An outbreak of *Campylobacter jejuni* infections associated with consumption of unpasteurized milk

Pepin County, Wisconsin
September 22, 2014

Final Report
December 18, 2014

Pepin County Health Department
740 7th Avenue West
P.O. Box 39
Durand, WI 54736

and

Wisconsin Division of Public Health
Bureau of Communicable Diseases

1 West Wilson St.
Madison, WI 53703-2659

CONTRIBUTORS

Pepin County Health Department

- Heidi Stewart, Health Officer/Director
- Terri Reiland, Assistant Director/ Public Health Nurse

Wisconsin Division of Public Health

Bureau of Communicable Diseases

- Jeffrey P. Davis, MD, Chief Medical Officer and State Epidemiologist

Communicable Diseases Epidemiology Section

- Suzanne Gibbons-Burgener, DVM, PhD, Section Chief
- Justin Kohl, MPH, Epidemiologist
- Rachel Klos, DVM, MPH, Epidemiologist,
- Traci DeSalvo, MPH, Epidemiologist
- Crystal Gibson, CDC/CSTE Applied Epidemiology Fellow
- Surveillance and Outbreak Support (SOS) Team

Wisconsin Department of Agriculture, Trade and Consumer Protection

Division of Food Safety

- Dr. Steven Ingham, Administrator,
- Peter Haase, Director, Bureau of Food Safety and Inspection
- Edward Lund, Dairy Sanitarian,

Division of Animal Health

- Dr. Paul McGraw, Administrator
- Dr. Libby Balzer, Veterinarian

Wisconsin State Laboratory of Hygiene

- David Warshauer, PhD, Associate Director, Communicable Disease Division
- Audrey Prieve, Manager, Communicable Disease Division
- Tim Monson, Microbiologist Supervisor, Communicable Disease Division
- Tam Van, PhD, Assistant Scientist, Communicable Disease Division
- Bacteriology Laboratory
    - Mike Rauch, Advanced Microbiologist
    - Ann Valley, Advanced Microbiologist
    - Jared Shelerud, Advanced Microbiologist
    - Kristin Gundlach, Senior Microbiologist
    - Holly Oxley, Senior Microbiologist
    - Sara Wagner, Microbiologist Entry
    - Juliann Whirry, Microbiologist Entry
- Virology Laboratory
    - Erik Reisdorf, Advanced Microbiologist
    - Jim Powell, Advanced Microbiologist
    - Tim Davis, Senior Microbiologist
    - T.J. Whyte, Senior Microbiologist

    - Tonya Danz, Senior Microbiologist
    - Tom Weiner, Senior Microbiologist
    - Rich Griesser, Microbiologist Entry
- Informatics
    - Mary Wedig, Programming and Planning Advanced
    - Barb Rosenthal, Information Systems Data Services Professional

Wisconsin Department of Agriculture, Trade and Consumer Protection
Bureau of Laboratory Services

- **Microbiology Laboratory**

Wisconsin Veterinary Diagnostic Laboratory

- Dr. Peter Vanderloo, Director
- Dr. Ogi Okwumabua, Chief of Microbiology Section

BACKGROUND

On Monday morning, September 22, 2014, the Pepin County Health Department (PCHD) was notified by a school nurse at Durand High School of an increase in absenteeism related to gastrointestinal illness among football players, student managers and football team coaches. Reported symptoms included diarrhea, nausea, vomiting, headache, abdominal cramps and fever. One student was hospitalized at the time of the initial report. The PCHD learned that approximately 50 football-affiliated individuals attended a "Thursday team dinner" off the school premises on Thursday, September 18. The PCHD notified staff in the Communicable Disease Epidemiology Section (CDES), Bureau of Communicable Diseases (BCD), Wisconsin Division of Public Health (DPH), and a joint investigation was launched.

During the investigation, PCHD and CDES staff were also informed of an increase in absenteeism among female volleyball team members related to gastrointestinal illness. Results of investigation of illness among students and staff not affiliated with the "Thursday team dinner" or football team are presented in Appendix B.

METHODS

Epidemiologic Investigation

Early case finding and hypothesis generating interviews

School staff provided the PCHD with contact information for all Durand High School football team players, coaches, and student managers. The PCHD conducted initial interviews with a sample of ill students to determine exposures (food, water, practices, other activities) that individuals affiliated with the football team may have had in common and shared those preliminary interviews with CDES staff. From these early interviews, PCHD learned that there was a team dinner on Thursday, September 18 held at a non-school venue. Team dinners are routinely held the evening prior to a football game. All team members, coaching staff and some parents who help coordinate the dinner are typically in attendance and eat at these pot-luck style meals. Food for the dinner is purchased, prepared and served by parents of the team members. A list of food and drink items served at this team dinner was obtained by the PCHD. At this dinner, there were multiple food items served including a chicken entrée, broccoli salad and other side

dishes, a variety of desserts including cookies, bars and brownies, and a variety of drinks including Kool-Aid, chocolate milk, and white milk.

Questionnaire design and administration

The CDES used information from the initial interviews conducted by PCHD to develop a standardized questionnaire that included questions regarding clinical signs and symptoms, sources of drinking water, food and drink items served at the team dinner, whether the students, managers and coaches consumed school lunch on Wednesday, Thursday or Friday (September 17-19), whether anyone else at the school or at home was known to be ill with signs or symptoms of gastrointestinal illness, and whether students attended other events with their teammates including football team practices (Appendix A). Parents who attended the team dinner were also interviewed using the same standard questionnaire, but omitting the questions regarding attendance of a football team practice and other school related exposures (e.g. shared water bottles, school lunch).

