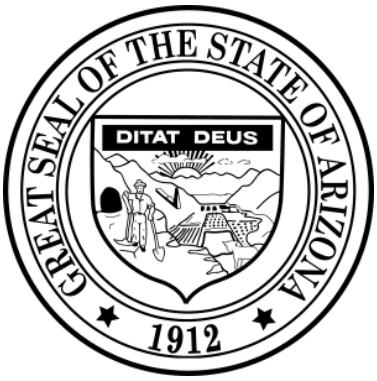

Gila River *Vibrio cholerae* Investigation

Upper Gila Watershed
Graham, Greenlee, and Gila Counties

Conducted jointly by
Arizona Department of Environmental Quality
Arizona Department of Health Services

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1.0 BACKGROUND INFORMATION

In July of 2006, the Arizona Department of Health Services (ADHS) received reports on two people infected with non-O1 *Vibrio cholerae (V. cholerae)*. An investigation was conducted to determine potential sources for their infections. Through interviews, it is was determined that one person swam in the Gila River on the San Carlos Indian Reservation in the vicinity of Bylas, Arizona prior to his illness. The swimmer reportedly had open wounds or abscesses at the time of swimming. No source of exposure was identified for the second case. Both people are recovering and no additional cases have been identified.

Based upon this information, initial environmental sampling was done by the ADHS in conjunction with the San Carlos Tribal EPA, Indian Health Service and Graham County Health Department on August 8, 2006 at two swimming locations. Samples collected from both locations tested positive for *V. cholerae non-O1* and *Escherichia coli* (*E. coli*) bacteria. Pulsed field gel electrophoresis (PFGE), a method of comparing the DNA of different bacterial isolates, was performed on the patient specimens and environmental samples to determine if they were related. The patient isolate did not match the water isolate, thus reducing the probability that the water was the definitive source of the infection; however, it is possible that testing is inconclusive, since environmental samples were obtained several weeks after the infection and multiple strains of *Vibrio spp*. may be present in the Gila River.

The strains of *V. cholerae* identified in both the patient and water samples were non-O1 and did not produce cholera toxin. ADHS staff emphasized that their initial testing and discovery of the *V. cholerae* bacterium was for non-O1 *Vibrio cholerae*. This particular serogroup of the bacterium is considered to be less infectious and less dangerous than O1 *V. cholerae* which was responsible for well-known, historical epidemics.

The American Public Health Association reports that:

> *Organisms of V. cholerae serogroups other than O1 and O139 have been associated with sporadic cases of foodborne outbreaks of gastroenteritis, but have not spread in epidemic form. They have been associated with wound infection and also, rarely, isolated from patients (usually immunocompromised hosts) with septicemic disease.*
>
> *Cases of non-O1/non-O139 gastroenteritis are usually linked to consumption of raw or undercooked seafood, particularly shellfish. In tropical endemic areas, some infections may be due to ingestion of surface waters. Wound infections arise from environmental exposure, usually to brackish water or from occupational accidents among fishermen, shellfish harvesters, etc. In high-risk hosts septicemia may result from a wound infection or from ingestion of contaminated seafood.*

According to the Center for Disease Control:

> *…The cholera bacterium may also live in the environment in brackish rivers and coastal waters. …. The disease is not likely to spread directly from one person to another; therefore, casual contact with an infected person is not a risk for becoming ill. (CDC, 2006)*

The literature also suggests, however, that *V. cholerae* may be transported by other vectors than the commonly recognized ones, including hypotheses about general mammalian transport (not limited to humans) and avian transport. The cholera bacterium has also been shown to attach to copepods or crustaceans in marine environments with a possibility that these hosts can also exist in riverine environments. While it prospers in brackish water environments, *V. cholerae* may persist in aquatic/riverine environments in less habitable circumstances. It also shows an affinity for persisting in biofilms that may form in aquatic environments with high nutrient concentrations (Rector, 2006).

