Supporting Information

Prioritizing Pathogens for Potential Future Regulation in Drinking Water

Rebecca Hoffman, Marilyn M. Marshall, Mark C. Gibson*, Paul A. Rochelle

*Senior Scientist
Clancy Environmental Consultants, Inc.
5413 Broadmoor Street
Alexandria, VA 22315
(703) 310-4108
mgibson@clancyenv.com

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TABLE S1. Brief description of preliminary CCL pathogens

Pathogen	Description
	Viruses
Adenoviruses	Primarily cause acute infections of the respiratory and gastrointestinal tracts and the eye
Astroviruses	Common cause of gastrointestinal illness in adults and children
Caliciviruses	Includes Norovirus, major cause of epidemic acute and mild gastrointestinal illness in adults and children
Enteroviruses	Includes coxsackieviruses and echoviruses; common human viruses associated with various clinical syndromes, ranging from minor fever to severe, potentially fatal gastrointestinal illness
Hepatitis A virus	Common cause of mild, acute liver disease followed by jaundice (hepatitis)
Hepatitis E virus	Causes serious, but acute liver disease, usually in adults
Rotavirus	A leading cause of severe gastrointestinal illness (diarrhea) among infants and young children
	Bacteria
Arcobacter butzleri	Common cause of acute gastrointestinal illness, originally classified as a species of *Campylobacter*
Aeromonas hydrophila	Commonly found in environmental waters and chlorinated water; causes mild to severe gastrointestinal disease, most commonly in young children and immunocompromised persons
Campylobacter jejuni	Can often be isolated from healthy cattle, chickens, and other animals and sometimes environmental waters; very common cause of acute gastrointestinal illness
Escherichia coli (O157)	Commonly excreted in the feces of cattle and other ruminants; toxin-producing bacterium that often causes severe gastrointestinal illness, bloody diarrhea, and can lead to kidney failure, especially in young children and the elderly
Helicobacter pylori	Sometimes found in the environment; capable of colonizing the human gut that can lead to chronic ulcers and cancer
Legionella pneumophila	Can often be found in environmental waters, including hot water systems; can cause pneumonia-like respiratory diseases when inhaled (Legionnaires' disease)
Mycobacterium avium	Commonly found in the environment; causes respiratory infection when inhaled or swallowed, the symptoms of which can be severe and similar to tuberculosis
Plesiomonas shigelloides	Can be isolated from freshwater, freshwater fish and shellfish, and from many animals; causes mild to severe gastrointestinal illness

TABLE S1. Brief description of preliminary CCL pathogens

Pathogen	Description
Salmonella enterica	Most human infections can be traced back to contaminated food products; usually causes mild self-limiting gastrointestinal illness, but which can be severe and include fever and profuse sweating
Shigella sonnei	Frequently found in water polluted with human feces; causes mild self-limiting gastrointestinal illness and bloody diarrhea
Vibrio cholerae	Occurs naturally in the plankton of fresh, brackish, and salt water; causes severe gastrointestinal illness that often results in death (cholera)
Yersinia enterocolitica	Most human infections can be traced back to contaminated food; causes a variety of symptoms depending on the age of the person infected, most often fever and gastrointestinal illness in children
	Protozoa
Blastocystis hominis	Common parasite that can cause various gastrointestinal disorders, including diarrhea and abdominal pain
Cyclospora cayetanensis	Human intestinal parasite that causes acute or prolonged gastrointestinal illness after consumption of fecally-contaminated food or water
Entamoeba histolytica	Common parasite in developing countries that can cause short- and long-term gastrointestinal illness
Isospora belli	Parasite can be found worldwide, especially in tropical and subtropical areas, can cause gastrointestinal disease in immunocompromised persons
Microsporidia	Highly diverse intracellular parasites that can cause a wide variety of illnesses, most commonly diarrhea, mainly in severely immunocompromised persons
Naegleria fowleri	Parasite found in shallow, warm surface and groundwater that causes primary amebic meningoencephalitis, which is usually fatal
Toxoplasma gondii	Parasite of cats, but can be carried by most mammals, usually causes minor and self-limiting “flu-like” symptoms but can have serious or fatal effects in a developing fetus or in immunocompromised persons
	Fungi
Aspergillus fumigatus group	Commonly found in soil and decaying organic matter and can cause significant respiratory illness in immunocompromised persons
Exophiala jeanselmei	Widely distributed in soil, plants, and decaying wood material, can cause various infections in humans, usually skin-related
Fusarium solani	Common plant pathogen that can cause various infections in humans, usually skin-related

Scoring waterborne disease outbreaks

Waterborne disease outbreak (WBDO) scores (0–5) in the alternative approach were determined using a decision tree format (Figure S1). In scoring outbreaks, the alternative approach used only published reports in the U.S. Centers for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Report (MMWR) or citations published in peer-reviewed literature. The microbial workgroup feels it is inappropriate to cite non peer-reviewed literature such as conference proceedings papers as supporting documentation for waterborne disease outbreaks, as was done in the USEPA process. Also, the alternative approach did not consider outbreaks occurring before 1974 because implementation of the original SDWA in that year is widely viewed as a turning point in the improvement of drinking water quality throughout the U.S. Moreover, the alternative process specifically advocates focusing on outbreaks occurring from 1980 onwards in the U.S. because of limited data availability for many earlier outbreaks. Furthermore, outbreak data from U.S. territories (e.g., 1994 *Vibrio cholerae* outbreaks in Northern Mariana Islands [*1*]) were carefully screened for usefulness, while those occurring in the developing world were not considered because the inclusion of outbreak (and occurrence) information from undeveloped countries—often with vastly different laws, regulations, and practices concerning drinking water treatment—would significantly distort the evaluation process.