On Tuesday, September 22 CDES staff began conducting telephone interviews of all students, parents and staff affiliated with the football team using a standardized questionnaire. On September 23 the PCHD learned that some of the milk served at the dinner was unpasteurized and was provided by a parent who obtained it from his/her own farm (Farm A). Thus, store-bought chocolate milk and unpasteurized white milk were provided at the dinner. Parents who coordinated the dinner reported that there were three 1/2 gallon jugs of store-bought chocolate milk and a 5 gallon cooler filled with unpasteurized milk. The unpasteurized white milk was served after the store-bought chocolate milk was gone. Empty ½ gallon jugs from the store-bought chocolate milk and plastic pitchers were used to mix the unpasteurized white milk with chocolate flavoring. There was no pasteurized white milk available at the team dinner. The questionnaire was modified to collect additional information about the type of milk consumed. Interviewees were asked to respond directly to the questions. Interviews concluded on Wednesday, October 1. In total, 65 interviews were conducted with individuals associated with the football team.

Cohort study

A retrospective cohort analysis was conducted on the exposure data collected among football-affiliated students, parents and coaches (attendees) who became ill and those who did not become ill to measure the association between disease and food and environmental exposures.

Case definitions

Clinical case definition: Diarrhea (3 or more loose stools within 24 hours) with illness onset during September 20-25 in an individual who attended the Thursday team dinner.

Laboratory-confirmed case: Illness that met the clinical case definition in an individual with laboratory confirmed *Campylobacter jejuni* infection.

Probable case: Illness that met the clinical case definition in an individual who either did not have a stool specimen tested or was tested but the specimen was not positive for *Campylobacter jejuni*.

Data preparation and analysis

CDES staff created a line list using MS Excel 2010 to record demographic information, clinical signs and symptoms, and laboratory results. Exposure information from the telephone interviews was entered into an Epi Info 7.0 database and a retrospective cohort analysis was conducted. Descriptive statistics and relative risks (including 95% confidence intervals) were computed using Epi Info 7.0. Statistical significance of any exposure's association with illness (p-values) was tested using the Chi-squared method. Mid-p exact one tailed p-values were reported except when the expected value of a cell was less than 5; Fisher's exact one tailed p-values were reported. Individuals were excluded from the cohort analysis if they reported an illness that did not meet the case definition.

The analysis of exposures related to football team practices and school-associated exposures (e.g. school lunch, shared water bottles at practice) included all football team players, managers and coaches regardless of illness. This analysis did not include parents.

The analysis of exposure variables associated with the Thursday team dinner included only attendees of the dinner (players, managers, coaches and parents) who were either not ill or who had illnesses that met the case definition for a confirmed or probable case.

The milk was not labeled or posted as raw or unpasteurized, and participants may not have been aware that they were consuming unpasteurized milk. To assess whether unpasteurized milk was consumed, a variable was derived from multiple items included on the questionnaire. An individual was considered to have consumed unpasteurized milk if he/she reported consuming white milk or white milk mixed with chocolate flavoring.

Epidemic Curve

An epidemic curve was constructed to assess the magnitude and timing of the outbreak and determine incubation periods.

Laboratory Investigation

Bacteriologic testing of human specimens

Clinical staff at the Chippewa Valley Hospital in Durand ordered testing of stool specimens from some patients who presented in their facility. Those stool specimens were sent to the Sacred Heart Hospital microbiology laboratory for testing. Any stool specimens that were positive for any reportable pathogens were forwarded to the Wisconsin State Laboratory of Hygiene (WSLH) for further testing that included serotyping and pulsed-field gel electrophoresis (PFGE).

The PCHD supplied stool collection kits and coordinated collection of stool specimens from ill individuals and forwarded those specimens to the WSLH. All stool specimens collected through PCHD were forwarded to the WSLH for microbiologic testing that included serotyping and PFGE.

Stool specimens from 30 symptomatic football-team affiliated individuals were submitted to the WLSH to be tested for enteric pathogens. Early in the investigation, to determine the likely etiology of the gastrointestinal (GI) illness using a broad array of diagnostic targets, 12 specimens were tested using the Luminex xTAG® GPP multiplex gastrointestinal pathogen PCR diagnostic assay which detects 14 of the more common GI pathogens: adenovirus 40/41, *Campylobacter*, *Cryptosporidium*, *Entamoeba histolytica*, *Escherichia coli* O157, Enterotoxigenic *E. coli*, *Giardia lamblia*, norovirus, rotavirus A, *Salmonella*, Shiga toxin-producing *E. coli*, *Shigella*, *Vibrio cholerae* and *Yersinia enterocolitica*. The WSLH then used

culture, PCR or direct fluorescence antigen testing to confirm results of any specimens that were positive for pathogens by the Luminex xTAG® GPP assay. All outbreak-related stool specimens were cultured for the 4 most common bacterial GI pathogens per standard WSLH Bacteriology Laboratory outbreak stool testing protocol: *E. coli* O157, *Shigella*, *Salmonella* and *Campylobacter*, and screened for Shiga toxins using the Meridian Premier® EHEC Enzyme Immunoassay (EIA). Specimens that tested positive for Shiga toxins using Luminex xTAG® GPP assay or Meridian Premier® EHEC EIA were cultured for Shiga toxin-producing *E. coli.* Specimens that tested negative for the 4 primary bacterial pathogens by culture and for Shiga toxins were tested for norovirus using real time PCR. All bacterial isolates, either recovered from stool specimens at the WSLH or received from clinical laboratories, were subtyped using PFGE to assess their genetic relatedness.

Environmental Investigation

On September 23, CDES staff notified the Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) of the outbreak and its possible association with consumption of unpasteurized milk based on preliminary findings of the epidemiologic investigation. The farm owners' names and location of the farm that provided the unpasteurized milk were provided.

On September 24, a DATCP Food Safety Inspector who is certified to inspect licensed bulk milk weighers and samplers visited the farm and collected one milk sample directly from the bulk tank according to the Bulk Milk Weigher and Sampler Protocol. The sample was shipped overnight and delivered the morning of September 25 to the DATCP Bureau of Laboratory Services in Madison where it was tested for *Campylobacter*, *E. coli* O157 and Shiga-toxin producing *E. coli* using the US FDA Bacteriological Analytic Manual (BAM) methods. Samples were tested for Shiga toxin producing *E. coli* using real-time PCR. For *Campylobacte*r detection, the BAX real time PCR kit was also used.

