

# **Molecular Identification of Fecal Contamination in the Elks Run Watershed, Jefferson County, West Virginia, 2016–17**

Open-File Report 2019–1064

U.S. Department of the Interior  
U.S. Geological Survey

**Cover.** Photograph showing Elks Run watershed in Jefferson County, West Virginia, by W. Bane Schill, U.S. Geological Survey.

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By W. Bane Schill and Deborah D. Iwanowicz

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DAVID BERNHARDT, Secretary

**U.S. Geological Survey**  
James F. Reilly II, Director

U.S. Geological Survey, Reston, Virginia: 2019

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## Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
Length		
mile (mi)	1.609	kilometer (km)
Area		
acre	4,047	square meter (m <sup>2</sup> )
acre	0.4047	hectare (ha)
acre	0.4047	square hectometer (hm <sup>2</sup> )
acre	0.004047	square kilometer (km <sup>2</sup> )
square mile (mi <sup>2</sup> )	2.590	square kilometer (km <sup>2</sup> )

## Conversion Factors—Continued

International System of Units to U.S. customary units

Multiply	By	To obtain
Volume		
liter (L)	33.81402	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
Mass		
gram (g)	0.03527	acre-foot per day (acre-ft/d)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as

$$^{\circ}\text{F} = (1.8 \times ^{\circ}\text{C}) + 32.$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as

$$^{\circ}\text{C} = (^{\circ}\text{F} - 32) / 1.8.$$

## Datum

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

## Abbreviations

AllBac	marker measures fecal <i>Bacterioides</i> levels from many mammalian sources
BoBac	marker measures bovine-associated <i>Bacteroides</i>
cfu	colony forming units
cytb	cytochrome b
°C	degrees Celsius
FUSA	Facilities Use and Service Agreement
HF183	marker measures human associated <i>Bacteroides</i>
HPyV	human polyomavirus
mL	milliliter
mtDNA	mitochondrial deoxyribonucleic acid
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
USGS	U.S. Geological Survey





# Molecular Identification of Fecal Contamination in the Elks Run Watershed, Jefferson County, West Virginia, 2016–17

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## Abstract

The U.S. Geological Survey conducted a study using modern methods of molecular analysis aimed at attempting to identify the source(s) of fecal contamination that had been identified in previous studies conducted by the West Virginia Conservation Agency in the Elk Run watershed, Jefferson County, West Virginia. Water samples from multiple sites showing elevated fecal coliform counts were analyzed using molecular markers associated with general mammalian fecal contamination (AllBac), human *Bacteroides* (HF183), bovine *Bacteroides* (BoBac), and human polyomavirus (HPyV). Samples were also analyzed by quantitative polymerase chain reaction (qPCR) for human and bovine cytochrome b (mitochondrial DNA marker). A headwater site (Elk Branch at Shenandoah Junction) was found to be severely affected by both human and bovine contamination in May 2017. Although many of the molecular marker levels as well as *Escherichia coli* numbers had declined by a repeat sampling in June 2017, total coliform bacterial numbers remained high. Examination of the data indicated that this site had probably been affected by two separate contamination events, an influx of bovine contamination close to the time of the May sampling and a human contamination event that had occurred earlier. Samples from all sites contained bovine mitochondrial DNA, whereas only one revealed relatively high levels of human mitochondrial DNA. The Elk Run watershed appears to be widely affected by bovine influences with human influence episodically playing a role. Surface runoff caused by rain events exacerbates both.

## Introduction

The Elks Run watershed is entirely in Jefferson County, West Virginia, is 7.9 miles in length, and has a northern tributary called Elk Branch. The Elks Run watershed encompasses about 18.1 square miles (11,598 acres). Approximately 1,500 people who live in the municipalities of Bolivar and Harpers Ferry get their drinking water from Elks Run, the

only stream in Jefferson County, West Virginia, that serves a municipal water supply. The principal land use in the Elks Run watershed is farming; however, developed areas that include houses, towns, and roads make up 1,726 acres of the entire watershed. Eight hundred eighty-one acres of the watershed surround Elk Branch. Cropland totals 1,859 acres, whereas pasture makes up 2,309 acres of the watershed. Of this agricultural acreage, 1,184 acres of cropland and only 332 acres of pasture surround Elk Branch (<https://elksrunwatershed.org/about-elks-run/>).

