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# Bacteriological methods for distinguishing between human and animal faecal pollution of water: results of fieldwork in Nigeria and Zimbabwe

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*Bacteriological techniques have traditionally been used to detect faecal pollution of drinking water supplies. Recently, methods have been developed to distinguish between human and animal faecal pollution in temperate climates. The present study assessed the applicability and practicality of these methods in tropical countries. Fieldwork in Nigeria and Zimbabwe has shown that animal faecal pollution can reliably be identified by the detection and enumeration of* Rhodococcus coprophilus *using modified M3 agar, whereas human faecal contamination can be identified by the detection of sorbitol-fermenting bifidobacteria. Each of these organisms was detected only in the faeces of the type (human or animal) that it was meant to indicate. Although* Streptococcus bovis *has been used in the past in mainly temperate countries to distinguish animal from human faecal contamination, the present study has shown that this organism is not a reliable indicator of animal pollution in the tropics because it was excreted by a proportion of the human population in both Nigeria and Zimbabwe. Water sources known to be contaminated by human or animal excreta were examined for these indicator organisms. The results correlated with the results obtained from examining human and animal faecal specimens for these organisms. The role of these bacteriological methods in water pollution control programmes is discussed.*

Bacteriological techniques for distinguishing between human and animal faecal pollution are a valuable tool in water pollution control programmes because they are useful in tracing the sources of faecal pollution of drinking-water supplies, and they can help in assessing the overall adequacy of protection of small rural water supplies, especially in developing countries. Several bacteriological methods have been described for the differentiation between human and animal faecal pollution (*1-6*). However, these methods were developed and have been tested only in temperate climates. The indicator bacteria commonly used for detecting animal faecal pollution are *Rhodococcus coprophilus* (*2*, *7*) and *Streptococcus bovis* (*3*, *6*, *8-10*), whereas bifidobacteria, especially the sorbitol-fermenting strains, are used to detect human faecal pollution (*4*, *5*). Selective media for isolating and enumerating these organisms have been described (*2-6*). Since dietary and geographical factors can affect the numbers and types of gut bacteria (*11*), we have investigated the applicability of these methods in two tropical countries, Nigeria and Zimbabwe.

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## THE STUDY AREAS

### *Afikpo and Ohaozara localities, Imo State, Nigeria*

Field investigations were conducted in Afikpo and Ohaozara local government areas in February and March 1983. These two locations were chosen as they were included in the Imo State Government/UNICEF Rural Drinking Water Supply and Sanitation Project, the main aims of which are to provide a steady, accessible supply of good quality water and to improve sanitation to a reasonable level through the construction of ventilated improved pit latrines.

Afikpo (05°55′N, 07°56′E) is situated about 6 km west of the river Azu. The headquarters of the Ohaozara local government area is at Ubiozara and the investigations were carried out at Okposi, Uburu, and Ubioazara. Okposi (06°04′N, 07°48′E) is situated close to Uburu, which is about 3 km from the Ubioazara local government area office.

Human faecal samples were obtained in the two local government areas, and faeces from sheep and cattle were obtained from the Afikpo abattoir. Water samples were collected from the Esu river, as well as ponds (Ata, Oba Nta, Anyuro and Ama-Nkanu) and boreholes (local reference numbers, TBH2 to TBH5) in the Ohaozara local government area.

Table 1. Ranges and geometric mean counts of indicator bacteria per gram of human and various animal faeces obtained from two study areas in Nigeria and Zimbabwe