CDES staff sent a letter to DATCP on October 3 requesting sampling of cow manure at the farm that supplied the unpasteurized milk. On October 6, a DATCP veterinarian visited the farm and collected 65 fecal specimens from dairy cows on the farm. The samples were shipped to the Wisconsin Veterinary Diagnostic Laboratory (WVDL) in Madison where they were sub-sampled. Upon receipt of the specimens at WVDL, a representative sample from each specimen

was sent by WVDL staff to the WSLH for Shiga-toxin testing using EIA. The WVDL Microbiology Section tested the specimens for *Campylobacter* using standard American Society of Microbiology bovine fecal culture procedures. *Campylobacter* isolates were then further identified using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS). The isolates from all specimens that were positive for *Campylobacter jejuni* at the WVDL were sent to the WSLH for PFGE subtyping.

RESULTS

Epidemiologic Investigation

Case finding, assessment and exclusions

Sixty-five individuals were interviewed using the standard exposure questionnaire. Among those interviewed 53 were students, 7 were coaches and 5 were parents affiliated with the football team. The 65 individuals interviewed included 26 with laboratory confirmed cases of *Campylobacter jejuni* infection, 12 with probable cases, 18 who were not ill, 5 who did not attend the team dinner, and 4 who reported symptoms of gastrointestinal illness that did not meet the clinical case definition. Illnesses not meeting the clinical case definition were excluded from the retrospective cohort analyses. For analysis of the Thursday team dinner exposures, 5 interviewees (3 ill, 2 well) who did not attend the team dinner were excluded from that analysis.

Clinical and descriptive epidemiologic features

Among the 38 individuals with confirmed and probable cases (case attendees), ages ranged from 14 to 49 years (median=16 years). Case attendees included 33 students, 5 coaches and no parents. The majority of interviewees were ill at the time of interview thus duration of illness data was available for only 11 case attendees (median duration=4 days). The most commonly reported signs and symptoms of illness among the case attendees were diarrhea, headache, fever, chills and sweats (Table 1). Table 2 presents the complete data including symptom information for each of the 38 case attendees. Sixteen of the 38 case attendees reported visiting a healthcare provider, including 10 who were hospitalized or received care at an emergency department. Measured temperature among those who reported having a fever ranged from 99.9°F to 105.0°F (median=102.0°F).

Table 1. Signs and symptoms of gastrointestinal illness among case attendees

Sign or symptom	Number (N=38)	Reported sign or symptom (%)
Diarrhea	36	95%
Headache	34	89%
Fever*	34	92%
Chills	34	89%
Sweats	34	89%
Abdominal cramps	31	82%
Fatigue	26	68%
Body ache	26	68%
Nausea	25	66%
Muscle ache	24	63%
Vomiting	11	29%
Bloody diarrhea*	8	23%

*Attendees with missing information: fever (1), bloody diarrhea (3)

Table 2. Signs, symptoms and dates of illness onsets among case attendees

No.	Onset Date	Well Date	N	V	D	BD	AC	Fe	Ch	Sw	HA	BA	MA	Fa
1	9/21/14	**	+	+	+	-	-	+	+	+	+	+	+	+
2	9/20/14	**	+	-	+	-	+	+	+	+	+	+	+	+
3	9/20/14	9/28/14	+	+	+	-	+	+	+	+	+	+	+	+
4	9/23/14	9/24/14	+	-	+	-	+	-	-	-	+	-	-	+
5	9/21/14	**	+	-	+	+	+	+	+	+	+	+	+	+
6	9/23/14	**	-	-	+	-	+		+	+	+	-	+	+
7	9/22/14	9/24/14	-	-	+	-	-	+	+	-	-	-	-	+
8	9/22/14	9/26/14	-	-	-	-	+	+	+	+	+	+	+	+
9	9/21/14	**	+	+	+	+	+	+	+	+	+	+	+	+
10	9/22/14	**	-	-	+	-	+	+	+	+	-	+	-	-
11	9/21/14	**	-	-	+	-	+	+	+	+	+	+	+	+
12	9/22/14	**	+	-	+	-	+	+	+	+	+	-	-	+
13	9/21/14	**	+	-	+	-	-	+	+	+	+	+	+	-
14	9/20/14	**	+	-	+	-	+	+	+	+	+	+	+	-
15	9/21/14	**	+	+	+	-	+	+	+	+	+	+	-	+
16	9/21/14	9/25/14	+	+	-		-	+	+	+	+	+	+	-
17	9/22/14	**	-	-	+	-	-	-	-	-	-	+	+	-
18	9/21/14	9/23/14	+	-	+	-	+	+	+	+	+	+	+	+
19	9/22/14	**	+	+	+	+	+	+	+	+	+	+	+	+
20	9/21/14	9/26/14	-	-	+	-	+	+	+	+	+	-	-	+
21	9/21/14	**	+	-	+		+	+	+	+	+	+	-	+
22	9/21/14	**	+	-	+	-	+	+	+	+	+	-	-	-
23	9/20/14	**	+	+	+	-	+	+	+	+	+	-	+	+
24	9/21/14	**	+	+	+	+	+	+	+	+	+	+	-	+
25	9/21/14	**	+	-	+	-	+	+	+	+	+	-	-	-
26	9/21/14	**	+	+	+	-	-	+	+	+	+	-	-	+
27	9/21/14	9/25/14	+	+	+	+	+	+	+	+	+	+	+	+
28	9/22/14	**	-	-	+	+	+	+	+	+	+	+	+	+
29	9/22/14	9/25/14	+	+	+	-	+	+	+	+	+	+	+	+
30	9/24/14	**	-	-	+	-	+	+	+	+	+	-	-	-
31	9/24/14	**	-	-	+	-	+	+	+	+	+	+	+	+
32	9/20/14	9/24/14	+	-	+		+	+	+	+	+	+	+	+
33	9/25/14	**	-	-	+	-	+	-	-	+	-	-	-	-
34	9/21/14	**	+	-	+	-	+	+	+	+	+	+	+	+
35	9/21/14	**	+	-	+	-	+	+	-	-	+	-	-	-
36	9/21/14	**	+	-	+	+	+	+	+	+	+	+	+	-
37	9/22/14	9/23/14	-	-	+	+	-	+	+	+	+	+	+	-
38	9/21/14	**	-	-	+	-	+	+	+	+	+	+	+	+
		Totals	**25**	**11**	**36**	**8**	**31**	**34**	**34**	**34**	**34**	**26**	**24**	**26**