Many surface waters and groundwaters in the eastern region of the United States are contaminated with fecal pollution resulting in adverse effects including degradation of water quality, increased health risks, and changes in aquatic ecosystem dynamics (Verhougstraete and others, 2015). This is particularly true in karstic areas such as the Elks Run watershed where underground conduits may funnel contaminants from distant and unobvious sources. Population explosions of microbes responsible for eutrophication often adversely affect aquatic species directly by the release of toxins or through disease. The Elks Run watershed, like many other rural areas, has experienced recent development. Residential septic systems exclusively provide sanitary services, and the failure of these systems can be a source of fecal contamination. Alternatively, contamination can result from cattle and other livestock having access to streams or from runoff from pastures and farmyards. From a managerial viewpoint, it is important to know if fecal contamination is from human or animal sources so that limited mitigation resources can be used most effectively. Over the last few years, molecular analyses of environmental samples using polymerase chain reaction (PCR) and (or) metagenomics approaches have been increasingly applied to determine the makeup of bacterial consortia, fecal source tracking, identification of invasive species, and estimation of biodiversity. These specialized molecular approaches are often not readily available to local, State, and nongovernmental organizations. The U.S. Geological Survey (USGS) is a leader in developing and applying these advanced assays (Mathes and others, 2007; Schill and Mathes, 2008). Previous studies sponsored and (or) conducted by the West Virginia Conservation Agency (<https://elksrunwatershed.org/map/>)

identified fecal contamination in Elks Run using standard microbiological methods. While standard culture methods can identify the presence of indicators of fecal pollution (fecal coliform bacteria or *Escherichia coli*), the human or animal source cannot be determined merely by the culture of bacterial fecal indicator species. In 2016, the USGS and the West Virginia Conservation Agency entered into a Facilities Use and Service Agreement (FUSA) to provide molecular analysis services for the study of fecal contamination source(s) in the Elks Run watershed. Three types of analyses were conducted to determine relative levels of *Bacteroides* bacteria that reflect total, human, and bovine fecal contamination. *Bacteroides* are strictly anaerobic and normally do not survive for long periods in the environment. Coliform bacteria that are routinely used for fecal monitoring are, in contrast, aerobic and can colonize stream sediments and plants and therefore be detected long after their injection into surface waters. Human polyomavirus is primarily excreted in urine and is specific as a marker of contamination from human sources. Mitochondrial DNA can find its way into surface waters by fecal contamination, the body fluids of human and animals, or simply through contact, such as bathing. Free DNA is quite ephemeral in the environment, and therefore detection reflects recent events. Taking into consideration the characteristics of these analyses and the persistence of the targets in the environment allows for the determination of the source of the contamination and provides information on how to best mitigate it. Ten sampling sites of interest based on previous microbiological testing were identified by the West Virginia Conservation Agency for this study (table 1, fig. 1). The purpose of this report is to present the results of molecular identification of fecal contamination sources for water samples collected during 2016–17 from the Elks Run watershed.

## Methods

### Field Methods

All samples were collected by USGS personnel on two occasions during May–June 2017 and none were collected within 48 hours following rain events. Water samples (2 liters for molecular analysis) were collected midstream in both horizontal and depth dimensions using a peristaltic pump with inlet attached to an extension pole. Care was taken not to disturb and collect sediment. A second (125-milliliter; mL) sample was collected for coliform analysis into a sterile bottle. All samples were transported to the laboratory on ice and processed within 3 hours. The collection apparatus (pump and tubing) was decontaminated between samplings by pumping Clorox Clean-Up Cleaner and Bleach (The Clorox Company, Oakland, California) in three boluses of 100 mL each followed by pumping a gallon of clean tap water to clear the disinfectant. Two liters of source water were pumped to waste at each site before sample collection began to further ensure that disinfectant residue was removed and the collected sample was truly representative of the source.

