Wolf Creek Bacteria Study

Fecal Indicator Bacteria Source Identification

Overview

A 12-week study on the Wolf Creek watershed, developed, funded, and conducted by the Central Valley Regional Water Quality Control Board (Central Valley Water Board or Board) in 2024, provides important information on water quality and the sources of fecal pollution contributing to elevated bacteria levels in the creek. Study results indicate that ruminants, a category of grazing mammals, are the largest and most consistent source of fecal pollution. Dogs were also a source in some areas, but humans, horses, and birds were not found to be a source of fecal contamination to Wolf Creek.

Geography

Wolf Creek, a northern Sierra foothills watershed in Nevada County, flows for 23 miles from the southwest slope of Banner Mountain through the city of Grass Valley and southward to its confluence with the Bear River. The elevation range for Wolf Creek is

between 3300 feet at the source to 1000 feet at the confluence with the Bear River. The Wolf Creek watershed consists primarily of mixed coniferous forest in the upper half of the watershed, with mixed conifer and oak woodland dominating the lower half of the watershed. The average annual precipitation in Grass Valley is over 50 inches, with most of the precipitation occurring from November through April. The area was heavily mined for gold, beginning shortly after the discovery of gold in the American River in 1848. The surface landscape around the creek was highly altered in the search for gold in placer deposits. Some of the most productive underground mines, such as the Empire and North Star Mines, following rich gold-bearing quartz veins, were also located within the Wolf Creek watershed. Primary land uses in the watershed today include urban to rural residential, commercial districts in Grass Valley, artisan fruit and vegetable agriculture, and small-scale livestock ranching.

Background

In 2018, the State Water Resources Control Board (State Board) developed statewide bacteria water quality objectives (https://www.waterboards.ca.gov/bacterialobjectives/) to protect recreational users from the effects of pathogens. Pathogens are small organisms, such as bacteria and viruses, that can cause illness. The primary source for pathogens in our waterways is fecal pollution from humans and other animals. Since it is not practical to monitor for the large variety of possible pathogens, fecal indicator bacteria are commonly used as evidence of contamination and an increased risk of illness. The indicator bacteria used for freshwater is *Escherichia coli* (*E. coli*), a species of bacteria that is abundant in the feces of warm-blooded animals, making it an excellent indicator organism.

Wolf Creek and its tributary, French Ravine, are listed as impaired due to indicator bacteria on California's 303(d) List. Wolf Creek is also identified in the State Board's Onsite Wastewater Treatment Systems (OWTS) policy as likely being impacted by OWTS discharges, such as those from poorly sited or failing septic systems. The Central Valley Water Board conducted monitoring on Wolf Creek in 2015-2016, which confirmed elevated levels of *E. coli* indicator bacteria and exceedances of statewide water quality objectives.

The goals of the 2024 study were to:

- evaluate the impairment status of Wolf Creek and its tributaries
- determine if Wolf Creek and its tributaries are impacted by OWTS discharges.

Study Design

The study focused on 14 miles of the creek, from the North Star Mining Museum at the southern city limits of Grass Valley, to Wolf Creek at Wolf Road, near Higgins Corner. The monitoring was conducted for 12 weeks of the dry season, from June 19 to September 4, 2024, to coincide with historical high indicator bacteria levels.

Board staff selected nine monitoring stations (Figure 1, Table 1) along the mainstem Wolf Creek and four tributaries (South Wolf Creek, Cherry Creek, French Ravine, and Rattlesnake Creek) to provide information on spatial trends and potential sources of fecal pollution. Stations were distributed upstream and downstream of suspected sources, including the tributary streams to Wolf Creek.

Board staff collected samples weekly for *E. coli* analysis and subsequent microbial source tracking (MST) through identification of genetic source markers. Board staff conducted *E. coli* analysis in-house using Standard Method 9223B (https://www.nemi.gov/methods/method_summary/5583/), a most probable number (MPN) method. Board staff filtered and froze all samples for possible MST analysis. When *E. coli* results exceeded the established threshold of 100 MPN/100mL, then Board staff submitted the corresponding sample filter for MST analysis by the subcontracting laboratory, Cel Analytical.

Cel Analytical performed the MST analysis using markers that identify genetic signatures from specific groups of animals. Given the surrounding land use, Board staff selected genetic markers for humans (*HF-183*), ruminants (*BacCow* and *Rum2Bac*), and horses (*HoF597*) at the onset of the study. For the latter third of the study (weeks 9 – 12), Board staff added additional analyses for avian (Avian AFD) and canine (*BacCan*) markers.

