Short Communication: Turbidity as an Indicator of *Escherichia coli* Presence in Water Troughs on Cattle Farms

R. P. Smith,¹ G. A. Paiba, and J. Ellis-Iversen

Veterinary Laboratories Agency, and Centre of Epidemiology and Risk Analysis, Woodham Lane, New Haw Addlestone, Surrey KT15 3PB, United Kingdom

ABSTRACT

Studies have shown that water-drinking troughs are an important source of *Escherichia coli* infection on cattle farms, and a study was designed to provide farmers with an easy-to-use tool to monitor trough contamination and help determine when to empty and clean water troughs. A total of 164 water troughs were sampled on 33 cattle farms, and the on-farm turbidity tester results were found to be significantly correlated with laboratory turbidity results. Turbidity was associated with *E. coli* concentration, although the association was not linear. Emptying the troughs within a week of sampling was shown to reduce the turbidity score, but no linear association between time of emptying and *E. coli* concentration was discovered. A turbidity score of 4 was set as a cutoff point for when to clean a trough, yielding a sensitivity of 0.94 and a specificity of 0.03 for identifying a level of *E. coli* concentration that was more likely to contain *E. coli* O157 (>5,800 cfu/100 mL).

Key words: *Escherichia coli*, drinking water, turbidity, England and Wales

Zoonotic pathogens such as verocytotoxic *Escherichia coli* O157 (VTEC O157) often result in asymptomatic infections in farm animals; thus, the farmer is often unaware of the presence of human pathogens in the stock. Simple methods of assessing the risk of exposure to such organisms would assist farmers in reducing the transmission among farm stock and may result in reducing the risk of onward transmission to people. Dirty drinking troughs may act as environmental reservoirs for VTEC O157 and other pathogens and allow recirculation of infection among animals. The presence of *E. coli* in dirty trough water has been associated with the presence of *E. coli* O157 in cattle, and *E. coli* O157 has been reported to persist for up to 8 mo in water trough sediment and still be infectious to calves after

6 mo (Lejeune et al., 2001a,b; Ellis-Iversen et al., 2005). Lejeune et al. (2001b) showed that water troughs with *E. coli* O157 present had significantly greater *E. coli* concentrations, and Rice and Johnson (2000) showed that the indicator wild-type *E. coli* was always present in troughs when the pathogenic strain *E. coli* O157 was also present.

Turbidity testing is generally used to estimate the suspension of OM in water by measuring the degree of light reflection caused by the suspended particles. Turbidity testing is used as an indicator of when to clean swimming pools, because high levels of turbidity can indicate high concentrations of bacteria and other OM, which can reduce the effectiveness of disinfectants. In a recent study, turbidity was also found to be an indicator of *Eimeria* cyst concentration in water (Ellis-Iversen et al., 2005).

This study aimed to develop and test a predictive tool that can be used by farmers to estimate the level of *E. coli* contamination of drinking trough water and thus to decide when to empty and clean such troughs. The objectives were 1) to investigate whether water turbidity can be used as a predictor for the presence of presumptive *E. coli* in water troughs; and 2) to assess the accuracy of an on-farm turbidity testing tool and recommend when a trough should be cleaned. Lejeune et al. (2001b) showed that troughs contaminated with *E. coli* O157 had, on average, 1.76 *E. coli* (log₁₀)/mL, which equates to 5,800 cfu/100 mL. This information was used to set a visual turbidity score (VTS) cutoff from which the trough is, on average, more likely to contain the O157 pathogenic strain of *E. coli*.

The data for this study were collected on 33 farms that had recently been tested and found to have at least one cow shedding VTEC O157 in its feces, which was monitored as part of a different study (Ellis-Iversen et al., 2007). The farms represented most regions of England and Wales and were sampled between March 16 and June 7, 2004 (3 farms were sampled twice).

The on-farm turbidity tester consisted of a 1-L glass jug onto which an equally graduated test strip of increasing darkness was attached [marked from 1 (clear) to 10 (black)]. Each sample was collected by drawing

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¹Corresponding author: r.smith@vla.defra.gsi.gov.uk

Figure 1. The relationship between water turbidity measured in a laboratory by spectrophotometry [fluorescent turbidity units (FTU)] and the concentration of *Escherichia coli* in the sample.

the bottle through the water in the trough, without stirring the water, and resuspending the sludge at the bottom of the trough. The VTS was scored as the lowest number visible when the jug was filled with water, and 5 readings were taken from different water troughs at each farm visit. The water samples were then sent to the National Laboratory Service in transport flasks containing 18 mg of sodium thiosulfate, to counteract bacterial numbers being reduced by the possible presence of chlorine, and tested within 24 h of collection. A subsample of 100 to 150 mL was tested for turbidity and up to 1 L was tested for *E. coli* enumeration. Turbidity was measured by using a tungsten spectrophotometer (Environment Agency, 1981) and expressed as fluorescent turbidity units. Presumptive *E. coli* colonies were enumerated by membrane filtration and spread plating (Environment Agency, 2002) and reported as colony-forming units per 100 mL, with a lower limit of detection of 10 colonies/100 mL.

Because some samples had a zero count, one was added to all counts to enable the log-conversion of all samples to reduce the effect of outliers. Visual turbidity score, laboratory turbidity, and bacterial concentrations were described by using Microsoft Excel scatter plots and compared by linear regression analysis clustered on farm and including visit month as an a priori confounder. The average VTS and enumeration results were also compared against individual water trough management practices (which were recorded on a sample collection form) by using either *t*-tests or Mood's median test. Geometric means were used to provide summaries of the skewed enumeration data. All analyses were completed by using STATA 9 (Stata Corp., College Station, TX) software.