2.0 METHODS AND RESULTS

2.1 OVERVIEW

The San Carlos Apache Tribe, ADHS, and Graham County requested assistance from the Arizona Department of Environmental Quality (ADEQ) in locating the source of the cholera strain and in determining associated *E. coli* levels on the Gila River.

The ADEQ Total Maximum Daily Load (TMDL) unit responded to this request with three sampling trips during the weeks of August 21, September 4, and October 2, 2006. Each collection effort coincided with a different range of the receding hydrograph for the Gila River and its major tributaries. Sampling geographic extent expanded as the investigation unfolded due to the findings in previous trips.

2.1.1 August Sample Collections

The first sampling trip tested river water at ten locations from the Highway 70 crossing of the Gila River on the San Carlos Apache Reservation to the Solomon Bridge northeast of Safford. Site selection rationales were guided by two objectives: testing the upstream extent of the *Vibrio* presence and bracketing of potential contributing sources, including the waste water treatment facilities or ponds of Safford, Thatcher, Pima, and Bylas. Results from sample collection effort #1 are detailed in Table 1.

Table 1. Site locations and results, August 2006

Site Description	Location (DMS)	Discharge, Cubic ft/sec	*E. Coli*, MPN per 100 ml	*V. Cholerae*, Presence/Absence	*V. cholerae*, MPN per liter
Gila below Solomon Bridge	32 49 40.7 / 109 37 53.2	4350 E	2169	Present	330
Gila below Safford WWTP	32 51 59.7 / 109 44 52.8	4350 E	1915	Present	2400
Gila below Thatcher Bridge	32 52 41.5 / 109 46 04.6	4350 E	1585	Present	330
Gila at Pima Bridge	32 54 50.2 / 109 49 33.3	4700 E	5794	Present	330
Gila at Ft. Thomas River Rd	33 02 55.3 / 109 57 57.5	4400 E	1725	Present	2400
Gila at Geronimo	33 05 27.5 / 110 01 49.4	4400 E	1300	Present	100
Gila at SCAT Reservation Boundary	33 05 37.8 / 110 03 23.9	4300 E	2755	Present	2400
Swimming location, Gila at mid-town Bylas	33 07 02.1 / 110 06 23.3	4300 E	1850*	Present	2400
Gila above Bylas WWT ponds	33 08 06.9 / 110 07 02.0	4300 E	2359*	Present	2400
Gila at Hwy 70 Crossing below Bylas (A)	33 09 47.3 / 110 08 03.5	4400 E	1758	Present	330

* - Minimum value from dilution; undiluted sample exceeded 2419.2 MPN (Colilert limit)

E – Estimated based on interpolation from bracketing USGS stations.

2.1.2 September Sample Collections

A second sample collection effort was undertaken after the Labor Day holiday when no clear "smoking gun" point source could be identified from the first collection effort. Results from the first round of sampling showed unexpected geographic extent and no discernable pattern of variation from site to site. High levels of *V. cholerae* (Non O1 serotype) occurred sporadically at a number of different locations, with dips in the densities present at intermediate sites. Sampling geographic extent was extended to the Arizona state line near Duncan while retaining sites on the reservation where the problem was first identified; Bonita Creek and the San Francisco River, two perennial tributaries to the Gila, were also sampled in their lower reaches. Results from the second sampling round are detailed in Table 2.