The alternative approach scores only outbreaks in water intended for use as drinking water or serving as a drinking water source. Thus, waterborne disease outbreaks in water explicitly used for recreational purposes (swimming pools, spas, nonpotable waters) were excluded from consideration. For example, a *Cryptosporidium* outbreak in 2001 infecting 358 patrons of a waterpark in Illinois was attributed to feces from an infected patron; furthermore, the waterpark's records indicated that one fecal or vomit accident every 1–2 days was usual in 2001 (*2*). Clearly, such swimming pool related outbreaks will not be controlled by regulations imposed at drinking water treatment plants and so they should not be used to score this attribute. In contrast, the USEPA's scoring approach included outbreaks linked to recreational water, swimming pools and hot tubs, as well as outbreaks in U.S. territories and developing countries—although the latter were weighted lower than outbreaks in more affluent countries. For the alternative approach, factors including the number of outbreaks attributed to a particular pathogen, the degree of water

treatment applied to the water serving as the source of the outbreak, and certainty of the evidence (i.e., was an actual pathogen identified during the outbreak or was it only epidemiologically linked?) were used to determine the overall outbreak score. In addition, the magnitude of the outbreak was considered in determining the score, since it may not be appropriate to give the same regulatory consideration to an outbreak affecting two people (even with a severe health outcome) as an outbreak affecting thousands of people. Lastly, outbreak data with only a serological association of waterborne disease were not considered because of the difficulty in determining the relationship between seropositivity and clinical disease in the absence of supporting water quality data (*3*).

Scoring microbial occurrence

Occurrence scoring (0–5) also used a decision tree approach (Figure S2) and only peer-reviewed data documenting pathogen occurrence in U.S. waters used for or intended for human consumption. As for scoring waterborne disease outbreaks, the microbial workgroup feels it is inappropriate to cite non peer-reviewed literature as supporting documentation for pathogen occurrence. The alternative approach differentiates occurrence in treated drinking water from occurrence in untreated drinking water by taking into account that many pathogenic microorganisms listed on the preliminary CCL (PCCL) are widely and even historically known to be easily controlled with chlorine disinfection (e.g., *Vibrio cholerae*) that is prevalent throughout the U.S. Subsequent steps in the scoring process discriminate frequency of detection and higher scores were assigned to pathogens for which there were multiple occurrence reports by multiple, independent research groups. Pathogens for which there was only a single report by a single laboratory or multiple reports by a single laboratory were scored lower pending independent confirmation.

The alternative approach also assigns higher scores to pathogens when organisms have been cultured directly from the water source as opposed to molecular detection because molecular-based methods may be more prone to false positives and can detect naked DNA in the absence of intact, infectious organisms (*3*). The microbial workgroup feels that until there are standard practices in place to ensure that all molecular laboratories perform their testing in properly

engineered laboratory space and apply strict contamination measures to their processes, molecular testing results for waterborne pathogen occurrence should not receive equal weight as culture-based results, as in the USEPA approach. Notably, the National Drinking Water Advisory Council CCL Workgroup report (*4*): "The Work Group noted a…key consideration in the CCL process should be that, in general, false negatives should be avoided when going from the Universe to the PCCL and **false positives should be avoided when going from the PCCL to the CCL** [emphasis added]."

Scoring health effects

Health effects were also scored on a scale of 1–5 using a decision tree template (Figure S3). For each microorganism, the most common health outcome for the general population and non-severely immunocompromised sensitive subpopulations (e.g., children, pregnant women, elderly individuals) was determined. A single health effects score was developed for the PCCL pathogen under evaluation. If death was the most common health outcome (e.g., *Naegleria fowleri*) the health effect score was 5. If death was not the most common health outcome, the microbial workgroup subsequently scored five subcriteria (require medical attention to recover, severity, infectious dose, secondary spread, sequellae) to obtain a consensus-based overall health effects score.

For most microorganisms, a small percentage of the exposed population, even within a subpopulation, can have more severe health outcomes than the majority of the exposed population. While this effect was acknowledged by the microbial workgroup, health effects scoring was still assigned based on consensus following determination of the most common outcome to avoid overly weighting exceptional cases. Furthermore, it was assumed that because the population and subpopulations under consideration are within the U.S., all individuals are well nourished and do not have chronic microbial infections (which are commonly observed in developing nations) that could exacerbate a microbial infection with any PCCL microbe. As in the USEPA approach, severely immunocompromised populations (e.g., those with primary or acquired severe immunodeficiency, organ transplant recipients) were not considered in the health effects scoring. Although these individuals comprise sensitive subpopulations, nearly all pathogens would be

assigned high health effect scores if severely immunocompromised subpopulations were considered, which would devalue the scoring process for this key attribute.