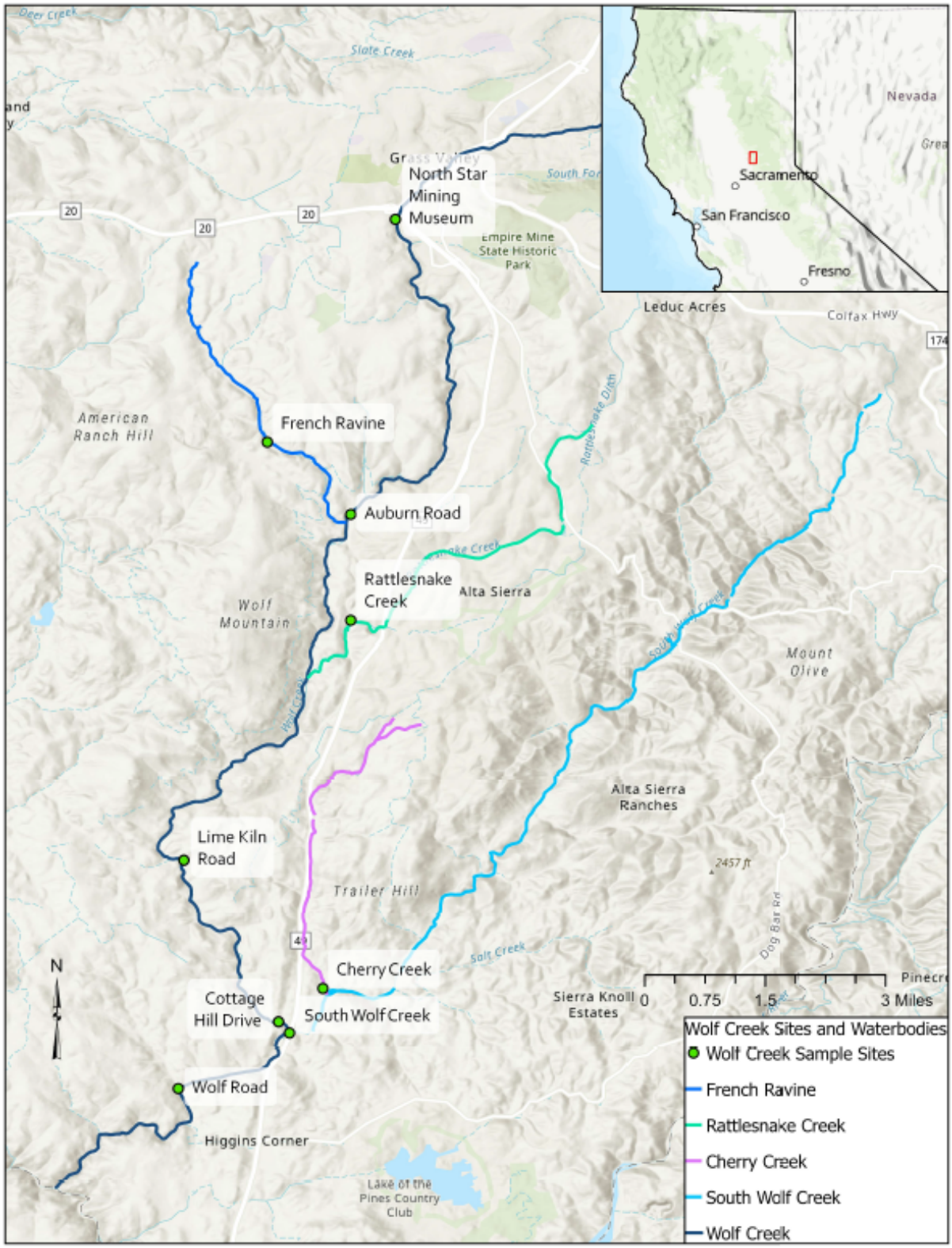

Figure 1. Wolf Creek Study Area and Sampling Sites

Table 1. Sampling Site Details

Station Name	Station Label	Station Code	Latitude	Longitude	Tributary or Mainstem
Wolf Creek at the North Star Mining Museum	North Star Mining Museum	516NEV109	39.208	-121.07	Mainstem
French Ravine at Hidden Valley Road	French Ravine	516NEV114	39.168	-121.093	Tributary
Wolf Creek at Auburn Road	Auburn Road	516NEV107	39.155	-121.078	Mainstem
Rattlesnake Creek at Auburn Road	Rattlesnake Creek	516NEV115	39.136	-121.078	Tributary
Wolf Creek at Lime Kiln Road	Lime Kiln Road	516NEV104	39.093	-121.108	Mainstem
Cherry Creek at Sharmiden Way	Cherry Creek	516NEV113	39.07	-121.083	Tributary
Wolf Creek at Cottage Hill Drive	Cottage Hill Drive	516NEV103	39.064	-121.091	Mainstem
South Wolf Creek above the confluence with Wolf Creek	South Wolf Creek	516NEV102	39.062	-121.089	Tributary
Wolf Creek at Wolf Road	Wolf Road	516NEV101	39.052	-121.109	Mainstem

Study Results

All samples were analyzed for *E. coli* (Table 2) and compared to the statewide bacteria water quality objectives for freshwater. The statewide bacteria objectives contain two methods of assessing *E. coli* results: the geometric mean (100 colony forming units (CFU) or most probable number (MPN) per 100 milliliters (mL)) and the statistical threshold value (STV) (320 MPN/100mL). MPN and CFU are different methods to estimate the number of bacteria in a water sample.

To assess impairment status, Board staff applied the six-week rolling geometric mean *E. coli* objective, which is the designated objective when five or more *E. coli* results are available within a six-week period. While the geometric mean is the more appropriate objective given the sampling frequency, it is worth noting that only nine out of 108 samples, six samples on tributary creeks and three samples on mainstem Wolf Creek, exceeded the STV.