Table 2. Site locations and results, September 2006

Site Description	Location (DMS)	Discharge, Cubic ft/sec	*E. Coli*, MPN per 100 ml	*V. Cholerae*, Presence/Absence	*V. cholerae*, MPN per liter
Gila at NM state line	32 43 28 / 109 05 57	1700 E	232	Absent	<32
Gila below Duncan WWTP	32 44 38 / 109 08 14	1650 E	386	Present	40
Gila at Old Safford Bridge near Clifton	32 57 54 / 109 18 29	928	6131	Present	40
San Francisco River above Clifton	33 07 56 / 109 16 58	500 E	602	Present	100
San Francisco River below Clifton	33 00 28 / 109 18 52	514	1020	Present	330
Gila River above Bonita Creek	32 53 37.7 / 109 28 39.2	1400 E	302	Present	100
Bonita Creek above Gila River	32 53 45 / 109 28 45	<5	162	Present	<32
Gila below Solomon Bridge	32 49 40.7 / 109 37 53.2	1530 E	1935*	Present	330
Gila below Safford WWTP	32 51 59.7 / 109 44 52.8	1400 E	741	Present	330
Gila below Thatcher Bridge	32 52 41.5 / 109 46 04.6	1325 E	910	Present	330
Gila at Pima Bridge	32 54 50.2 / 109 49 33.3	1315 E	1109	Present	40
Swimming location, Gila at mid-town Bylas	33 07 02.1 / 110 06 23.3	1160 E	1478	Present	330
Gila at Hwy 70 Crossing below Bylas (B)	33 09 59.6 / 110 08 09.0	1150 E	1454	Present	330
Gila at BLM campground nr Winkleman	33 01 22 / 110 44 13	330 E	55	Present	2400

* - Minimum value from dilution; undiluted sample exceeded 2419.2 MPN (Colilert limit)
E – Estimated based on interpolation from bracketing USGS stations.

2.1.3 October Sample Collections

ADEQ followed up on sampling on the Gila one month later to test whether the decline of the Gila River hydrograph from an active monsoon season to near historic flow norms would bring about a reduction in *E. coli* and *V. cholerae* counts. The geographic extent of sampling was the same as the September sample collection effort, with fewer stream sites but the addition of six sampling locations on the San Carlos Reservoir, as requested by the San Carlos Apache Tribe and Graham County. Results were analyzed by two different laboratories using the same protocols while in consultation with one another. *E. coli* results from the third sample collection effort uniformly met Arizona water Quality Standards at all lake and stream sites. *Vibrio* counts were low and generally less than the previous sampling. One site (Gila at Pima Bridge) showed a small increase in *Vibrio* counts from September to October. Two stream and three reservoir sites showed either no presence of the *Vibrio* bacterium or amounts below quantification levels. An additional three stream sites showed quantifiable results only at reporting limit thresholds. Results from the third collection effort are summarized in Table 3.

Table 3. Site locations and results, October 2006

Site Description	**Location (DMS)**	**Discharge, Cubic ft/sec**	***E. Coli*, MPN per 100 ml**	***V. Cholerae*, Presence/Absence**	***V. cholerae*, MPN per liter**
Gila below Duncan WWTP	32 44 38 / 109 08 14	71 E	65.7	Present	20
Gila at Old Safford Bridge near Clifton	32 57 54 / 109 18 29	150	108.6	None Detected	<20
San Francisco River below Clifton	33 00 28 / 109 18 52	88	11.8	Present	210
Gila below Solomon Bridge	32 49 40.7 / 109 37 53.2	200 E	31.0	Present	20
Gila at Pima Bridge	32 54 50.2 / 109 49 33.3	277 E	89.7	Present	50
Swimming location, Gila at mid-town Bylas	33 07 02.1 / 110 06 23.3	245 E	52.7	Present	20
Gila at BLM campground near Winkleman	33 01 22 / 110 44 13	330 E	54.9	None Detected	<20
San Carlos Reservoir, Site 1	33 11 01.7 / 110 31 20.9	N.A.	0	Present	39
San Carlos Reservoir, Site 2 (Dam)	33 10 35.4 / 110 31 26.2	N.A.	1.0	Present	100
San Carlos Reservoir, Site 3	33 10 31.4 / 110 30 31.4	N.A.	1.0	Present	330
San Carlos Reservoir, Site 4	33 10 28.5 / 110 28 23.8	N.A.	0	Present	<32
San Carlos Reservoir, Site 5	33 11 55.6 / 110 28 44.8	N.A.	0	Present	<32
San Carlos Reservoir, Site 6	33 11 30.4 / 110 29 39.9	N.A.	2.0	Present	<32

* - Minimum value from dilution; undiluted sample exceeded 2419.2 MPN (Colilert limit)
E – Estimated based on interpolation from bracketing USGS stations

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3.0 DISCUSSION

3.1 *Vibrio cholerae*

Available literature on the prevalence and densities of *Vibrio* in inland riverine environments was sparse. The conventional wisdom behind the understanding of *V. cholerae* has been accrued and distilled from the knowledge base of the O1 serotype historically responsible for epidemics. Non-O1 serotype is not well-known and has not been studied in-depth.