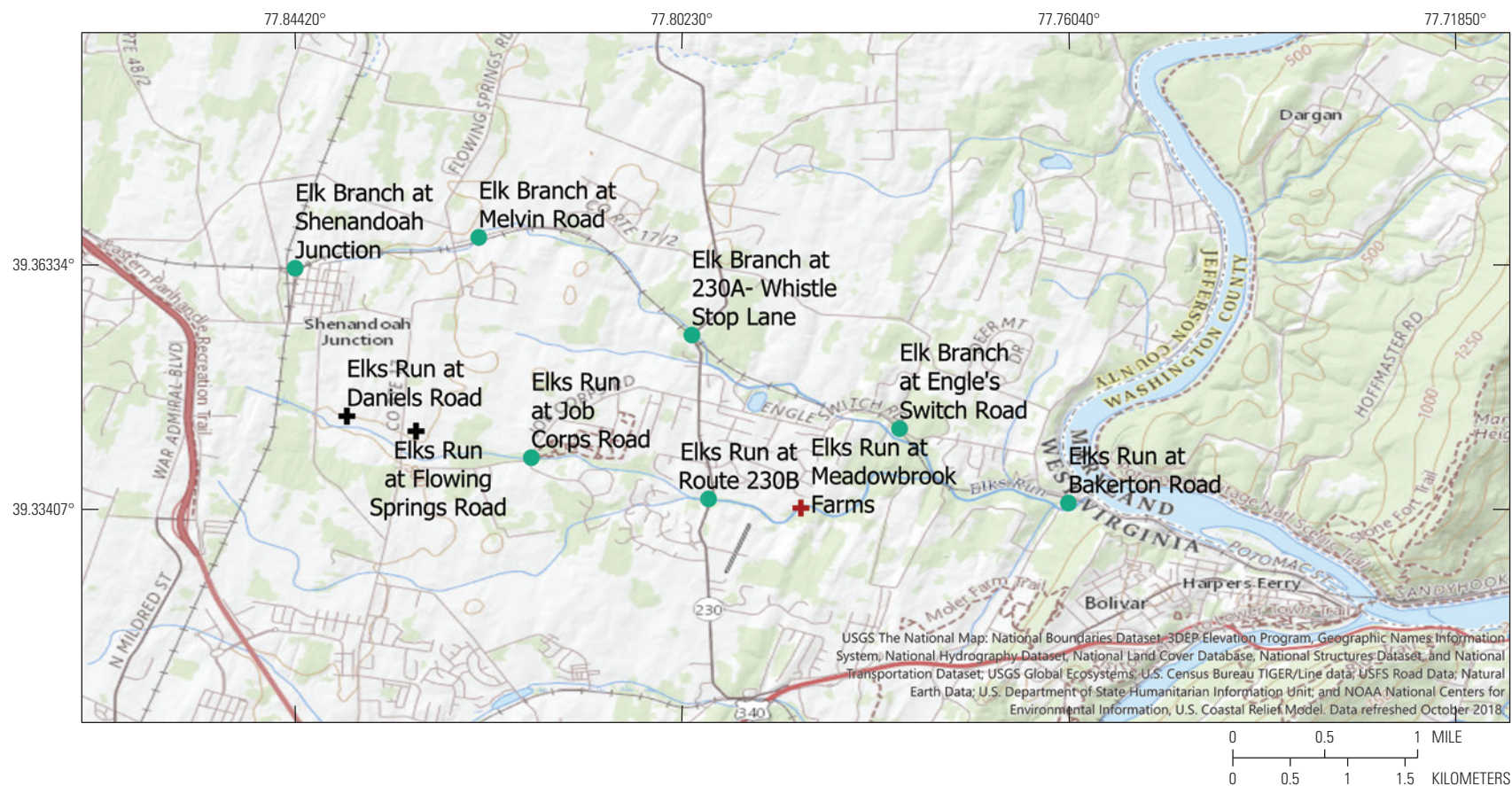
### Laboratory Methods

The Hach U.S. Environmental Protection Agency (EPA) Approved Method 10029 was used to determine coliform bacterial contamination in exceedance of the West Virginia one-time 400 colony forming units per 100 mL (cfu/100 mL) criterion. Three sample water volumes (1 mL, 10 mL, and 100 mL)

**Table 1.** Sampling site names, locations, and numbers of *Escherichia coli* measured in the Elks Run watershed, Jefferson County, West Virginia, 2017.

[*E. coli*, *Escherichia coli*; cfu, colony forming units; mL, milliliter]

Site	Short name	Latitude (in degrees)	Longitude (in degrees)	Dates sampled	<i>E. coli</i> (estimated)
Elks Run at Daniels Road	Daniels Road	39.34403	77.83860	Not sampled (dry)	Not sampled
Elks Run at Flowing Springs Road	Flowing Springs Road	39.34240	77.83110	Not sampled (dry)	Not sampled
Elks Run at Job Corps Road	Job Corps Road	39.33953	77.81860	June 13 and 22, 2017	900 cfu/100 mL
Elks Run at Route 230B	230B	39.33506	77.79940	June 13 and 28, 2017	700 cfu/100 mL
Elks Run at Meadowbrook Farms	Meadowbrook Farms	39.33407	77.78940	Not sampled (no access)	Not sampled
Elks Run at Bakerton Road	Bakerton Road	39.33462	77.76040	June 13 and 22, 2017	500 cfu/100 mL
Elk Branch at Shenandoah Junction	Shenandoah Junction	39.36000	77.84420	May 19 and June 28, 2017	33,000 cfu/100 mL
Elk Branch at Melvin Road	Melvin Road	39.36334	77.82430	June 13 and 22, 2017	260 cfu/100 mL
Elk Branch at 230A- Whistle Stop Lane	Whistle Stop Lane	39.35276	77.80120	June 13 and 28, 2017	2,890 cfu/100 mL
Elk Branch at Engle's Switch Road	Engle's Switch Road	39.34268	77.77870	June 13 and 22, 2017	900 cfu/100 mL



**Figure 1.** Sampling sites in the Elks Run watershed, Jefferson County, West Virginia, 2016–17.



were filtered through 0.45-micrometer filters that were transferred to Petri-pad dishes; the pads were saturated with 2 mL of m-ColiBlue24 (Hach Company, Loveland, Colorado) selective/differential growth medium. Sealed culture dishes were then incubated at  $35 \pm 0.5$  degrees Celsius ( $^{\circ}\text{C}$ ) for 24 hours and photographed. Colonies were counted using Image-J, a freeware program (Schneider and others, 2012) to determine fecal coliform (as blue *Escherichia coli* colonies) and other coliform (red) colonies. Samples containing greater than 400 cfu/100 mL were subjected to molecular analyses to detect human and (or) bovine contributions. Both samples (that were collected at different times) were pooled and analyzed if only one of the two replicate samples exceeded this threshold.