Table 3 summarizes the geometric mean calculations for all sample sites. Since the objective is a rolling geometric mean based on a six-week interval, there were sufficient data collected during the study to calculate nine distinct geometric means (weeks 1 – 5, weeks 1 – 6, weeks 2 – 7, etc.). Several sites consistently exceeded the geometric mean objective. French Ravine exceeded the objective for all nine geometric mean periods. South Wolf Creek exceeded the objective for eight geometric mean periods. Cherry Creek and Wolf Creek at Lime Kiln Rd each exceeded the objective for seven geometric mean periods. The sites with the fewest geometric mean exceedances were Wolf Creek at the North Star Mining Museum with zero, Rattlesnake Creek with two, and Wolf Creek at Auburn Road with three. Geometric mean exceedances trended downward from eight out of nine sites during the second geometric mean period (weeks 1 – 6) to only two out of nine sites for the final two geometric mean periods of the study (weeks 7 – 12 and weeks 8 – 13). These results are depicted as proportions of exceedances of the geometric mean bacteria water quality objective per site in Figures 2A and 2B.

Table 2. E. coli Test Results by Sample Site

Station Label	6/19	6/26	7/2	7/10	7/17	7/24	7/31	8/7	8/14	8/21	8/28	9/4
North Star Mining Museum	7.4	14.8	14.6	19.9	13.5	29.2	770.1	22.8	34.5	18.3	13.2	9.8
French Ravine	83.3	125.9	111.2	2419.6	547.5	290.9	218.7	127.4	93.3	172.3	61.3	123.4
Auburn Road	218.7	228.2	93.4	172.3	56.5	178.9	44.1	88.2	46.4	83.3	61.3	110
Rattlesnake Creek	160.7	290.9	145	98.5	34.5	60.9	43.5	93.2	16.9	56.3	53.8	63.1
Lime Kiln Road	172.2	238.2	101.7	113.7	147	228.2	88.2	178.2	60.9	128.1	48.7	59.4
Cherry Creek	290.9	133.3	178.9	613.1	365.4	275.5	261.3	86	21.1	114.5	98.7	20.4
Cottage Hill Drive	285.1	547.5	307.6	517.2	63.8	224.7	49.5	46.4	24.6	22.6	48.8	44.3
South Wolf Creek	49.5	60.9	52.1	248.1	214.2	547.5	133.3	155.3	191.8	98.7	325.5	143.9
Wolf Road	155.3	115.3	139.1	137.4	67.7	124.6	78.9	116.9	49.6	53.8	104.3	90.9

Table 3. Geometric Mean Results by Period Including Counts of Samples Exceeding the Bacteria Objective