Disputed/different PCCL pathogens

As indicated in Table S3 below (see also Table S2), using the alternative approach, an alternate microbial CCL3 was developed listing eight microbes, five of which were in agreement (non-disputed) with the USEPA's draft CCL3. However, there are substantial differences between the results of the two approaches, resulting in nine PCCL organisms or groups of related organisms for which the two approaches did not concur (i.e., disputed PCCL pathogens). As discussed in the accompanying article, the AWWA microbial workgroup unanimously voted that including the bacterial pathogens *Salmonella enterica*, *Shigella sonnei*, and *Vibrio cholerae* that are widely and historically known to be sensitive to chlorination is inappropriate. Each of the remaining six PCCL pathogens is briefly described below, including an overview of their scoring and postscoring evaluation under the alternative approach. Three examples of completed scoring templates for waterborne disease outbreaks, occurrence in water, and health effects for a viral, bacterial, and protozoan PCCL pathogen are also provided (Figures S4–S6).

On alternate microbial CCL3 but not on USEPA draft CCL3

Enteroviruses. These common human viruses are associated with various clinical syndromes, ranging from minor febrile (fever) illness to severe, potentially fatal conditions. There are 10–15 million symptomatic enterovirus infections annually in the U.S. and 30,000–50,000 hospitalizations each year (*5*, *6*). Enteroviruses were responsible for at least one waterborne disease outbreak in the developed world (*7*) and were detected in U.S. source waters and treated drinking water (*8*, *9*). A variety of coxsackieviruses and echoviruses have been identified using molecular methods in municipal well water (*10*). The alternative approach resulted in an overall score of 7.5 for enteroviruses, ranking them 11th out of the 22 evaluated PCCL microbes, and just above the breakpoint score of 7.25 for inclusion on the alternate microbial CCL3. Three of the five experts on the microbial workgroup voted to retain enteroviruses on the alternate microbial CCL3.

***Mycobacterium avium*.** This opportunistic pathogen causes pulmonary disease and is capable of disseminating throughout the body and leading to debilitating and life-threatening infection. The incidence rates of pulmonary mycobacteriosis appear to be increasing in the U.S. and the developed world (*11*). Because *M. avium* is more resistant to most water disinfectants than other waterborne bacteria (*12*, *13*), it is reasonable to assume that these biofilm-associated organisms may continue to pose risks to treated water supplies (*14*). In addition, the degree of *M. avium* resistance to disinfection remains uncertain. The alternative approach (total score = 9.0) ranked *M. avium* 7th on the list of PCCL pathogen. All five microbial workgroup members voted to retain *M. avium* on the alternate microbial CCL3.

Rotavirus. Infection with rotavirus is the most common cause of diarrhea in children worldwide. They are excreted in very large quantities in the feces of infected subjects, so they are present in relatively high concentrations in wastewater and environmental water (*15*, *16*, *17*). Rotaviruses have been detected by molecular methods in treated drinking water (*18*) and non-disinfected well water (*10*), and 13.8% of groundwater samples were positive in a large survey (*19*). They received an overall score of 8.5 and were ranked 9th by the alternative approach (Figure S4). Four of five microbial workgroup members voted to retain rotaviruses on the alternate microbial CCL3.

On USEPA draft CCL3 but not on alternate microbial CCL3

***Entamoeba histolytica*.** Although this amebic pathogen is very common in developing countries, it is rare in the U.S. and waterborne disease outbreaks are not anticipated in the U.S. MMWR-reported outbreaks have occurred either outside the U.S. (e.g., Palau) or in situations where multiple pathogens were recovered from stool samples making it impossible to determine which of the diarrheal agents actually caused the outbreak (*20*). There are no reports on the occurrence of *E. histolytica* in source or finished drinking water in the U.S. (occurrence score = 0). Coupled with an outbreak score of 1 and moderate health effect score of 3, this produced a total score of 4.0 (ranked 19th) for this organism placing it well below the threshold for inclusion on the alternate CCL3. None of the five microbial workgroup members voted for its reconsideration for inclusion on the alternate microbial CCL3.

***Helicobacter pylori*.** This bacterium is one of the most common infectious pathogens worldwide and is associated with a variety of upper gastrointestinal conditions, including chronic gastritis, peptic ulcers, and gastric malignancy (*21*). However, its prevalence is closely tied to socioeconomic conditions and it is more common in developing countries than in most affluent nations. Its route of transmission remains largely unknown and most attempts to recover the organism from environmental water samples have been unsuccessful (*22*). Establishing a link between drinking water and *H. pylori* infection, even if such a link exists, will be difficult given the bacterium's potential long-term health effects. Identifying waterborne disease outbreaks will also be difficult when considering the high proportion of the population that carries *H. pylori* in their gastrointestinal tract. Although infection may lead to significant health effects in some individuals, the lack of outbreak data and detection in water only by molecular methods resulted in an overall score of 6.5 (ranked 15th) (Figure S5). Thus, *H. pylori* was not included on the alternate microbial CCL3, though it did receive one postscoring vote for reconsideration for inclusion.