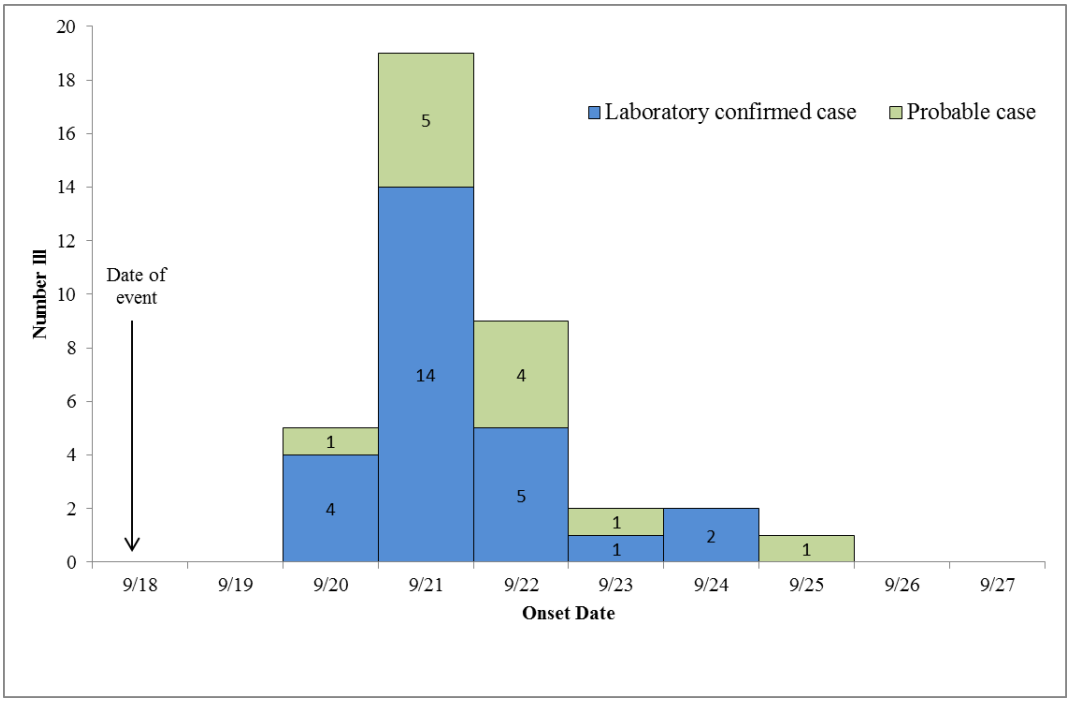
***Still ill when interviewed*

N=Nausea, V=Vomiting, D=Diarrhea, BD=Bloody Diarrhea, AC=Abdominal Cramps, Fe=Fever, Ch=Chills, Sw=Sweats, HA=Headache, BA=Body Ache, MA=Muscle Aches, Fa=Fatigue

Epidemic curve

Dates of illness onset among the 38 case attendees ranged from September 20 to September 25 (Figure 1) and the incubation period ranged from 2 to 7 days (median=3 days). The majority of illness onsets occurred during September 21-22.

Figure 1. Epidemic curve: Number of case illnesses by date of illness onset



Cohort Study

Fifty-seven individuals (41 ill and 16 non-ill) were included in the retrospective cohort analysis for all variables other than the exposures at the Thursday team dinner. None of the school related exposures (e.g. attendance at practice, shared water bottles at practice, school lunch etc.) were associated with illness.

Table 3. Attack rates associated with school-related exposures September 17-19.

Food / Drinks / Exposures	Persons who ATE Foods / HAD Exposures				Persons who did NOT eat foods / did NOT have exposures				Statistical Analysis		
	Ill	Well	Total	%Ill	Ill	Well	Total	%Ill	RR	95% CI	p
Wednesday											
Wednesday School Lunch	27	9	36	75%	6	3	9	67%	1.125	0.683, 1.853	0.4503*
Wednesday Attend Practice	41	14	55	75%	0	2	2	0%	und	und	0.0752*
Wednesday Shared Water Bottles at Practice	21	12	33	64%	4	1	5	80%	0.796	0.478, 1.323	0.4335
Thursday											
Thursday School Lunch	33	10	43	77%	6	5	11	55%	1.407	0.801, 2.473	0.0875
Thursday Attend Practice	40	14	54	74%	1	2	3	33%	2.222	0.445, 11.096	0.1873
Thursday Shared Water Bottles at Practice	28	11	39	72%	4	2	6	67%	1.077	0.592, 1.960	0.5673
Friday											
Friday School Lunch	31	11	42	74%	5	4	9	56%	1.328	0.721, 2.449	0.2404*
Friday Attend Practice	3	0	3	100%	34	16	50	68%	1.471	1.216, 1.779	0.3317*
Friday Attend Game	33	11	44	75%	8	5	13	62%	1.219	0.768, 1.935	0.1842
Friday Ride Bus to Game	24	11	35	69%	6	1	7	86%	0.800	0.549, 1.166	0.3396*
Apple at Friday Game	15	6	21	71%	17	5	22	77%	0.924	0.650, 1.316	0.3398
Granola Bar at Friday Game	25	9	34	74%	8	2	10	80%	0.919	0.635, 1.330	0.516*
Shared Water Bottles at Friday Game	22	7	29	76%	8	4	12	67%	1.138	0.726, 1.784	0.4049*
Water from Trainer's Office at Friday Game	5	0	5	100%	26	11	37	70%	1.423	1.154, 1.755	0.1997*
Subway Subs Before/After Friday Game	29	10	39	74%	4	2	6	67%	1.115	0.615, 2.022	0.5144*

* Fisher's 1-tailed p value used
und = undefined

Fifty-six individuals (38 ill, 18 non-ill) who attended the Thursday team dinner were included in the analysis of Thursday dinner food items and their association with illness. Table 4 includes results for all food and beverage exposures at the Thursday team dinner that were examined.