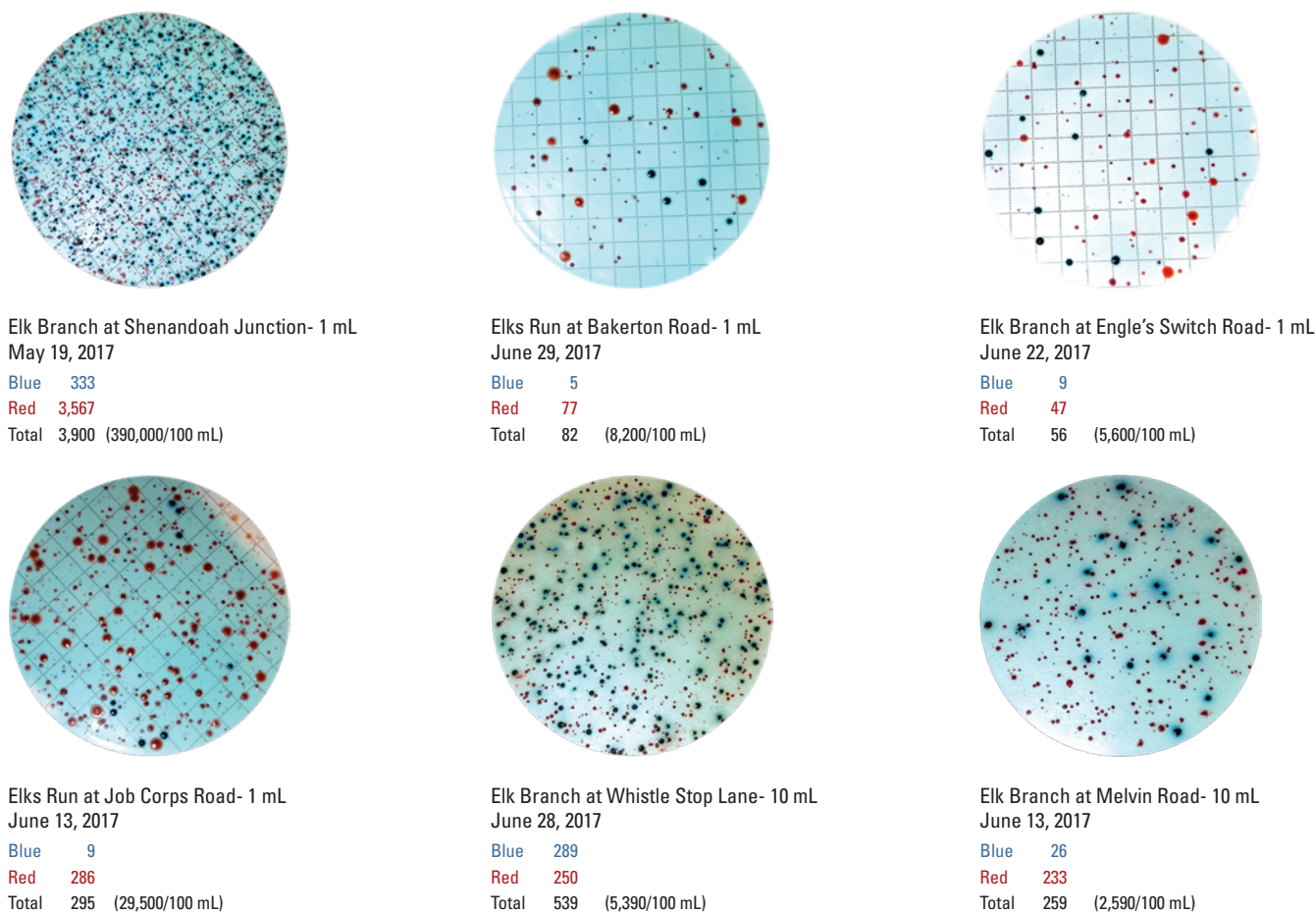
Bacteria, virus, and other cellular materials were collected from 2 liters of source-water samples by a flocculation/precipitation method newly developed by the USGS Leetown Science Center Molecular and Environmental Microbiology Laboratory. This method described by Schill and Galbraith (2019) is a modification of those described in Zhang and others (2010), Zhang (2012), and Zhang and others (2013) that eliminates the need to filter large volumes of water through membrane filters, which is time consuming. Settled flocs were collected, the flocculating agent was removed, and nucleic acids were extracted using the ZR Soil Microbe DNA MicroPrep procedure (Zymo Research Corp., Irvine, California). Purified nucleic acids were eluted in 200 microliters of elution buffer and stored at  $-20^{\circ}\text{C}$  pending analysis. Field blanks (tap-water samples processed through the collection apparatus in the field and extracted in the same way as actual samples) served as negative sample collection and extraction control blanks.