***Naegleria fowleri*.** Primary amebic meningoencephalitis (PAM) associated with exposure to *Naegleria fowleri* is a very rare condition but is associated with a high mortality rate. Although a small number of PAM-related deaths have been reported from contaminated recreational waters in the U.S., none have been reported from treated water supplies. Each of these cases involved bathing or swimming in warm freshwater and therefore should not be the focus of drinking water regulations (*23*). Moreover, the organism is sensitive to chlorine at very low levels (*24*). Even though *N. fowleri* was one of only two organisms assigned the maximum health effects score of 5, a catastrophic outcome for a very small number of individuals should not be the sole driver behind placing an organism on the CCL. However, since there has only been a single episode of disease linked to water intended for drinking and a single research group has reported detection in non-disinfected water using molecular methods, it received scores of 1 for both outbreaks and occurrence. Consequently, the total score of 7.0 (ranked 12th) was just below the threshold for inclusion on the alternate microbial CCL3 (Figure S6). Morever, none of the microbial workgroup members voted for its reconsideration for inclusion on the alternate microbial CCL3.

TABLE S2. PCCL pathogen scores using the USEPA approach[a]

PCCL Pathogen	EPA Score[b]
Naegleria fowleri	9.0
Legionella pneumophila	8.6
Escherichia coli O157:H7	8.2
Hepatitis A virus	8.2
Shigella sonnei	8.2
Helicobacter pylori	8.0
Campylobacter jejuni	7.5
Salmonella enterica	7.5
Caliciviruses	7.1
Entamoeba histolytica	7.1
Vibrio cholerae	7.1
Adenoviruses	6.6
Enteroviruses	6.6
Cyclospora cayetanesis	6.5
Mycobacterium avium	6.5
Rotavirus	6.5
Yersinia enterocolitica	6.4
Arcobacter butzleri	6.1[c]
Fusarium solani	5.9
Plesiomonas shigelloides	5.8
Hepatitis E virus	5.6
Toxoplasma gondii	5.2
Aspergillus fumigatus group	5.1[c]
Exophiala jeanselmei	5.1[c]
Aeromonas hydrophila	4.8
Astroviruses	3.4[c]
Microsporidia	3.4[c]
Blastocystis hominis	1.7[c]
Isospora belli	3.1[c]

[a] SOURCE: (*25*, *26*). [b] Sum (maximum of 10) of the higher of waterborne disease outbreaks score (1-5) or occurrence of the pathogen in water (1-3) and normalized adverse health effects score (1-5); the dashed line is the USEPA-identified "natural" breakpoint above which are the draft CCL3 contaminants. [c] Not evaluated by alternative approach.

TABLE S3. Comparison of alternate microbial CCL3 and USEPA draft CCL3 microbes

PCCL Pathogen[b]	USEPA Draft CCL3	Alternate Microbial CCL3	Disputed/Different	Alternate Approach Total Score (Max = 15)	Alternate Approach Rank by Scoring	Postscoring Evaluation[a]					
						Sum of Microbial Workgroup Votes	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5
Toxigenic *E. coli* (O157)				14.5	**1**	**5**	1	1	1	1	1
Legionella pneumophila				14.0	**2**	**5**	1	1	1	1	1
Shigella spp.				14.0	**3**	**0**	0	0	0	0	0
Salmonella enterica				13.5	**4**	**0**	0	0	0	0	0
Campylobacter-like organisms				13.5	**5**	**3**	1	0	1	0	1
Caliciviruses (Norovirus)				11.5	**6**	**5**	1	1	1	1	1
Mycobacterium avium				9.0	**7**	**5**	1	1	1	1	1
Hepatitis A virus				8.5	**8**	**3**	1	0	1	1	0
Rotavirus				8.5	**9**	**4**	1	1	1	0	1
Vibrio cholerae				8.0	**10**	**0**	0	0	0	0	0
Enteroviruses (Coxsackieviruses and Echoviruses)				7.5	**11**	**3**	1	0	1	0	1
Naegleria fowleri				7.0	**12**	**0**	0	0	0	0	0
Yersinia enterocolitica				7.0	**13**	**0**	0	0	0	0	0
Fusarium solani				6.5	**14**	**0**	0	0	0	0	0
Helicobacter pylori				6.5	**15**	**1**	0	0	0	0	1
Aeromonas hydrophila				6.5	**16**	**0**	0	0	0	0	0
Adenoviruses				5.5	**17**	**0**	0	0	0	0	0
Toxoplasma gondii				5.0	**18**	**0**	0	0	0	0	0
Entamoeba histolytica				4.0	**19**	**0**	0	0	0	0	0
Cyclospora cayetanensis				3.0	**20**	**0**	0	0	0	0	0
Hepatitis E virus				3.0	**21**	**0**	0	0	0	0	0
Plesiomonas shigelloides				2.0	**22**	**0**	0	0	0	0	0
						Totals	**8**	**5**	**8**	**5**	**8**

Key
- USEPA draft CCL3 microbes
- Alternate microbial CCL3
- Disputed/different PCCL microbes

[a] An expert-based postscoring evaluation was used to determine if a high-ranked (i.e., above the cut-off total score of 7.25 that is one-half of [14.5] the highest scored organism) PCCL pathogen should be retained on an alternate CCL3 or if a low-ranked PCCL pathogen should be reconsidered for inclusion (yes = 1, no = 0; 3 or more votes constituted a majority). [b] The alternative approach evaluated 22 of 29 PCCL pathogens.