Station Label	Period 1 (weeks 1-5)	Period 2 (weeks 1-6)	Period 3 (weeks 2-7)	Period 4 (weeks 3-8)	Period 5 (weeks 4-9)	Period 6 (weeks 5-10)	Period 7 (weeks 6-11)	Period 8 (weeks 7-12)	Period 9 (weeks 8-13)	Count >100 MPN/ 100 mL per Site
North Star Mining Museum	13.4	15.2	33.1	35.5	41	40.4	40.3	33.6	17.9	**0**
French Ravine	274	276.8	325.1	325.7	316.3	203.7	141.4	122.6	109.1	**9**
Auburn Road	135.3	141.8	108.6	92.7	82.5	73.1	74	68.3	74.5	**3**
Rattlesnake Creek	118.2	105.8	85.1	70.4	49.2	44.8	48.3	48.5	49.6	**2**
Lime Kiln Road	147.5	158.6	141.9	135.2	124.1	126.6	105.3	84.1	83.3	**7**
Cherry Creek	274.3	274.5	269.7	250.7	175.5	132.7	106.7	69.1	53	**7**
Cottage Hill Drive	275.4	266.2	198.8	131.8	86.5	51.3	49.1	37.5	35.4	**4**
South Wolf Creek	96.4	128.8	151.9	177.6	220.7	189.3	202.9	162.4	169	**8**
Wolf Road	118.3	119.3	106.6	106.8	90	77	82.7	78.5	78.4	**4**
Count >100 MPN/ 100 mL per Period	**7**	**8**	**7**	**6**	**4**	**4**	**4**	**2**	**2**	**44**

Figure 2A. Northern Sites Proportion of Exceedances of the Geometric Mean Bacteria Objective