One exposure variable was associated with illness among those who attended the Thursday team dinner. Consumption of any milk during the Thursday team dinner was associated with illness. Thirty-eight (100%) ill individuals and 12 of 18 (67%) well individuals reported consuming milk during the team dinner ($p = 0.0006$). Among the 38 ill individuals who reported consuming milk, 32 (84%) consumed some unpasteurized milk and 6 (16%) only consumed chocolate milk from a store-bought ½ gallon jug which could have contained pasteurized or unpasteurized milk. Twenty-seven (71%) of the ill individuals who reported consuming any milk consumed only unpasteurized milk.

A risk ratio and p-value could not be calculated for consumption of chicken alfredo because 100% of respondents reported consuming this item. Among those who did report eating

chicken alfredo, 67% developed an illness that met the clinical case definition. Among respondents who reported consuming unpasteurized milk, 76% developed an illness meeting the clinical case definition.

Table 4. Attack rates associated with consumption of food and beverages served at the Thursday team dinner on September 18.

Food / Drinks	Persons who ATE Foods				Persons who did NOT eat foods				Statistical Analysis		
	Ill	Well	Total	%Ill	Ill	Well	Total	%Ill	RR	95% CI	p
Any milk	38	12	50	76%	0	6	6	0%	und	und	0.0006*
Banana Bar	7	3	10	70%	29	15	44	66%	1.062	0.672, 1.679	0.5595*
Broccoli Salad	11	11	22	50%	26	7	33	79%	0.635	0.403, 0.999	0.0163
Brownies	8	5	13	62%	24	11	35	69%	0.897	0.552, 1.457	0.3280
Chicken Alfredo	38	18	56	68%	0	0	0	0%	und	und	0.1149*
Chocolate Chip Cookies	17	8	25	68%	18	8	26	69%	0.982	0.678, 1.424	0.4638
Cinnamon Roll	11	4	15	73%	26	14	40	65%	1.128	0.771, 1.650	0.4027*
Garlic Bread	36	17	53	68%	2	1	3	67%	1.019	0.448, 2.316	0.6957*
Garlic Butter	17	12	29	59%	18	6	24	75%	0.782	0.533, 1.147	0.1141
M&M Bars/Cookies	15	7	22	68%	21	10	31	68%	1.007	0.692, 1.464	0.4893
Nut Cookies	0	3	3	0%	34	13	47	72%	0.000	und	0.0285*
Peanut Butter Bars	11	3	14	79%	25	14	39	64%	1.226	0.855, 1.758	0.2587*
Pumpkin Bars	19	9	28	68%	18	9	27	67%	1.018	0.704, 1.472	0.4639
Watermelon	12	8	20	60%	24	10	34	71%	0.850	0.559, 1.291	0.2220
Ice in Drinks	1	1	2	50%	36	17	53	68%	0.736	0.182, 2.979	0.5515
Kool-Aid	8	6	14	57%	30	12	42	71%	0.800	0.489, 1.309	0.1730
Bottled Water	15	7	22	68%	19	8	27	70%	0.969	0.665, 1.411	0.4361
Tap Water	0	1	1	0%	22	1	23	96%	0.000	und	0.0833

* Fisher's 1-tailed p value used
und = undefined

Laboratory and Environmental Investigation

Bacteriologic and viral testing of human subjects

Thirty individuals submitted stool samples through their healthcare provider or directly to the PCHD, and the PCHD forwarded the specimens to WSLH. Of the 30 specimens submitted to the WSLH, 26 tested positive for *Campylobacter jejuni* and 3 tested positive for non-O157 Shiga toxin-producing *E. coli* (STEC) infections. All three of the non-0157 STEC-infected individuals also had concurrent *Campylobacter jejuni* infections.

During the course of laboratory testing, a blood specimen collected at the Chippewa Valley Hospital in Durand on September 22 from one case-patient who was hospitalized was

presumptively culture positive for a *Bacillus* species. The results of follow-up testing are described in Appendix C.

Environmental investigation

On September 30 the DATCP staff reported the bulk milk tank sample that was collected on September 24 from the farm that supplied the unpasteurized milk for the team dinner was negative for *Campylobacter* and Shiga toxin-producing *E. coli*.

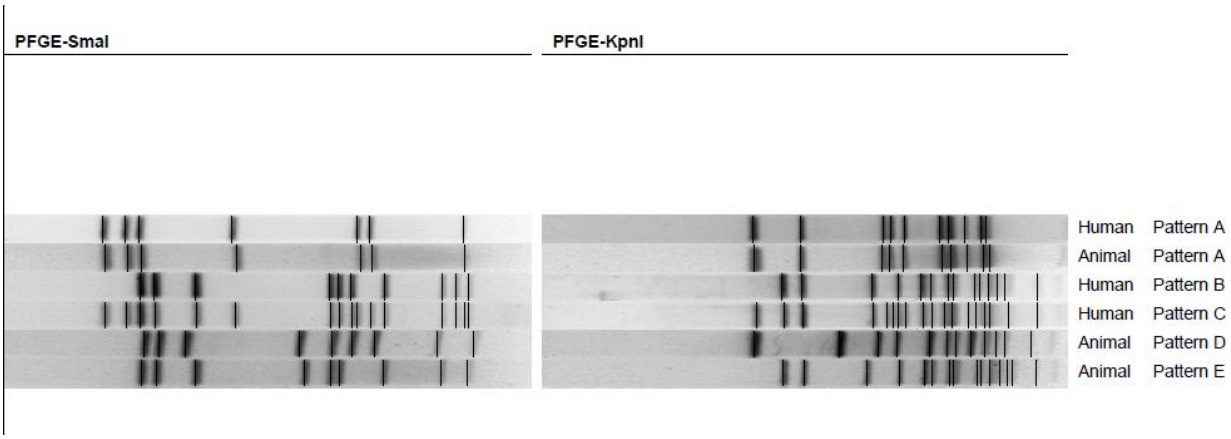
On October 14, the Wisconsin Veterinary Diagnostic Lab (WVDL) reported that 12 of 65 bovine fecal specimens collected on October 6 tested positive for *Campylobacter.* Ten of the specimens that tested positive for *Campylobacter* were positive for *Campylobacter jejuni* and the remaining 2 specimens were positive for *Campylobacter hyointestinalis.* Of the 10 bovine fecal specimens that were positive for *Campylobacter jejuni*, 9 were sent to the WSLH for PFGE subtyping.