Multiple molecular analyses were applied to detect human and (or) bovine fecal contributions to the fecal contamination of Elks Run watershed source waters. Several analyses targeted strains of *Bacteroides* that are known to be host-specific, including the HF183 human marker (Seurinck and others, 2005; Seurinck and others, 2006; Green and others, 2014) and the BoBac bovine marker (Layton and others, 2006). The AllBac marker (Layton and others, 2006) was used to detect fecal contamination from mammalian sources. Human polyomaviruses (HPyV) were detected by use of published methods (McQuaig and others, 2006; McQuaig and others, 2009; McQuaig and others, 2012) to further confirm human contamination. Additionally, the presence of human and bovine mitochondrial DNA (mtDNA) was determined to further examine influence (Schill and Mathes, 2008). All samples subjected to quantitative polymerase chain reaction analysis (qPCR) were done in quadruplicate with results expressed as the geometric mean. Human placental DNA and calf thymus DNA were included as positive controls in analyses of mtDNA to verify the proper performance of the assays. Amounts of the target(s) identified by qPCR were ranked on a scale of 1 to 1,000 for numerical comparisons, or 1 to 1,000,000 and plotted using a logarithmic scale for purposes of visual comparison.

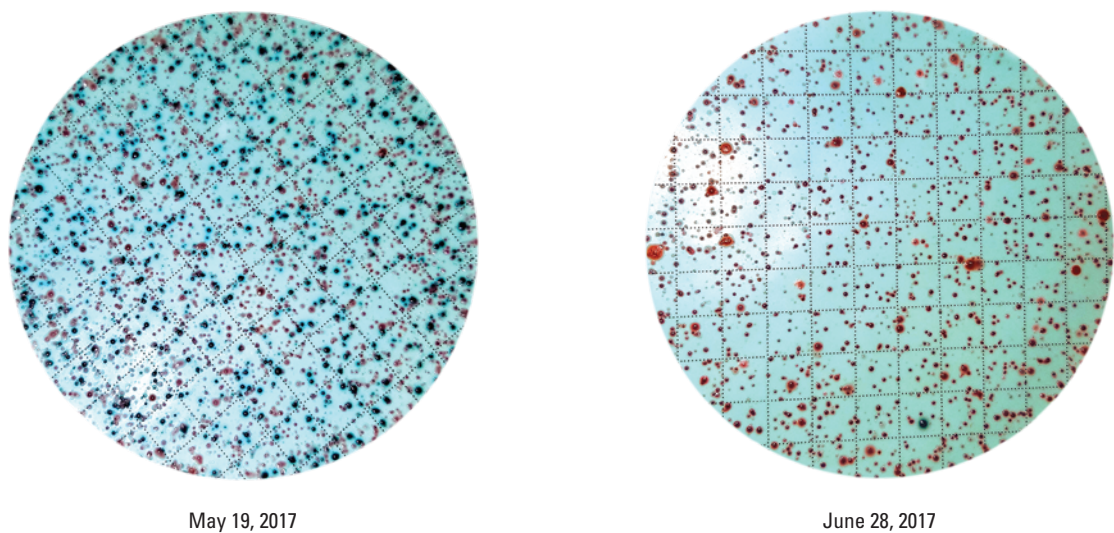
## Results

Two sites (Elks Run at Daniels Road and Elks Run at Route 230B) were dry throughout the study period. A third site (Elks Run at Meadowbrook Farms) visually appeared to be dry but had no public access. USGS personnel are required to have written permission from landowners to conduct studies on private property. Coliform bacterial contamination was detected at all sites, and fecal coliform numbers (as *Escherichia coli*) ranged from 260 cfu/100 mL (Elk Branch at Melvin Road) to 39,300 cfu/100 mL at the Elk Branch at Shenandoah Junction site (table 1, fig. 2). For purposes of completeness, molecular analyses were conducted on water samples from the Elk Branch at Melvin Road site even though fecal coliform bacteria did not reach the 400 cfu/mL criterion. The Elk Branch at Shenandoah Junction site was particularly interesting. A sample collected on May 19, 2017, carried approximately 390,000/100 mL of total coliform bacteria, of which 33,300 were *E. coli*. A repeat sample collected on June 28, 2017, still carried many coliform bacteria, but fecal coliform bacteria numbers had dropped to only 80 cfu/100 mL (fig. 3). Additionally, a strong sewage odor was noticeable during the May sampling that was absent in June. As expected, molecular analyses demonstrated very high levels of fecal markers in the May sampling at the Elk Branch at Shenandoah Junction site that dissipated by the June sampling. Interestingly, all *Bacteroides* molecular markers were highest in concentration in the May sample, thus indicating both bovine and human major fecal contamination. Human contribution was further supported by the presence of HPyV at higher levels in the May sample than any other sample/site. Bovine mtDNA as measured by qPCR of mitochondrial cytochrome b (cytb) was also found in high concentration in the May sample, although amounts of human mtDNA were not particularly high (table 2). It is unknown how far downstream the effects of this episodic event persisted, but elevated *Bacteroides* and cytochrome b (cytb) levels indicate that they may have stretched as far as Melvin Road about a mile downstream.

