Jan 26; 3(1):e19. <https://doi.org/10.1371/journal.pgen.0030019> PMID: 17257057
72. Liu B, Ye B, Yang L, Zhu X, Huang G, Zhu P, et al. Long noncoding RNA lncKdm2b is required for ILC3 maintenance by initiation of Zfp292 expression. *Nat Immunol*. 2017 May; 18(5):499–508. <https://doi.org/10.1038/ni.3712> PMID: 28319097
73. Kotov JA, Kotov DI, Linehan JL, Bardwell VJ, Gearhart MD, Jenkins MK. BCL6 corepressor contributes to Th17 cell formation by inhibiting Th17 fate suppressors. *J Exp Med*. 2019 Jun 3; 216(6):1450–1464. <https://doi.org/10.1084/jem.20182376> PMID: 31053612
74. Rahimi H, Ahmadzadeh A, Yousef-amoli S, Kokabee L, Shokrgozar MA, Mahdian R, et al. The expression pattern of APC2 and APC7 in various cancer cell lines and AML patients. *Adv Med Sci*. 2015 Sep; 60(2):259–63. <https://doi.org/10.1016/j.advms.2015.04.007> PMID: 26046517
75. He X, Zhang C, Shi C, Lu Q. Meta-analysis of mRNA expression profiles to identify differentially expressed genes in lung adenocarcinoma tissue from smokers and non-smokers. *Oncol Rep*. 2018 Mar; 39(3):929–938. <https://doi.org/10.3892/or.2018.6197> PMID: 29328493

76. Yamada M, Mori M. Thyrotropin-releasing hormone-degrading enzyme in human serum is classified as type II of pyroglutamyl aminopeptidase: influence of thyroid status. *Proc Soc Exp Biol Med*. 1990 Sep; 194(4):346–51. <https://doi.org/10.3181/00379727-194-43107> PMID: 1975097
77. Klecha AJ, Genaro AM, Gorelik G, Barreiro Arcos ML, Silberman DM, Schuman M, et al. Integrative study of hypothalamus-pituitary-thyroid-immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signaling pathway. *J Endocrinol*. 2006 Apr; 189(1):45–55. <https://doi.org/10.1677/joe.1.06137> PMID: 16614380
78. Mostafa MM, Rider CF, Shah S, Traves SL, Gordon PMK, Miller-Larsson A, et al. Glucocorticoid-driven transcriptomes in human airway epithelial cells: commonalities, differences and functional insight from cell lines and primary cells. *BMC Med Genomics*. 2019 Jan 31; 12(1):29. <https://doi.org/10.1186/s12920-018-0467-2> PMID: 30704470
79. Jonczyk MS, Simon M, Kumar S, Fernandes VE, Sylvius N, Mallon AM, et al. Genetic factors regulating lung vasculature and immune cell functions associate with resistance to pneumococcal infection. *PLoS One*. 2014 Mar 3; 9(3):e89831. <https://doi.org/10.1371/journal.pone.0089831> PMID: 24594938