Pulsed field gel electrophoresis (PFGE)

Twenty-six *Campylobacter jejuni* isolates collected from ill individuals had PFGE performed using two restriction enzymes (SmaI and KpnI). The main PFGE pattern combination noted among the human specimens was .0233(SmaI)/.1548(KpnI), referred to as PFGE Pattern A. A secondary PFGE pattern (.2619 SmaI / .0022 KpnI) was also identified, referred to as PFGE Pattern B. Among the 26 case-attendees' *Campylobacter jejuni* isolates, 19 were PFGE Pattern A isolates, 6 were Pattern B isolates, and one had an unrelated PFGE pattern, Pattern C. One of the case-attendees had multiple *Campylobacter jejuni* colonies detected that included PFGE Pattern A colonies and Pattern B colonies.

The WSLH results of PFGE testing of the 9 bovine *Campylobacter jejuni* isolates included 6 specimens that matched with PFGE Pattern A, the most common pattern found among the case attendee specimens. Two other bovine isolates had PFGE patterns that matched each other (Pattern D), but did not match any of the human isolate patterns. One bovine isolate had a PFGE pattern (Pattern E) with no human or bovine matches. Figure 2 illustrates the various PFGE patterns of the *Campylobacter jejuni* isolates and demonstrates the matching patterns of Human Pattern A and Bovine Pattern A.

Figure 2. PFGE patterns of the human and bovine isolates of *Campylobacter jejuni*



CONCLUSIONS

Thirty-eight outbreak associated cases were detected during this investigation of an outbreak of *Campylobacter jejuni* infections among football team affiliated individuals in Durand, Wisconsin. Analysis of data pertaining to foods consumed during the team dinner demonstrates that consuming milk during the team dinner was associated with illness. Thirty-eight (100%) ill individuals reported consuming milk during the team dinner and 71% of the ill individuals reported consuming only the Farm A unpasteurized milk.

Farm A bulk tank milk sampled six days after the team dinner was negative for *Campylobacter* and Shiga toxin-producing *E. coli*. Cattle can shed *Campylobacter* and Shiga toxin-producing *E. coli* intermittently, and contamination of milk may occur during and after milking. The bulk tank milk sample collected on September 24 was not representative of milk that was served during the team dinner. Collection and testing of bovine manure samples proved to be a better method to detect bacteria that were present at the Farm A.

Nine bovine manure samples collected from Farm A were positive for *Campylobacter jejuni*. PFGE subtyping of *Campylobacter jejuni* isolates from case attendees detected 3 outbreak associated patterns (Patterns A-C), Pattern A was the predominant pattern identified. PFGE subtyping of *Campylobacter jejuni* isolates from the bovine manure collected from Farm A also detected Pattern A. The results of the epidemiologic and laboratory investigations conducted by DPH, PCHD, DATCP, WVDL and WSLH determined that consumption of Farm A unpasteurized milk during the Thursday team dinner was associated with the occurrence of *Campylobacter jejuni* infections among football team-affiliated individuals.

Campylobacter infection affects the intestinal tract and is the most commonly reported cause of bacterial diarrheal illness in Wisconsin (Wisconsin DHS, 2006). Common signs and symptoms of *Campylobacter* infection include diarrhea (sometimes bloody), cramping, abdominal pain and fever; in rare cases infection can be severe and the bacteria can be isolated from the bloodstream.

Outbreaks of *Campylobacter jejuni* infections associated with the consumption of unpasteurized milk have been documented in Wisconsin and nationally (CDC, 2013). Cattle are a known reservoir for *Campylobacter* and Shiga toxin-producing *E. coli*, both of which were isolated from stool specimens collected from ill individuals during this outbreak. These organisms can be shed intermittently by cattle and can be found in their environments. Enteric pathogens commonly associated with unpasteurized milk include *Campylobacter*, Shiga-toxin producing *E. coli* (STEC) inclusive of *E. coli* O157 and non-0157 STEC, and other bacteria. During 1998-2011 a total of 148 outbreaks associated with unpasteurized milk consumption were reported to the CDC (CDC, 2013). The majority of illnesses associated with these outbreaks were caused by STEC, *Campylobacter* or *Listeria*. Notably, children are particularly affected by illness associated with consuming unpasteurized milk; among the 148 outbreaks reported to the CDC, 82% of outbreak reports with patient age data included illness among children or adolescents.

Campylobacter infections are usually caused by eating or drinking contaminated food or water, including unpasteurized milk from infected cows. *Campylobacter* infections can be prevented by proper food handling (e.g., prompt refrigeration of food at <40°F, disinfecting cutting boards and counters immediately after use), avoiding consumption of raw or undercooked meat, washing hands before and after food preparation and after handling animals (including pets), and avoiding consumption of unpasteurized milk.