Molecular marker levels are shown graphically in figure 4. Field blanks (mock samples of sterile water collected at the time and place of sampling) universally were extremely low for all markers, indicating little or no cross-contamination during field sample collection. No sites were found to be exclusively influenced by either human or animal contamination, and all sites contained some level of all the indicator targets tested. All molecular marker levels were found to be much higher at the Shenandoah Junction site in May than measured at any other time and (or) site sampled. Water samples from other time points and sites revealed patterns of contamination that varied according to the target of the marker. The AllBac marker measures fecal *Bacteroides* levels from mammalian sources. This marker revealed that the Elks Run 230B site was found to be the least impacted by fecal contamination, whereas other than the Shenandoah Junction site in May, the following sites were most impacted: Job Corps Road, Melvin Road, Engle's Switch Road, Whistle Stop Lane, and Bakerton Road.



**Figure 2.** Coliform bacteria from water samples at selected sites in the Elks Run watershed, Jefferson County, West Virginia. Photographs of membrane filtration coliform bacteria tests (U.S. Environmental Protection Agency (EPA) Approved Method 10029). Red colonies are coliform bacteria; blue colonies are *Escherichia coli* (fecal coliforms). (mL, milliliter)

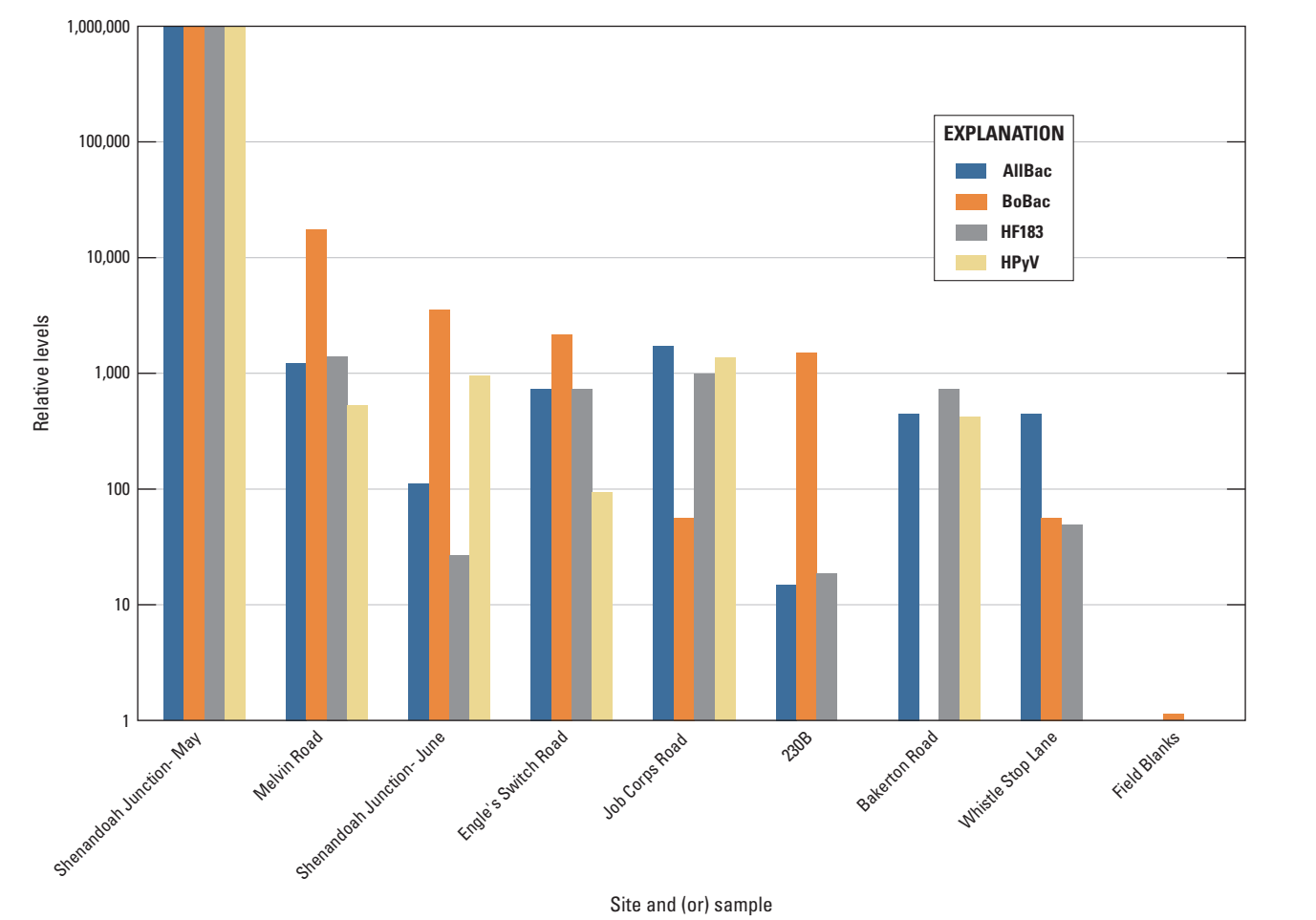


**Figure 3.** Coliform bacteria at the Elk Branch at Shenandoah Junction site, Jefferson County, West Virginia, May and June of 2017. *Escherichia coli* (fecal coliform) bacterial colonies (blue) and total coliform (red) bacteria were extremely elevated in the May 19, 2017, sample. Fecal coliform bacteria were greatly reduced by June 28, 2017, but total coliform bacterial numbers remained elevated.