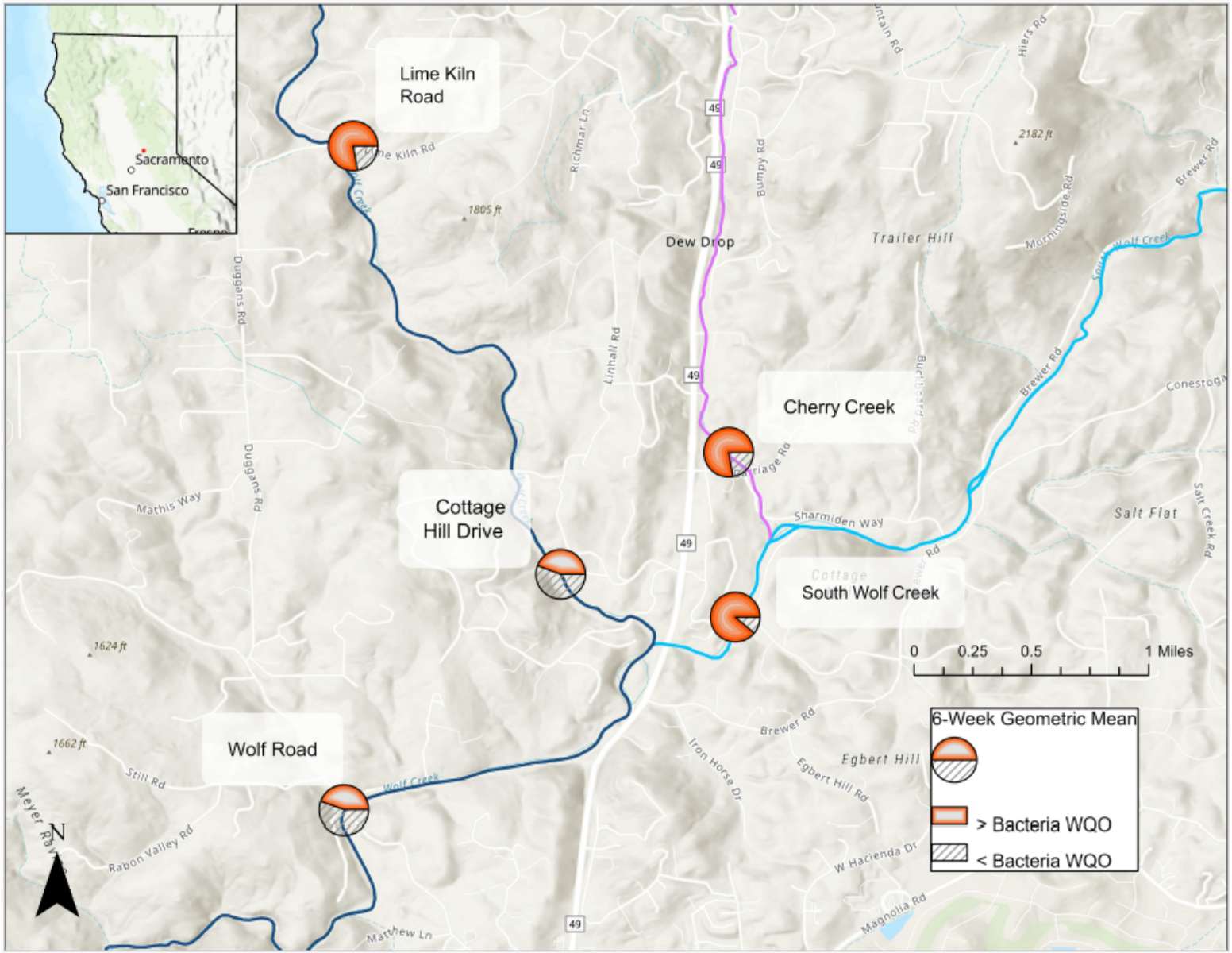

Figure 2B. Southern Sites Proportion of Exceedances of the Geometric Mean Bacteria Objective

MST Results

Board staff submitted samples for MST analysis when individual *E. coli* results exceeded the pre-determined threshold of 100 MPN/100mL. This threshold for MST analysis was based on previous Central Valley Water Board MST studies and provided greater confidence of sufficient bacterial presence to produce a quantifiable DNA signal.

Overall monitoring results show that 50% of the samples collected for indicator bacteria analysis (54 out of 108) exceeded the 100 MPN/100mL threshold for MST analysis. In the first six weeks of the study, 37 out of 54 samples exceeded the threshold for MST analysis, while only 17 out of 54 samples exceeded this threshold in the second half of the study.

MST samples were analyzed for genetic markers chosen to identify ruminant, human, horse, bird, and dog sources of fecal contamination. The MST results for this study (Figure 3) indicate that ruminants are the largest and most consistent source of fecal contamination. Wolf Creek and its tributaries had quantifiable detections for the ruminant marker, *BacCow*, in 26 out of 54 (48%) samples and quantifiable detections for the ruminant marker, *Rum2Bac*, in six out of 54 (11%) samples. The Cherry Creek site had the most ruminant detections, with six quantifiable detections of the marker *BacCow* and three quantifiable detections of the *Rum2Bac* marker. The South Wolf Creek, Wolf Creek at Cottage Hill Drive, and Rattlesnake Creek sites also had higher percentages of ruminant detections, with over 65% quantifiable detections for the *BacCow* marker. These summary results demonstrate the variability between the two ruminant markers, which could be influenced by a number of factors, including marker sensitivity and species specificity.

There were no quantifiable detections for the human marker, *HF183,* during the study. Five of the 54 samples submitted for MST analysis revealed trace signals of the human marker, with one sample above the detection limit but below the limit of quantitation, and four samples below the detection limit. The detection limit is the lowest concentration (i.e., the smallest amount) that can be detected by the method. The quantification limit is the lowest concentration a sample can be measured with reasonable accuracy and precision. Results that are between the detection and quantification limits are detectable but not quantifiable, meaning the targeted marker was present but at a concentration that was too low to determine. These trace results for the human marker suggest minimal human contribution.

There were no detections of the horse marker, *HoF597*, during the study.

Board staff added analyses for bird and dog markers to the MST analysis for the final four weeks of the study (13 samples). There were no detections for the bird marker, *AvianAFD.* Conversely, dogs were a consistent source of fecal contamination in some areas. Of the 13 samples tested, five samples (38%) revealed quantifiable detections for the dog marker, *BacCan.*