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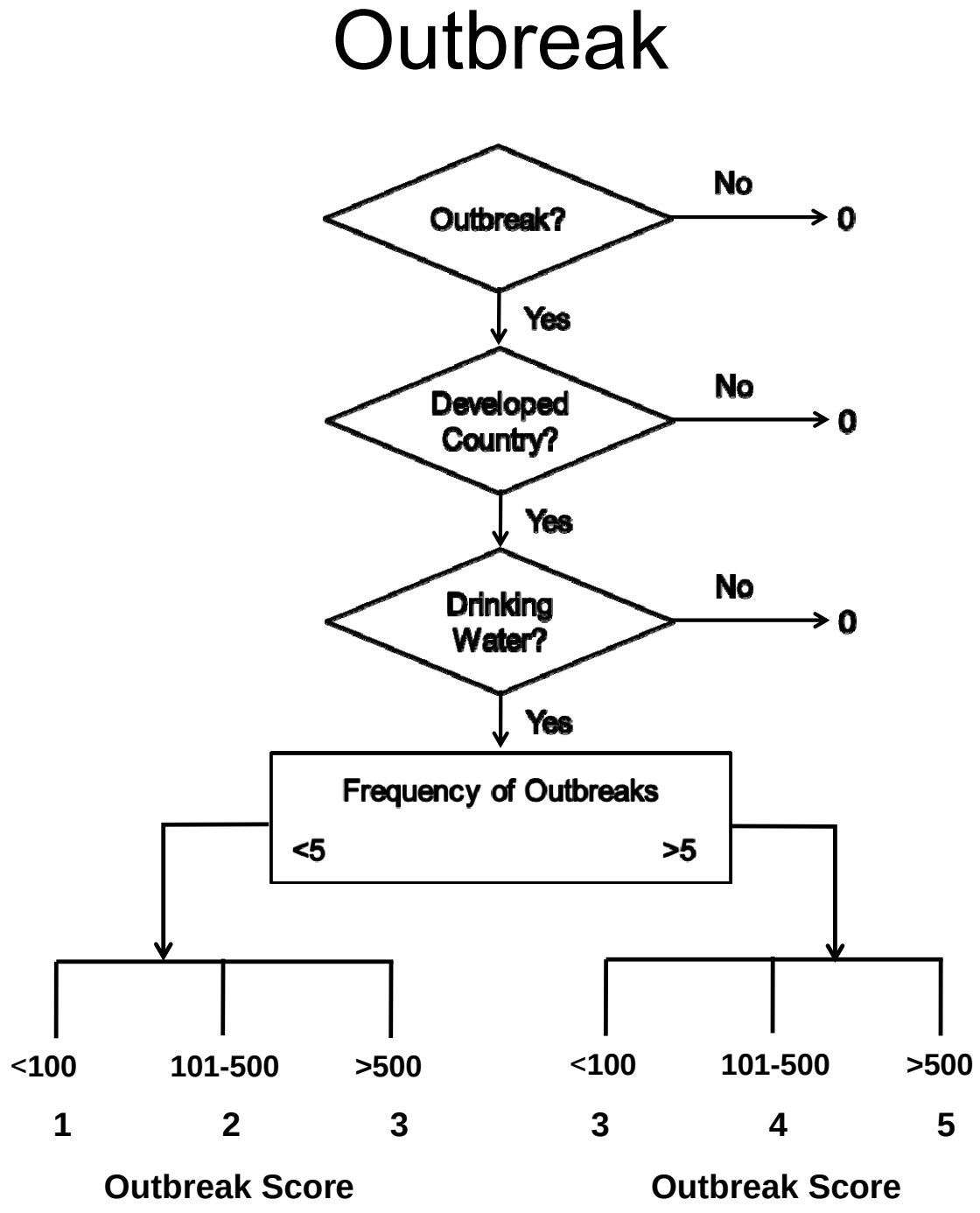
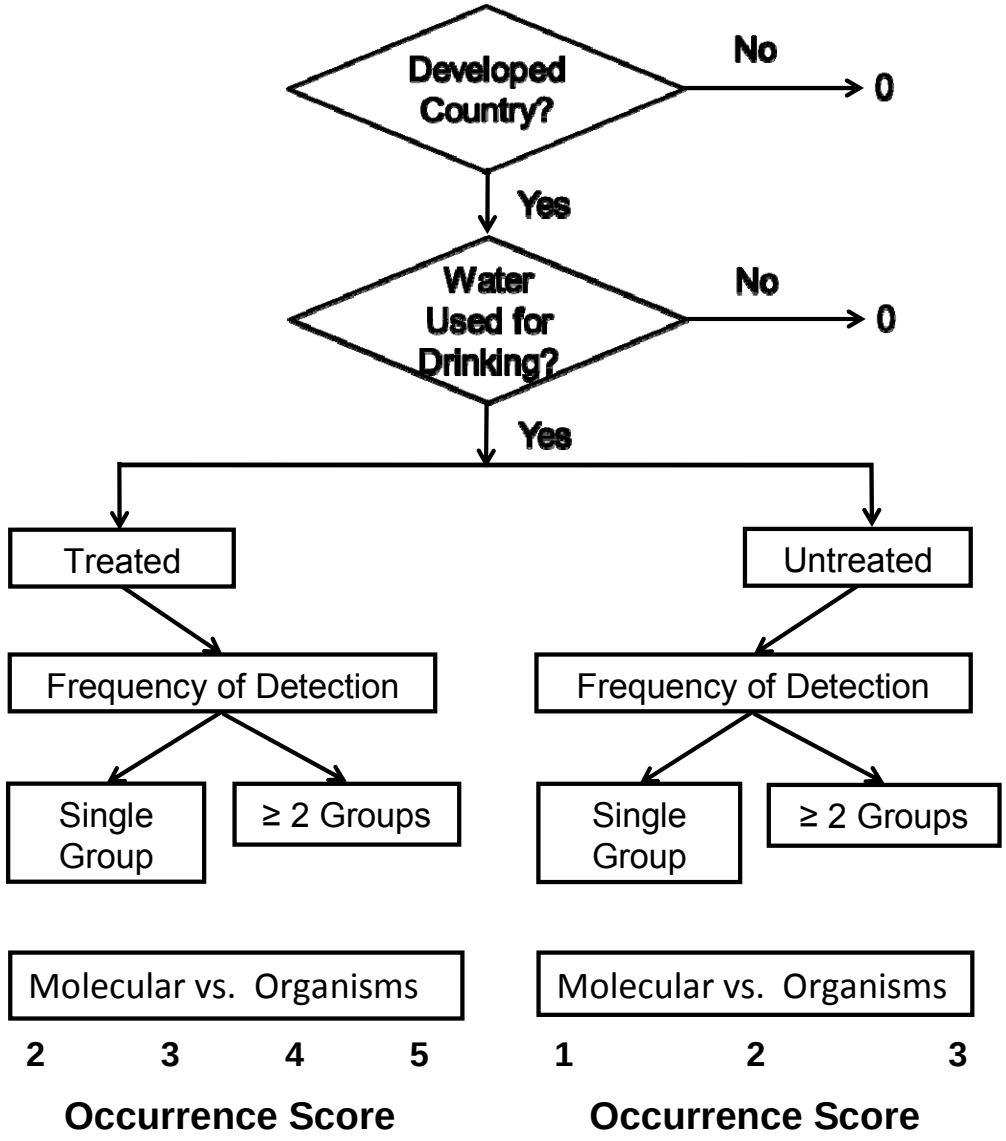