REFERENCES

Centers for Disease Control and Prevention. 2013. Available at: http://www.cdc.gov/Features/RawMilk/

Wisconsin Department of Health Services. 2006. Available at: http://www.dhs.wisconsin.gov/publications/P4/P42034.pdf

APPENDIX A: Investigation Questionnaire

DEMOGRAPHIC INFORMATION

Name: _____ Age: _____ Gender: M F

Parent/Guardian Name: _____

Address: _____

_____

City: _____ County: _____

State: _____ Zip: _____

Phone (home): _____ Phone (cell): _____

Occupation: _____

_____

High risk activities: ☐ Daycare ☐ Food handler ☐ Health care provider

(*Provide appropriate exclusion and education if individual is ill / was ill)*

Grade: _____

What is your role with the football team?

Coach / Player / Other: _____ / Not associated with the football team

Which teams do you play on or work with? Varsity JV C-Team Other:_____

CLINICAL INFORMATION

Have you (your child) been ill with gastrointestinal symptoms (vomiting, diarrhea, abdominal cramping, nausea, etc.) in the last 2 weeks? Y N *If no skip to **Other Ill Persons***

Onset Date: ____ / ____ / ____ **Onset Time: ____:____ AM PM**

Well Date: ____ / ____ / ____ **Well Time: ____:____ AM PM**

Nausea	Y	N	Vomiting	Y	N	Diarrhea *(3 or more loose stools 24 hours)*	Y	N
Bloody diarrhea	Y	N	Abdominal cramps	Y	N	Fever (_____^{o})	Y	N
Chills	Y	N	Sweats	Y	N	Headache	Y	N
Body aches	Y	N	Muscle aches	Y	N	Fatigue	Y	N
Other:	Y	N	_____					

Did you see a physician? Y N Date: ____ /____/ ____

Were you seen in an ER/ED Y N Where: _____

Were you hospitalized overnight? Y N Where: _____

Was a stool specimen collected? Y N Results: _____

If No, would you be willing to submit a stool specimen for free testing? Y N

OTHER ILL PERSONS

Has anyone in your household been ill with GI symptoms during the last two weeks? Y N

1) If yes, who: _____ Onset date: ____ / ____ / ____ Time: ____:____ AM PM

What were their symptoms(circle)? Nausea / Vomiting / Diarrhea / Abd. cramps/ Fever / Headache/ Other: _____

2) If yes, who: _____ Onset date: ____ / ____ / ____ Time: ____:____ AM PM

What were their symptoms(circle)? Nausea / Vomiting / Diarrhea / Abd. cramps/ Fever / Headache/ Other: _____

3) If yes, who: _____ Onset date: ____ / ____ / ____ Time: ____:____ AM PM

What were their symptoms(circle)? Nausea / Vomiting / Diarrhea / Abd. cramps/ Fever / Headache/ Other: _____

Do you know anyone else (friend, co-worker, teammate, classmate, etc.) who was ill with GI symptoms during this same time period? Y N

1) If yes, who: _____ Onset date: ____ / ____ / ____ Time: ____:____ AM PM

What were their symptoms(circle)? Nausea / Vomiting / Diarrhea / Abd. cramps/ Fever / Headache/ Other: _____

2) If yes, who: _____ Onset date: ____ / ____ / ____ Time: ____:____ AM PM

What were their symptoms(circle)? Nausea / Vomiting / Diarrhea / Abd. cramps/ Fever / Headache/ Other: _____

Did you observe or hear about anyone who became ill with vomiting or diarrhea in a public place (school, practice, bus etc)?

If yes who?_____When?_____Symptoms:_____

If yes who?_____When?_____Symptoms:_____

If yes who?_____When?_____Symptoms:_____

If yes who?_____When?_____Symptoms:_____

If yes who?_____When?_____Symptoms:_____

WEDNESDAY SEPTEMBER 17

Please describe your activities for **Wednesday September 17** especially activities in common with members of the football team (school, games, practices, other etc).

Did you attend practice on Wednesday? Yes / No

Did you drink from the shared water bottles at practice? Yes / No

Meal	**Where did you eat the meal? Circle: home / outside the home**	**Location Outside the home**	**What was eaten**	**Did you eat with anyone else**
Lunch	Home / outside the home			
Dinner	Home / outside the home			

Other (snack)	Home / outside the home			
Other comments:				

THURSDAY SEPTEMBER 18

Please describe your activities for **Thursday September 18** especially activities in common with members of the football team (school, games, practices, other etc).

Did you attend practice or a game on Thursday? Yes / No

9/18 Thursday Lunch

Lunch from home	Y N Unk	Specify what was eaten including beverages:
School Lunch	Y N Unk	Specify what was eaten including beverages:

Did you attend the 9/18 team dinner Thursday night Y/N? (Please specify foods eaten below)

EXPOSURES

Food Item	**Yes/No**	**Details**
Chicken Alfredo	Y N Unk	
Garlic Bread	Y N Unk	
Garlic Butter	Y N Unk	
Broccoli Salad	Y N Unk	
Watermelon	Y N Unk	
Cinnamon Roll	Y N Unk	
Pumpkin Bar w/ cream cheese frosting	Y N Unk	
Banana bar w/ cream cheese frosting	Y N Unk	
M & M Bars/cookies	Y N Unk	
Chocolate Chip Cookies	Y N Unk	
Nut cookies	Y N Unk	
Peanut Butter Bars	Y N Unk	
Brownies	Y N Unk	
Other	Y N Unk	Specify:

Other	Y N Unk	Specify:
Other	Y N Unk	Specify:
Drinks:	Y N Unk	
Shared Water Bottles at Practice	Y N Unk	
White Milk	Y N Unk	Source: *Igloo cooler/ Pitcher* or *store bought ½ gallon jug* or *unk* *Like whole milk/thick* or *more like skim milk/thin* or *unk* Other details (e.g., number of glasses from each source):
Chocolate Milk	Y N Unk	Source: *Igloo cooler/ Pitcher* or *store bought ½ gallon jug* or *unk* *chocolate syrup mixed in* or *from store bought container* or *unk* *Like whole milk/thick* or *more like skim milk/thin* or *unk* Other details (e.g., number of glasses from each source):
Koolaid	Y N Unk	
Water	Y N Unk	Specify (bottled or tap):
Ice in any of your drinks?	Y N Unk	Specify:
Other	Y N Unk	Specify:

FRIDAY SEPTEMBER 19

Please describe your activities for **Friday September 19** especially activities in common with members of the football team (school, games, practices, other etc).