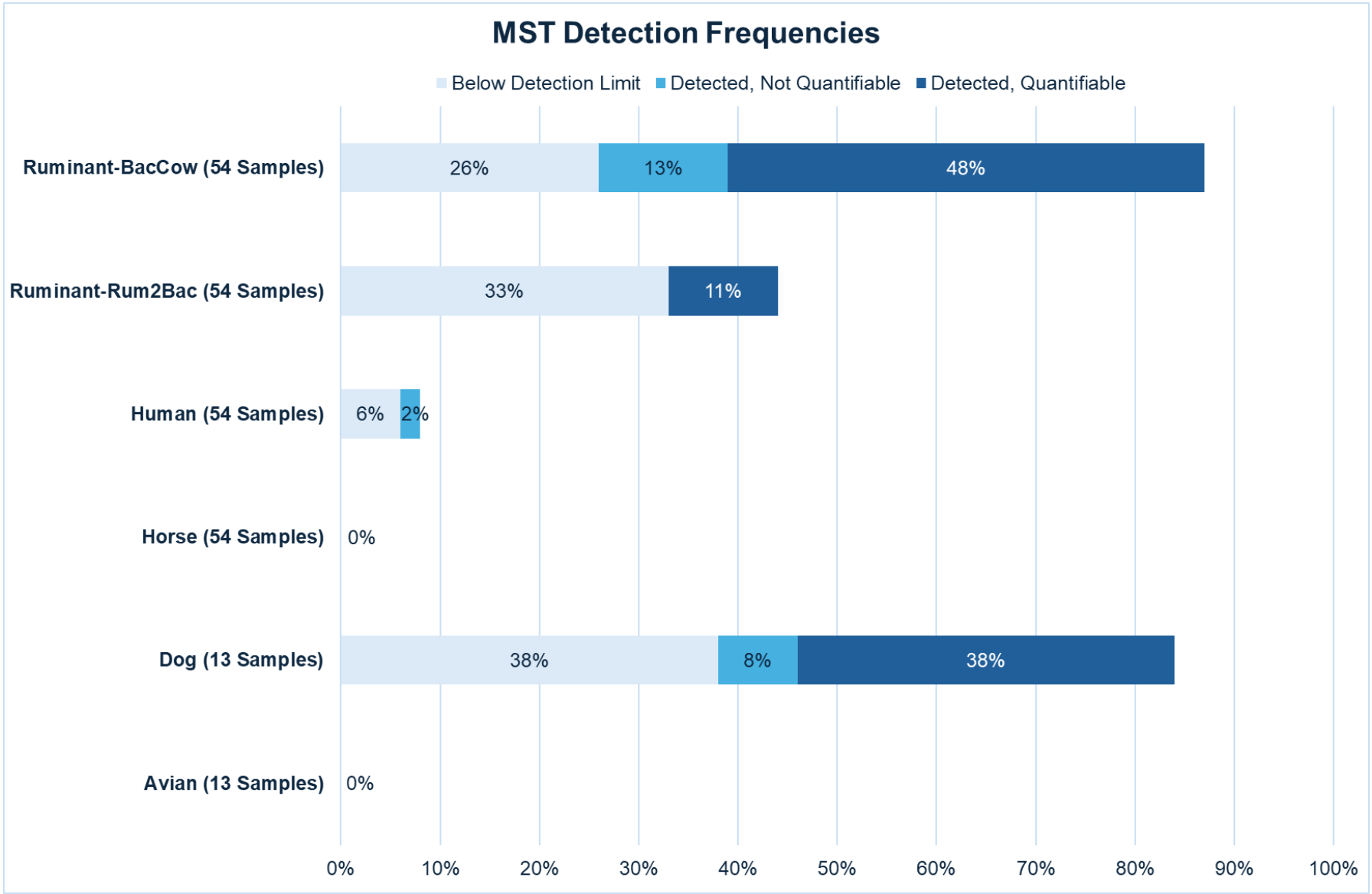

Figure 3. Frequency of Genetic Marker Detections

Conclusion

The study indicates that the indicator bacteria impairments in Wolf Creek and French Ravine still exist. During the 12-week period, all tributaries exceeded the statewide bacteria objectives, some more consistently than others, indicating that there is not an obvious single source of *E. coli* to Wolf Creek.

The study found that ruminant sources had the strongest genetic signature and were the most consistent source of fecal contamination in the creek and its tributaries. Ruminants can be domestic or wild grazing animals, and the markers tested do not point to a specific ruminant species. More study would be needed to determine the comparability and specificity of the markers used and to identify specific ruminant species that are contributing to fecal pollution in Wolf Creek and its tributaries. The marker for humans was detected infrequently and at levels that were not quantifiable or detected below the limit of detection, suggesting minimal impact of OWTS and other human activities to Wolf Creek and tributaries in the stretches tested for this study during the dry season. The dog marker was quantifiable in five out of 13 samples tested, which could warrant future study with a larger sample size. The genetic markers for birds and horses were not detected at any level during this study.

The local community and visitors to Wolf Creek should be aware of water quality conditions that may impact their health. Monitoring has shown that some areas of the creek and its tributaries tested frequently exceed bacteria objectives, indicating an increased risk of illness. More information and a link to the *E. coli* map for this project can be found on the SWAMP Recreational Water Quality Monitoring website (https://www.waterboards.ca.gov/centralvalley/water_issues/swamp/rbua/).