FIGURE S1. Decision tree template for evaluating outbreak.

Occurrence in Water



Note: An occurrence score is decreased by one if the frequency of detection is only 1, (i.e., only one group has reported this microbe in drinking water)

FIGURE S2. Decision tree template for evaluating occurrence.

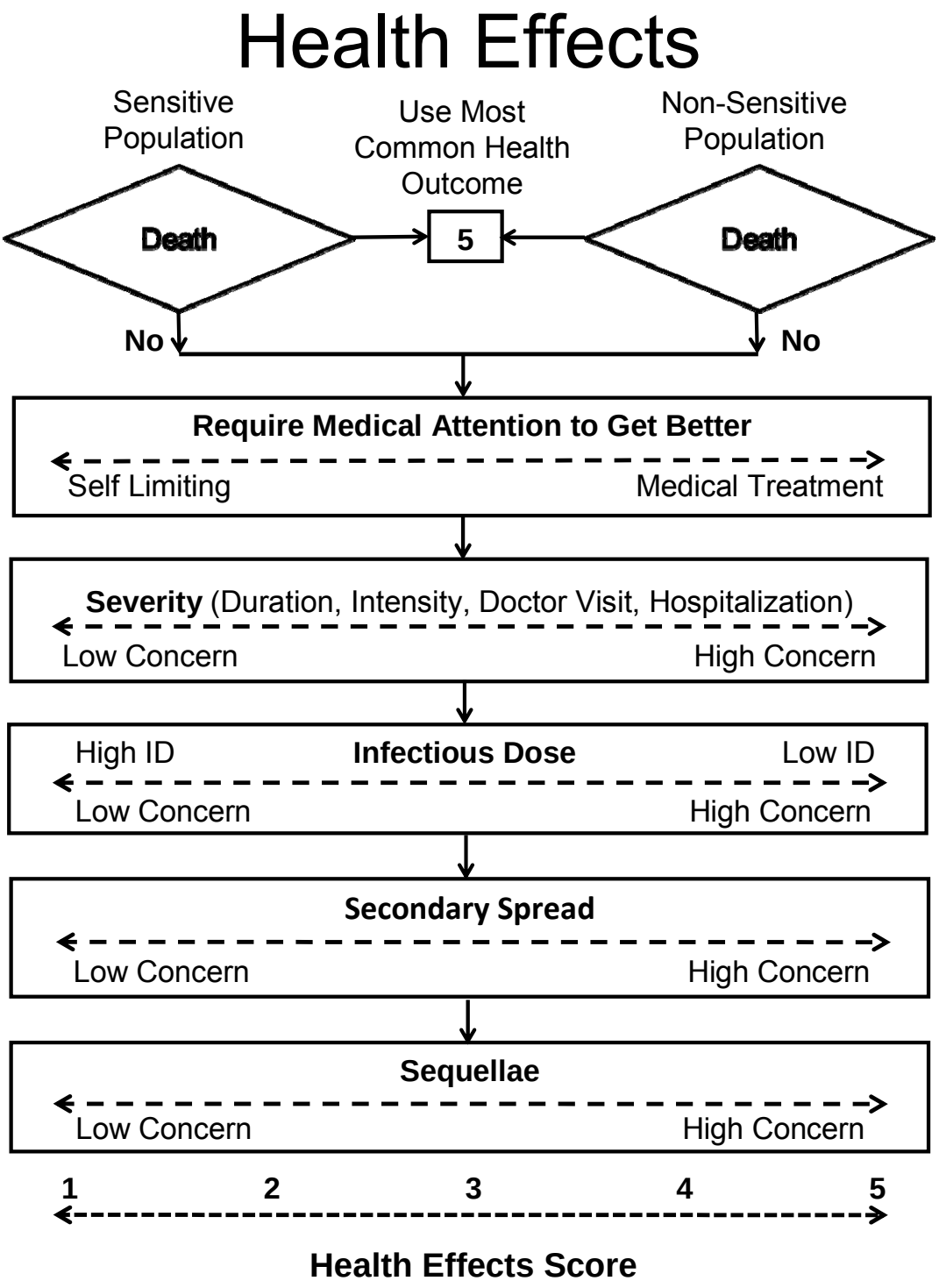


FIGURE S3. Decision tree template for evaluating health effects.

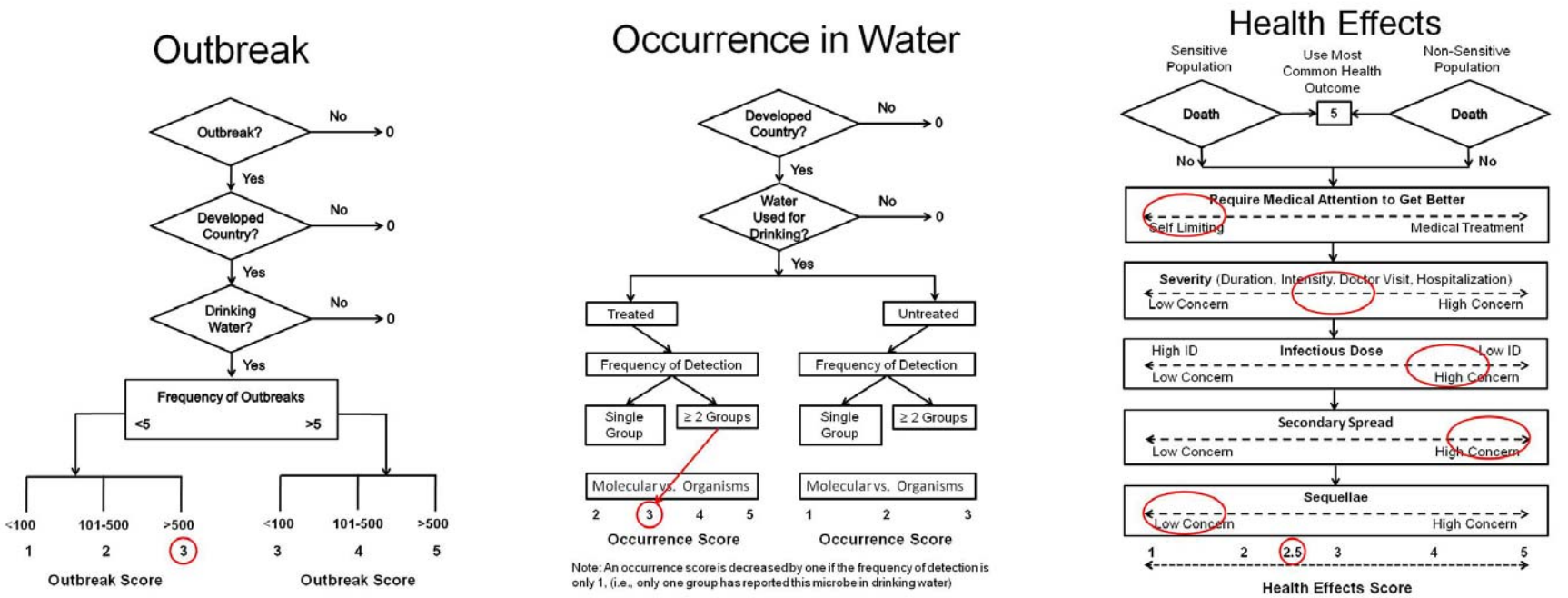


FIGURE S4. Rotavirus: Completed decision tree templates for outbreaks, occurrence in water, and health effects.