A total of 164 water trough samples were collected from 36 farm visits [mean 4.6 samples per visit (minimum 2, maximum 5)]. The linear regression model and the linear trend line showed that the turbidity of a

Table 1. Sensitivity and specificity scores for the effectiveness of an on-farm turbidity tester to detect concentrations of *Escherichia coli* linked to the presence of *E. coli* O157 in cattle water troughs

VTS ¹	<i>E. coli</i> >5,800 cfu/100 mL			VTS comparison	Sensitivity	Specificity
	Positive	Negative	Positive, %			
1	3	77	3.8	1 vs. 2–8	0.17	0.46
2	4	32	11.1	1–2 vs. 3–8	0.39	0.24
3	4	25	13.8	1–3 vs. 4–8	0.61	0.06
4	6	5	54.5	1–4 vs. 5–8	0.94	0.03
5–8	1	4	20.0			

¹Visual turbidity score.

Figure 2. Relationship between laboratory turbidity scores [fluorescent turbidity units (FTU)] and the turbidity tester results (visual turbidity score, VTS) for water trough samples. One outlier was removed from the graph (VTS 3, laboratory turbidity 97).

water sample and the log-transformed concentration of presumptive *E. coli* were significantly associated ($P = 0.006$), but the relationship was not linear and the coefficient of determination was low ($R^2 = 0.124$; Figure 1). The linear regression results of the VTS tool were strongly positively correlated with the turbidity measured in the laboratory ($P < 0.001$) and, although the linear coefficient of determination was low ($R^2 = 0.333$; Figure 2), it was considered a useful method of determining water turbidity.

The data were analyzed to identify a VTS cutoff point at which to empty and clean a water trough. Sensitivity and specificity were estimated for each increasing VTS score, against the remaining VTS, for whether more than 5,800 cfu/100 mL was detected (Table 1). The specificity of the test at all cutoffs was poor, but a comparison of VTS 1 to 4 against the others produced the highest sensitivity, and this was chosen as the cutoff because it was deemed more important to identify most samples with a substantial number of *E. coli* at the expense of false positive results. On average, 11% of samples with VTS 1 to 4 had concentrations of 5,800 or more *E. coli* cfu/100 mL, whereas VTS 5 to 8 had, on average, 20% of samples that were more likely to contain *E. coli* O157, as indicated by the research of Lejeune et al. (2001b).

The majority (72.3%) of water troughs used water from water mains (public water supply) and almost all (92.8%) were in current use. Troughs in current use had a significantly higher presumptive *E. coli* count than those not in use (Table 2). Water from water mains had, as expected, lower turbidity scores, but the geometric means showed a significantly higher average *E. coli* count than water not from water mains ($P < 0.001$).

The crude results indicate that water not from water mains had fewer positive samples but with larger *E. coli* counts. The use of water not from water mains is frequently supplemented by adding disinfectants post-tapping (Rutter et al., 2000). The large variation between *E. coli* counts could have been caused by inactivation of disinfectants by OM, UV light, or other factors in some of the troughs and by effective disinfection in the others.

Just over half (54.1%) of all troughs had been emptied in the week before a visit, and 25.7% had not been emptied in the last 3 wk. An analysis of these emptied troughs indicated that emptying the water trough within a week before sampling reduced the VTS but had no linear effect on *E. coli* counts, which may indicate that, although the troughs are regularly contaminated by *E. coli*, other turbidity elements are reduced when the water is refreshed. Straw was present in 36.6% of troughs, and 26.8% contained noticeable sediment or debris, but neither had an apparent effect on the turbidity, although *E. coli* counts increased with visible contamination. The 9.8% of troughs with algae or scum present had significantly higher VTS scores, and none of these troughs had been emptied in the week before the sampling visit.

The standing water in water troughs is often suspected as an on-farm source of microorganism recycling among groups of cattle, and pathogens such as VTEC O157, *Campylobacter*, *Cryptosporidium*, *Giardia lambda*, and *Eimeria* have all been associated with and isolated from water (Slifko et al., 2000; Lejeune et al., 2001a,b; Nygard et al., 2004; Ellis-Iversen et al., 2005). Although the VTS tool did not accurately predict *E. coli*

Table 2. Water trough sample results presented against individual water hygiene practices

Factor	VTS, ¹ median	P-value, Mood's test	Presumptive <i>Escherichia coli</i> , geometric mean/100 mL	P-value, <i>t</i> -test	Troughs, ² n
Water main		0.130		<0.001	155
Yes	1.0		315		
No	2.0		126		
In use		0.842		<0.001	152
Yes	2.0		296		
No	2.0		29		
Straw present		0.884		<0.001	164
Yes	2.0		348		
No	2.0		202		
Sediment present		0.229		<0.001	164
Yes	1.0		360		
No	2.0		214		
Algae or scum present		0.011		0.005	164
Yes	2.5		233		
No	1.0		248		
Trough emptied		0.004		<0.001 ³	75
Last week	1.0		270		
1–3 wk	1.0		105		
>3 wk	2.0		190		

¹VTS = visual turbidity score.²The VTS analysis excluded 1 sample and *E. coli* excluded 2 samples because of missing results.³ANOVA test used.

concentrations, the VTS had a strong positive relationship with the laboratory turbidity test score. Recommending to farmers that water troughs be emptied and cleaned when the VTS is greater than 4 may help to reduce concentrations of *E. coli* linked to the presence of *E. coli* O157 and might also reduce the presence of other pathogens in water.

Emptying and cleaning water troughs is a laborious task for farmers, and an on-farm VTS tool could aid them in deciding when to empty a water trough. However, to achieve a high probability of emptying troughs likely to contain a high level of *E. coli*, many troughs that do not contain this level of *E. coli* would also be emptied. The association between VTS and other pathogens needs investigation.

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