Contamination of Bacteriological Media by *Leptospira biflexa*

J. J. BRENDLE and A. D. ALEXANDER

Division of Veterinary Medicine, Walter Reed Army Medical Center, Washington, D.C. 20012

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Contamination of media with a strain of *Leptospira biflexa* was traced to the deionized water supply. The leptospiral contaminant appeared in media sterilized by filtration through 0.45- and 0.22-µm pore size membrane filters.

The substitution of water deionizing systems for water distillation systems as a laboratory water supply, combined with the current use of filter sterilization techniques for the preparation of heat-labile tissue culture and bacteriological media, present a chance for contamination by *Leptospira biflexa*. *L. biflexa* comprise the water or nonpathogenic leptospiras which are omnipresent in natural fresh waters. The contamination of stock leptospiral cultures with an *L. biflexa* strain and its subsequent isolation from leptospiral media and the deionized water supply is reported.

In routine microscope agglutination tests for leptospiral agglutinins in convalescent sera from human patients presumptively infected with serovar (syn. serotype) *icterohaemorrhagiae*, reactions were obtained with 17 out of 18 live screening antigens of diverse serovars. The antigens were grown in an albumin-fatty acid medium (9). Since the test is relatively serovar specific (1), a battery of selected antigens is conventionally used to assure detection of antibodies which may be produced by any of the large number of different serovars. All but one of the antigens were from pathogenic (*L. interrogans*) serovars. The exceptional antigen was from an *L. biflexa* strain, Patoc 1, which was included because of its genus-specific cross-reactivity with positive human sera (1). The observed reactions were contrary to the more restrictive cross-reaction patterns usually obtained with positive human sera. Therefore, repeat tests were done on the same sera and on additionally selected human sera positive for diverse serovars. The identical multiple reactions were obtained even with sera which previously reacted with few antigens. The 18 diagnostic antigens were then tested with their homologous antisera. Agglutination occurred only with the Patoc 1 antiserum. When all of the respective 18 antisera were tested against each of the 18 antigens, only the antisera to Patoc 1 agglutinated all antigens. Six of the 18 contaminated antigens were arbitrarily selected and tested against a group of serologically different *L. biflexa* antisera. The cross-reaction patterns (Table 1) of all six antigens were identical. It was apparent that the antigens were contaminated with the same leptospira and that the leptospiral contaminant had overgrown the original cultures.

Approximately 1 month later, a contaminating leptospira was found in a batch of albumin-fatty acid medium (9) prepared a few days previously. Since the contaminated medium and the medium for maintenance of the conventional antigens had been prepared with deionized water and then sterilized by filtration through a 0.22-µm pore size membrane filter, attention was directed to the water supply as a common source of contamination. The water deionizing system passed the tap water from the city water supply in sequence through tanks with an activated charcoal prefilter and three mixed resin beds. The resins in the deionizer were locally prepared, also with city tap water. Samples of water from the deionizer were obtained from the initial flow after standing overnight and after a 2.5- and 5-gallon (9.463- and 18.925-liter) run-off. At the same time, tap water in the building and deionized water in a carboy kept in the laboratory were sampled. Portions of each sample were filtered through either a 0.45- or 0.22-µm membrane filter. Each filtrate (1 ml) was inoculated into five tubes of Fletcher medium (9). To obviate the possibility of leptospiral contamination, all media were prepared with distilled water. After sterilization by filtration, they were tyndalized at 56 C for 1 h on each of three consecutive days.

Leptospiras were isolated from the 0.45-µm membrane filtrate of the initial sample of deionized water. No leptospiras were recovered from the other samples. The deionized-water isolate (designated strain WRAIR) was compared serologically with the isolates from contaminated antigens and contaminated medium in conven-
Table 1. Serological reactions of leptospiral isolates with L. biflexa serovars

| Antiserum to serovar (strain) | Titer with test isolates |
|-------------------------------|-------------------------|
|                               | Contaminant from antigens* | Contaminant from media | Isolate from deionized water |
| andamana (CH 11)              | —                      | —                      | —                      |
| codice (CDC)                  | —                      | —                      | —                      |
| Undetermined (LT 490)         | —                      | —                      | 1,600 6,400 800 |
| Undetermined (Ghent)          | —                      | —                      | 200 100 100 |
| patoc (Patoc 1)               | —                      | —                      | 1,600 3,200 400 |
| sao paulo (Sao Paulo)         | —                      | —                      | 200 200 100 |
| semaranga (Veld. S-173)       | —                      | —                      | —                      |
| Undetermined (Waz 3)          | 100                    | 100                    | 100                    |
| Undetermined (Turtle A183)    | —                      | —                      | —                      |
| Undetermined (Wa Reiden)      | —                      | —                      | 6,400 6,400 1,600 |
| illini (3055)                 | —                      | —                      | —                      |

* Identical reactions obtained with contaminating leptospiras found in six different antigens.
* Negative at 1:100 dilution.

Definitively traced to the laboratory tap water supply. It may have originated from the tap water at the local facility where the resins were prepared. The finding of L. biflexa in tap water is not unusual. Nonpathogenic leptospiras have been commonly isolated from the tap water of municipal water supply systems in this and other countries (3). In fact, Walker (10) reported the isolation of L. biflexa from the tap water of this institute in 1927. Another factor allowing leptospiral contamination was the ability of these organisms to penetrate bacterial retaining filters. This characteristic is also well recognized (8). Therefore, the findings in this report are not surprising. The preparation of bacteriological or tissue culture medium containing heat-labile components but no antibiotics present special risks of leptospiral contamination. The contamination of a tissue culture media with L. biflexa is described in a separate report (J. Tumilowicz, A. D. Alexander, and D. K. Stafford, In Vitro, in press).

To eliminate leptospiral contamination, we suggest that media sterilized by filtration be prepared with distilled water or boiled deionized water, or that completed media be heated at 56 C for 1 h (7).

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