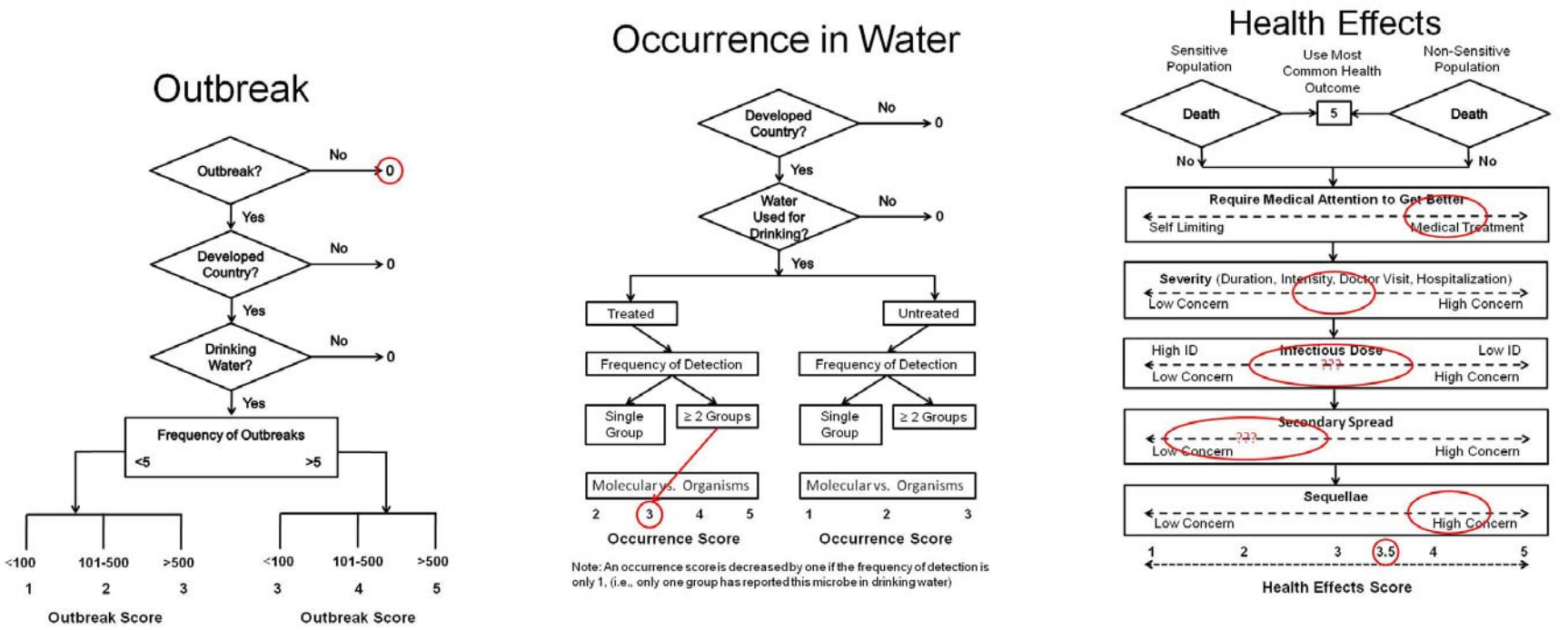


FIGURE S5. ***Helicobacter pylori*: Completed decision tree templates for outbreaks, occurrence in water, and health effects.**

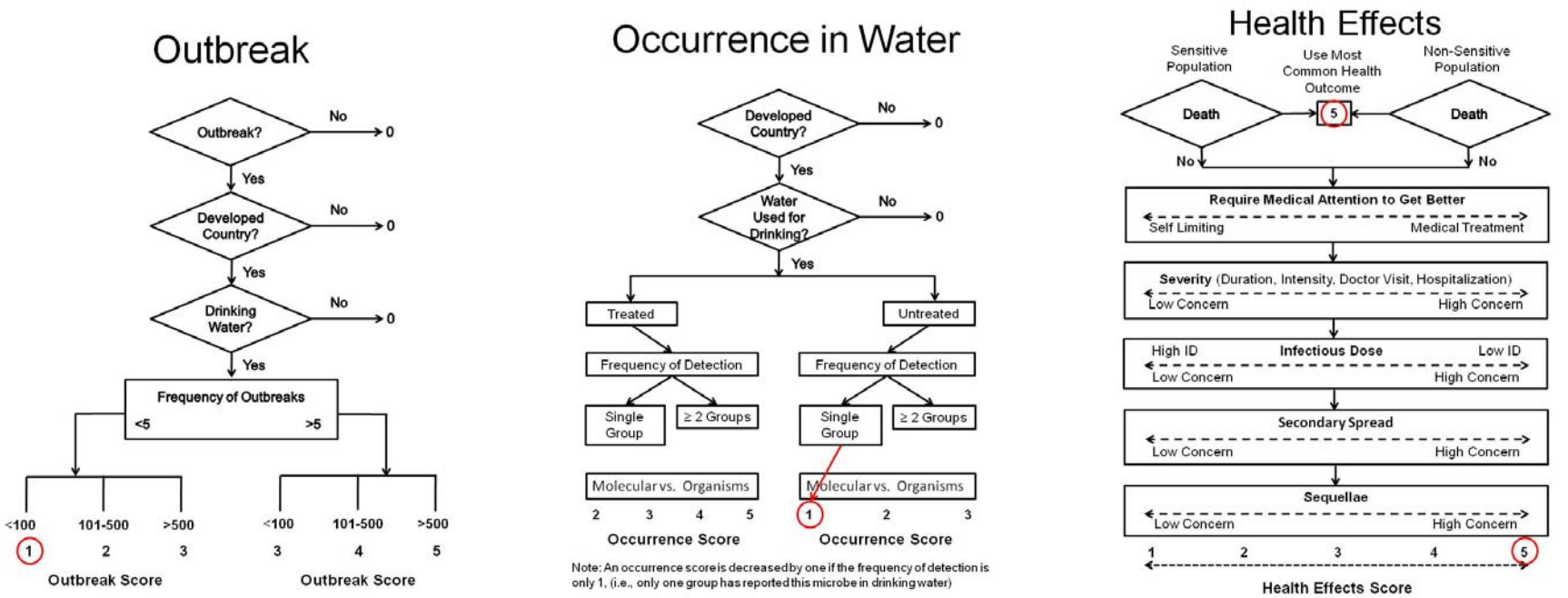


FIGURE S6. ***Naegleria fowleri*: Completed decision tree templates for outbreaks, occurrence in water, and health effects.**