Conventional assumptions about *V. cholerae* include the following: *V. cholerae* is found in estuarine (brackish) or marine (saline) environments; *V. cholerae* is carried by shellfish and exposure occurs through ingestion of seafood or the drinking of contaminated water; where *V. cholerae* exists in other environments, it is associated with sewage spills or known exposure to human feces. (CDC, 2006; American Public Health Association)

A number of sources discovered contested the conventional assumptions and suggested that *V. cholerae* is more widespread than previously thought while not necessarily holding to the conclusions listed above. Perez-Rosas and Hanzen (1989) found high densities of *V. cholerae* present at pristine inland sites at the highest points in a watershed in Puerto Rico. While higher densities were present at sewage outfalls on the Mameyes River, their findings showed that *Vibrio* was not necessarily correlated with a saline environment or with known sewage exposure. Rhodes, Schweitzer, and Ogg (1985) isolated Non-O1 serotype *Vibrio* from a horse, a lamb, and two American bison that died in the early 1980s in western Colorado, suggesting that herbivores might act as carriers or possible vectors for the transport of the organism. Rhodes, Smith, and Ogg (1986) followed up with a study that found *V. cholerae* at 21 of 24 river, stream, canal, and ditch sites tested in Mesa County, Colorado. *Vibrio* was found at sites with both high (>17mmols Na+) and low (<5mmols Na+) salinity. They concluded that *V. cholerae* appeared to be indigenous to the natural waters of the area. Ogg, Ryder, and Smith (1988) followed this study with one that isolated V. cholerae from the feces of 20 species of aquatic birds in Colorado and Utah in 1986 and 1987. While speculation on the transforming of O1 serotype into the Non-O1 serotype in the intestinal tract of waterfowl was briefly raised in the paper, evidence was not conclusive as to whether *Vibrio* might be indigenous to the native riverine flora or transported into inland aquatic environments. Their findings lend support to the view that aquatic birds may be a possible vector for the migration of the *Vibrio* bacterium to inland waters.

ADEQ's results in the course of this investigation lend support to some of the alternative hypotheses offered above. The Gila River and its tributaries in the study area can be considered neither brackish nor saline (specific conductivity values range from 212- 444 μs/cm). The area investigated is well inland (200-300 miles) from the nearest coastal area. No consistent numerical correlation was noted with *E. coli*, the state water quality indicator organism for bacteriological quality and an indicator of mammalian fecal contamination. Water temperatures were uniformly warm in the course of sampling, ranging from 19.5 to 26.3 degrees Celsius (*Vibrio* has not been observed in the research detailed above at temperatures less than 10 degrees Celsius). No geographic consistency was observed in the location of high *Vibrio* densities during the investigation, nor did any individual site show results over time that was consistent with a point source problem (i.e. resistant to decline with the receding hydrograph for the Gila River).

3.2 *Escherichia Coli*

The Gila River and its tributaries flowed at record levels for the monsoon season this year. As is typically seen in flood events, *Escherichia coli* counts rose to high levels exceeding Arizona water quality standards along the Gila River and persisted at high levels consistently at several sites through the first two sample collection efforts. Only when flows receded to near historic norms in early October did *E. coli* levels come back into compliance with state standards. This pattern has been frequently observed by ADEQ in Arizona streams statewide during high-flow conditions, with a higher likelihood of occurrence in high-order main-stem streams like the Gila. The correlation with flows was more readily apparent for *E. coli* than for *Vibrio* in this investigation.