Did you attend practice on Friday night? Yes / No
Did you attend the game on Friday night? Yes / No

If you attended the game, did you ride up on the bus with the other players? Yes / No

9/19 Friday Lunch

Lunch from home	Y N Unk	Specify what was eaten including beverages:
School Lunch	Y N Unk	Specify what was eaten including beverages:

9/19 Friday

EXPOSURES

Food Item	**Yes/No**	**Details**
Subway Subs before game	Y N Unk	Specify what was on the sub: Meat: Veggies: lettuce, tomatoes, green peppers, spinach, olives, jalepenos, onions, banana peppers, cucumbers, other: Sauce: *Purchased by coach* or *purchased by self*
Subway Subs after game	Y N Unk	Specify what was on the sub: Meat: Veggies: lettuce, tomatoes, green peppers, spinach, olives, jalepenos, onions, banana peppers, cucumbers, other: Sauce: *Purchased by coach* or *purchased by self*
Granola Bar at Game	Y N Unk	
Apple at Game	Y N Unk	
Other	Y N Unk	Specify:
Other	Y N Unk	Specify:
Other	Y N Unk	Specify:
Other	Y N Unk	Specify:
Drinks:	Y N Unk	
Shared Water Bottles at Practice/game	Y N Unk	Specify:
Water from managers office	Y N Unk	
Other	Y N Unk	Specify:
Other	Y N Unk	Specify:
Other	Y N Unk	Specify:

What did you do after the game was cancelled on Friday night?

Were there any other activities or social gatherings you participated in with other members of the football team during 9/17-9/19 (Wednesday-Friday)?

Additional Comments: _____

_____

_____

_____

_____

_____

THANK YOU FOR YOUR TIME!

Appendix B: Non-Football Related Illness

BACKGROUND

During the investigation of gastrointestinal illness among Durand High School football players, PCHD and CDES staff were informed of an additional increase in absenteeism from September 18 to September 28 that was related to gastrointestinal illness, particularly among female volleyball team members. Reported symptoms included diarrhea, vomiting, headache, abdominal cramps and fever.

METHODS

Epidemiologic and Laboratory Investigation

The PCHD provided CDES staff with contact information for non-football affiliated students who reported having gastrointestinal illness. CDES staff conducted interviews with ill students using a standardized questionnaire to identify food or other exposures that individuals not directly affiliated with the football team may have had in common, including contact with football team members and staff. CDES staff created a line list using MS Excel 2010 to record demographic information, clinical signs and symptoms, and laboratory results.

Stool samples were collected from a sample of symptomatic non-football affiliated students and were submitted to the WSLH to be tested for the 4 most common bacterial gastrointestinal pathogens per standard WSLH Bacteriology Laboratory outbreak stool testing protocol: *E. coli* O157, *Shigella*, *Salmonella* and *Campylobacter*, and screened for Shiga toxins using the Meridian Premier® EHEC Enzyme Immunoassay (EIA). Specimens were also tested for the viral gastrointestinal pathogens norovirus, rotavirus, sapovirus and astrovirus using real-time PCR.

RESULTS

Epidemiologic and Laboratory Investigation

Eighteen individuals were interviewed by CDES staff. Clinical signs and symptoms among those reported are presented in Table 1. While diarrhea was reported by 11 individuals, none reported at least 3 episodes during 24 hours and consequently did not have illness that met the clinical case definition that was used during the investigation of the football team outbreak.

In addition, no clear and consistent pattern of contact with football team-affiliated individuals could be determined.

Two stool samples were submitted by symptomatic individuals not affiliated with the football team. Both samples were negative for all 8 bacterial and viral pathogens that were tested for at the WSLH.

Table 1. Signs and symptoms of gastrointestinal illness among non-football affiliated individuals

Symptom	Number (N=18)	Reported symptom (%)
Diarrhea	11	92%
Abdominal Cramps	11	92%
Fever	10	83%
Chills	9	75%
Vomiting	9	75%
Nausea	8	67%
Sweats	7	58%
Bloody Diarrhea	0	0%
Headache	0	0%
Body Aches	0	0%
Muscle Aches	0	0%
Fatigue	0	0%

CONCLUSION

The epidemiologic and laboratory investigations did not reveal a clear source for gastrointestinal illnesses among individuals at Durand High School who were not affiliated with the football team.

Appendix C: *Bacillus anthracis* laboratory testing

During the morning of September 24, 2014, the Pepin County Health Department (PCHD) and the Division of Public Health (DPH) were notified that a culture of a blood specimen collected on September 22, 2014 from one case-patient who was hospitalized was presumptively positive for a *Bacillus* species. The clinical laboratory report stated the isolated *Bacillus* species was likely a contaminant, but staff at the clinical laboratory could not definitively rule out *Bacillus anthracis* because they did not have the test methods in their facility. On the same day, the clinical laboratory reported that a culture of a stool specimen collected on September 22, 2014 from the same patient was positive for a *Campylobacter* species. The blood and stool isolates were sent by courier to the Wisconsin State Laboratory of Hygiene (WSLH) later that day for confirmation, species identification and further testing. On September 24, 2014 the WSLH reported that real time PCR results of testing the *Bacillus* isolate were negative for the presence of all three *Bacillus anthracis* DNA target sequences. These results ruled out infection with *Bacillus anthracis* (anthrax). The submitting laboratory, the patient's healthcare provider and PCHD and DPH staff were immediately notified of the results. The *Campylobacter* isolate was later confirmed as *Campylobacter jejuni* at the WSLH and was linked by pulsed field gel electrophoresis subtyping to other epidemiologically-linked *Campylobacter jejuni* isolates.