**Table 2.** Relative amounts of molecular markers at each site and (or) sample collected in the Elks Run watershed, Jefferson County, West Virginia, 2017.

[AllBac, marker measures fecal *Bacterioides* levels from mammalian sources; BoBac, marker measures bovine-associated *Bacterioides*; HF183, marker measures human-associated *Bacterioides*; HPyV, human polyomavirus; cytb, cytochrome b; see table 1 for full site (sample) names and dates]

Site (sample)	AllBac	BoBac	HF183	HPyV	Bovine cytb	Human cytb
Job Corps Road	1.71	0.06	0.99	1.44	431.35	45.92
230B	0.02	1.5	0.02	0.07	1.68	121.81
Bakerton Road	0.45	0	0.74	0.49	824.85	34.18
Shenandoah Junction- May	1,000	1,000	1,000	1,000	1,000	59.74
Shenandoah Junction- June	0.12	3.52	0.03	1.02	6.32	3.37
Melvin Road	1.23	17.42	1.41	0.6	730.15	1,000
Whistle Stop Lane	0.45	0.06	0.05	0.07	204.87	17.2
Engle’s Switch Road	0.74	2.18	0.74	0.16	617.92	61.69
Field blank(s)	0.01	0	0	0.07	0.88	6.28

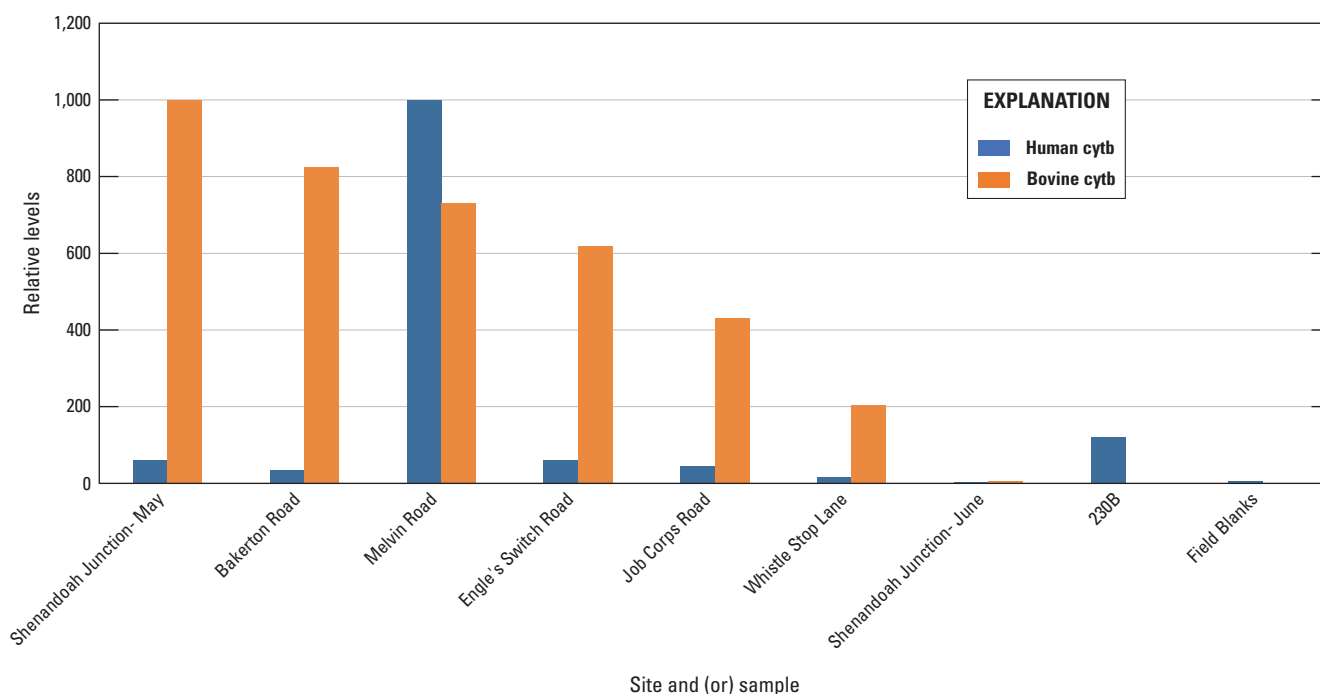


**Figure 4.** *Bacterioides* and human polyomavirus (HPyV) relative marker levels at sampled sites in the Elks Run watershed, 2016–17. Levels of all markers are graphed as log-scaled values relative to those in the Elk Branch at Shenandoah Junction sample collected in May 2017 and field blanks (sterile tap water) that were prepared in the field at the times of sample collections. *Bacterioides* markers tested include AllBac (total fecal *Bacterioides*), BoBac (bovine-associated *Bacterioides*), and HF183 (human-associated *Bacterioides*).