E. coli also showed some geographic associations with the locations of agricultural fields in the Duncan-Sheldon-York corridor and again in the Safford Valley from the Solomon area to Pima. Levels generally were lower at sites within the Gila Box Riparian National Conservation Area. Not all *E. coli* problems noted in this investigation can be provisionally attributed to agricultural practices; testing on the San Carlos Apache Reservation, where agriculture is limited, showed high levels upstream of agricultural fields. Additionally, the Clifton area was contributing a portion of the *E. coli* load to the Gila, as evidenced by *E. coli* counts above and below the town of Clifton on the same day of sampling.

The most notable result to emerge from *E. coli* sampling on the Gila River, and a marker for additional concentrated investigation in an upcoming TMDL project, was the dramatically escalating count noted in a two to three hour period when samples were taken successively at the New Mexico state line (232 MPN/100 ml), below the Duncan waste water treatment ponds (386 MPN/100 ml), and at the Old Safford Bridge near Clifton (6131 MPN/100 ml). Results point to a heavy source loading in this stretch of the Gila. Further research will be conducted.

E. coli results for the San Carlos Reservoir rank very well when compared to other Arizona lakes and reservoirs (Fitch, 2006).

4.0 CONCLUSIONS

- *E. coli* and *Vibrio* levels generally followed the hydrograph of the Gila River this monsoon season. High flows could generally be correlated with poorer bacteriological water quality as evidenced by water quality standards violations for *E. coli* and sporadic high counts of *V. cholerae*.
- Though both species were responsive to discharge, associations between *V. cholerae* and *E. coli* were correlated only in their co-existence in the Gila River aquatic ecosystem (presence or absence). They do not appear to be numerically correlated with one another. Within any given sample collection effort, high *Vibrio* densities were found with relatively low *E. coli* results as well as vice versa.
- All *Vibrio* detections but one occurred in waters having a temperature of greater than 20 degrees Celsius. Were sampling to continue through winter months, it would be expected that *Vibrio* would disappear with the onset of colder water temperatures. However, the literature suggests that *Vibrio* appears cyclically on an annual basis, and that this is a part of the normal behavior of the pathogen.
- Non-O1 *V. cholerae* isolated in Arizona waters could not be related to salinity; while chemical analyses were not conducted on Gila River water samples, in situ conductivity

readings did not exceed 444 μs/cm and total dissolved solids readings, where recorded, did not exceed 285 mg/l. The lower limit suggested for salinity in references is a total dissolved solids value of 1000 mg/l (Hem, 1985).

- Two areas appear to be contributing disproportionately to the *E. coli* problem – the lower reach of the San Francisco River, including Clifton, and the reach of the Gila River between Duncan and Three Way (approximately 10 miles south of Clifton). Although premature for any conclusion, it is a prevalent agricultural area and it is understood that farmers in this area apply manure to the fields as fertilizers. The possibility of impact from the agricultural practices along the Gila River in this stretch is a consideration.

At this point and based on all results compiled from ADEQ's investigation, we are not able to conclusively point to any particular source for *V. cholerae non-O1*. Initial hypotheses that waste water treatment ponds (WWTPs) played a pivotal role were not borne out by consistent high densities in the same locations from one sampling effort to the next. The spatially diffuse and temporally variable results from this investigation suggest alternatives other than a cut-and-dried point source like a single WWTP outfall. As the investigation proceeded, initial assumptions that a point source (or multiple point sources) could be isolated began to evolve into a consideration that *V. cholerae non-O1* might be indigenous to the Gila River aquatic environment and could well be present every summer season. References in the literature mentioned a possible role for sediment as a reservoir for the bacteria; it is a possibility that the *Vibrio* organism waits in the sediments of the Gila River for warm water conditions and begins to multiply when conditions are favorable. It is also possible that *Vibrio* is liberated from sediments and entrained in the water column by the high flows of monsoon storms on an annual basis.

Another possibility is that waterfowl in the area are transporting the *Vibrio* bacteria from coastal areas or on migratory flyways and depositing infected feces into the Gila River aquatic ecosystem. Speculation has turned recently to the consideration that waste water treatment ponds in the area, all of which are close to the Gila River, may draw migratory waterfowl for their attractive habitats and calm water. If this is the case, the river may be receiving its *Vibrio* load from birds traveling back and forth between the river and WWTPs. Based on current data, it appears the organism is prevalent throughout the aquatic ecosystem, with higher concentrations making sporadic appearances in areas that are close to populated areas of eastern Arizona and may share a link, though not necessarily a causal link, to the practices and improvements of the communities of the Duncan and Safford Valleys and the San Carlos Apache Tribe. Further research would shed light on the causative mechanisms responsible for increases of *V. cholerae non-O1*.

5.0 RESPONSE

5.1 Recommendations

ADHS and ADEQ jointly recommended to the affected Arizona counties (Graham, Greenlee, and Gila) and the San Carlos Apache Tribe that swimming advisories be posted at all easily-accessible points along the Gila after the first sampling trip confirmed that *Vibrio cholerae* was present in the Gila River. For several reasons, the advisories were based on the concurrent *E. coli* water quality standard exceedances. The reasons included the following:

1.) *According to ADHS, Non O1 serotype V. cholerae is not toxigenic, but only pathogenic*. The form of *V. cholerae* found in the Gila River was not the epidemic form that spreads rapidly throughout a population.
2.) *Arizona has no water quality standards for V. cholerae*. Arizona, like many states, has chosen to put in place water quality standards for only indicators of bacterial contamination. *E. coli*, the bacterium of most common concern for water contamination, is the chosen indicator species for Arizona water quality standards.
3.) *No epidemiological data on safe exposure levels to V. cholerae non O1 was available.* Anecdotal suggestions from EPA authorities and wide-ranging tabular values for infectious doses of O1 *V. cholerae* provided the only available guidance.
4.) No long-term data studies on V. cholerae were available to inform the setting of numeric thresholds for V. cholerae in inland waters.
5.) *Data that were available on infectious doses of V. cholerae were based on ingestion rates and did not address exposure through dermal routes*.

The results from the third sampling trip necessitated a different approach. While *V. cholerae* was still present at low levels at a number of sites, *E. coli* levels had fallen back into compliance with state water quality standards. For this reason, the Arizona Department of Environmental Quality rescinded its recommendation that swimming advisories be posted on the basis of exceedances of *E. coli* state water quality standards in October. The Arizona Department of Health Services concurrently issued a general “safe swimming” advisory warning against the immuno-compromised or at-risk populations swimming in the Gila River with open cuts or sores.

5.2 Actions

Graham, Greenlee, and Gila counties and the San Carlos Apache tribe responded to the state’s recommendation by posting all commonly-used approaches to the river with prominent signs warning against swimming for the duration of the *E. coli* spike. Public service announcements were made on local cable-access TV channels, and flyers were posted at public locations throughout the area, including convenience stores, post offices, and other commonly-frequented establishments. The State of Arizona augmented this effort through ADEQ’s Communication Office by issuing a notice to Eastern Arizona residents regarding elevated *V. cholerae* and *E. coli* levels in the Gila River. The notice was posted at the take-out beaches and recreational areas of the Gila Box National Riparian Conservation Area. At the counties’ and tribe’s request, an effort towards initiating long-term studies of this problem was made by contacting Dr. Charles Gerba of the University of Arizona and asking him to consider investigating the issue. Dr. Gerba expressed interest and is currently considering the matter. Additionally, the ADEQ Monitoring Unit of the Surface Water Section has incorporated *V. cholerae* presence/absence testing and enumeration into its rotational basin monitoring design for FY 07 on a provisional basis to begin to ascertain whether *V. cholerae* appears elsewhere in Arizona streams.

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