Levels of HF183 (human-associated *Bacteroides*) were found to mirror those of AllBac except for the Whistle Stop Lane site that had similar levels to the Shenandoah Junction site (June sample) and the 230B site. Collectively, the human-associated fecal *Bacteroides* marker HF183 was higher at the Shenandoah Junction (May), Melvin Road, Job Corps Road, Engle's Switch Road, and Bakerton Road sites than at the Whistle Stop Lane, Shenandoah Junction (June), and 230B sites. BoBac (bovine-associated *Bacteroides*) levels were found to be relatively elevated at the 230B site as compared to HF183, indicating that fecal contamination at this site is more from bovine sources than human sources. Conversely, HF183 was more prominent at the Job Corps Road site than the BoBac marker, indicating more human influence at this site. Human polyomavirus (HPyV) is passed primarily in the urine and is a specific indicator of human contribution. HPyV levels were second highest at the Job Corps Road site adding support to this site being mostly human influenced.

Mitochondrial DNA analysis provides additional perspective on the influence of humans and animals on surface waters. Although mtDNA can be introduced into water by fecal contamination, body fluids (for example saliva) and shed skin cells from bathing also are common sources. Mitochondrial DNA degrades and (or) is removed from surface waters much more quickly than bacterial or viral particles and thus is reflective of relatively recent contamination (Harwood and others, 2014; He and others, 2016). The levels of human and

bovine mtDNA observed at sampling sites in the Elks Run watershed are shown in figure 5. Combining and comparing these results with those found using the fecal *Bacteroides* and HPyV markers indicates that the Shenandoah Junction site in May was mostly influenced by bovine contamination at the time of sampling. Human contamination had apparently happened earlier as evidenced by the human mtDNA marker level probably having decayed somewhat from its unknown initial value but with bacterial and viral human markers still very much present at the time of sampling. By the time of the June sampling at the Shenandoah Junction site, both the bovine and human mtDNA markers had declined to nearly background levels. The human bacterial marker HF183 had also declined in concentration, but the HPyV viral marker remained high, probably reflective of the relative stabilities of the markers (viral > bacterial > mitochondrial). Most sites had recent significant bovine influence as revealed by elevated bovine cytb (mitochondrial DNA) levels. Additionally, most sites showed evidence of recent human influence but to a much lower level than bovine. The Melvin Road site was an exception with much higher human as opposed to bovine mtDNA levels.



**Figure 5.** Human and bovine cytochrome b (cytb, mtDNA) marker levels. Graphed levels are scaled relative to the highest observed and to the field blank(s) collected at the time of sampling. The highest bovine cytb level measured was at the Shenandoah Junction site in May, whereas the highest human cytb level was measured at the Melvin Road site.



## Summary

Surface water and groundwater are often affected by fecal contamination, the source of which is difficult to pinpoint and eliminate. This is particularly true in karstic areas such as the Elks Run watershed where underground conduits may funnel contaminants from distant and unobvious sources. Elks Run and its tributary, Elk Branch, in the Elks Run watershed in Jefferson County, West Virginia, have been and are being influenced by episodic fecal contamination, especially from surface runoff as a result of rain events. No samples analyzed in this study were collected within 48 hours of rain events. Nevertheless, most samples contained relatively high numbers of coliform and fecal coliform bacteria as determined by standard membrane filtration methods. Molecular methods were used to ascertain the source—human or bovine. To achieve this, a newly developed method was used for sample preparation that avoids the need for tedious filtration of large water volumes. Three analyses were conducted to determine relative levels of *Bacteroides* bacteria that reflect total, human, and bovine fecal contamination. *Bacteroides* are strictly anaerobic and normally do not survive for long periods in the environment. Coliform bacteria that are routinely used for fecal monitoring are, in contrast, aerobic and can colonize stream sediments and plants and therefore be detected long after their injection into surface waters. Human polyomavirus is primarily excreted in urine and is specific as a marker of contamination from human sources. Mitochondrial DNA can find its way into surface waters by fecal contamination, the body fluids of humans and animals, or simply through contact, such as bathing. Free DNA is ephemeral in the environment, and therefore detection reflects recent events.

Evidence of human and bovine fecal contamination was found at all sampling sites in the Elks Run watershed. A major episodic contamination event was observed at Shenandoah Junction in May 2017, with levels of all markers being orders of magnitude above those from other sites. By late June, however, molecular marker levels had declined, even though total coliform bacteria numbers were still high. It is unknown how far downstream the effects of this episodic event persisted, but *Bacteroides* levels indicate that they may have stretched as far as Melvin Road about a mile downstream.

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For additional information, contact:  
Director, Leetown Science Center  
U.S. Geological Survey  
11649 Leetown Road  
Kearneysville, WV 25430

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