| Samples | | | | |
|---|---|---|---|---|
| Source | No. examined | *Escherichia coli* | Faecal streptococci | *Streptococcus bovis* |
| Human: ON[a] | 12 | $1.7 \times 10^7$–$8.4 \times 10^8$ ($1.3 \times 10^8$)[b] | $5.5 \times 10^5$–$5.8 \times 10^8$ ($1.7 \times 10^7$) | 0–$9.6 \times 10^4$ (6) |
| Human: HZ[a] | 13 | $4.8 \times 10^4$–$7.0 \times 10^9$ ($1.3 \times 10^7$) | $7.0 \times 10^4$–$3.6 \times 10^7$ ($8.1 \times 10^5$) | 0–$5.0 \times 10^5$ (58) |
| Cattle: AN[a] | 5 | $3.6 \times 10^5$–$1.4 \times 10^6$ ($7.2 \times 10^5$) | $6.2 \times 10^5$–$9.0 \times 10^5$ ($7.9 \times 10^5$) | $2.4 \times 10^5$–$5.0 \times 10^5$ ($3.8 \times 10^5$) |
| Sheep: AN | 5 | $1.8 \times 10^8$–$5.1 \times 10^9$ ($8.9 \times 10^8$) | $1.3 \times 10^6$–$1.9 \times 10^7$ ($6.5 \times 10^6$) | $6.0 \times 10^5$–$4.1 \times 10^7$ ($7.1 \times 10^6$) |
| Elephant: HZ | 4 | $2.3 \times 10^5$–$3.6 \times 10^7$ ($1.9 \times 10^6$) | $4.0 \times 10^6$–$7.0 \times 10^7$ ($1.7 \times 10^7$) | $9.8 \times 10^5$–$5.0 \times 10^7$ ($8.1 \times 10^6$) |
| Buffalo: HZ | 4 | $2.3 \times 10^5$–$5.7 \times 10^5$ ($3.2 \times 10^5$) | $1.3 \times 10^6$–$4.5 \times 10^7$ ($5.1 \times 10^6$) | $8.5 \times 10^4$–$2.6 \times 10^7$ ($8.5 \times 10^5$) |
| Giraffe: HZ | 3 | $2.4 \times 10^5$–$2.6 \times 10^5$ ($2.5 \times 10^5$) | $1.3 \times 10^4$–$1.8 \times 10^4$ ($1.5 \times 10^4$) | $1.3 \times 10^3$–$1.8 \times 10^3$ ($1.5 \times 10^3$) |
| Impala: HZ | 4 | $1.9 \times 10^4$–$9.3 \times 10^7$ ($5.1 \times 10^6$) | $2.0 \times 10^4$–$9.4 \times 10^5$ ($3.2 \times 10^5$) | $6.4 \times 10^2$–$5.6 \times 10^5$ ($5.2 \times 10^4$) |
| Kudu: HZ | 2 | $1.3 \times 10^6$–$1.7 \times 10^6$ ($1.5 \times 10^6$) | $6.4 \times 10^5$–$7.3 \times 10^5$ ($6.9 \times 10^5$) | $1.2 \times 10^4$–$3.0 \times 10^5$ ($2.8 \times 10^5$) |
| Waterbuck: HZ | 3 | $3.5 \times 10^6$–$4.0 \times 10^6$ ($3.8 \times 10^6$) | $5.0 \times 10^5$–$5.2 \times 10^5$ ($5.1 \times 10^5$) | $4.9 \times 10^4$–$5.4 \times 10^6$ ($1.1 \times 10^5$) |
| Wildebeest: HZ | 4 | $1.8 \times 10^5$–$2.4 \times 10^7$ ($2.7 \times 10^6$) | $1.1 \times 10^5$–$5.3 \times 10^6$ ($1.7 \times 10^6$) | $1.2 \times 10^4$–$7.2 \times 10^4$ ($2.6 \times 10^4$) |
| Zebra: HZ | 1 | $2.7 \times 10^3$ | $1.1 \times 10^3$ | $2.8 \times 10^3$ |
| Ostrich: HZ | 3 | $1.4 \times 10^6$–$8.6 \times 10^6$ ($2.9 \times 10^6$) | $9.9 \times 10^4$–$8.5 \times 10^6$ ($5.9 \times 10^5$) | $1.2 \times 10^4$–$6.8 \times 10^4$ ($3.7 \times 10^4$) |

[a] ON = Ohaozara, Nigeria; HZ = Hwange National Park, Zimbabwe; AN = Afikpo, Nigeria.
[b] Figures in parentheses are geometric means.
[c] ND = not done.

### *Hwange National Park, Zimbabwe*

Hwange (formerly Wankie) National Park was formed in 1928 as a game reserve and 21 years later was proclaimed a national park. It covers an area of 14 545 km$^2$ and is located along the border with Botswana in the north-west of Zimbabwe to the south of Victoria Falls, extending from 18°53′S to 19°30′S and from 25°45′E to 27°25′E. It has only one wet season, from November to March, with occasional showers in October and April. Rainfall decreases in intensity westwards and is 625 mm per year, on average, in the main camp area. However, during the present study (November–December 1982) only about 5 mm of rain were recorded. The climate is cold (7–28°C, with ground frost) in June/July and the hot dry season is between August and November.

Surface waters are usually present in several rivers (Deka, Gwaai, Lukozi, and Sinamatella) and springs, but during the present study these sources were reduced to small ponds or water-holes as a result of a severe drought. To supplement the scarcity of water, dams and pans have been constructed throughout the National Park to provide water for the wild game. The pans are either natural clay basins holding water for some time during the wet season, which have been converted to permanent pools, or clay basins that have been deepened and are now supplied with water from boreholes (*12*). During visits to these water-holes and pans (Dom, Guvalala, and Nyamandhlovu) some animals invariably contaminate the water with their faeces. Samples of water were obtained from these pans and also from the Lukozi river. Fresh animal droppings were collected from around these pans and human faecal samples were collected from the staff of the National Park and Hwange Colliery Hospital.

Table 1: *continued from previous page*

| Bifidobacteria | | | | |
|---|---|---|---|---|
| Total | Sorbitol +ve | *Rhodococcus coprophilus* | *Micromonospora* | *Streptomyces* |
| $3.8 \times 10^8$–$6.6 \times 10^{10}$ ($4.7 \times 10^9$) | $9.5 \times 10^7$–$1.6 \times 10^9$ ($1.3 \times 10^9$) | 0 | 0 | 0–$5.0 \times 10^3$ (37) |
| $1.0 \times 10^6 \times 3.0 \times 10^{11}$ ($5.6 \times 10^9$) | $9.2 \times 10^5$–$6.1 \times 10^9$ ($1.2 \times 10^8$) | 0 | 0 | $1.2 \times 10^2$–$8.0 \times 10^4$ ($2.6 \times 10^3$) |
| 0 | 0 | $6.2 \times 10^3$–$7.8 \times 10^4$ ($2.1 \times 10^4$) | $6.0 \times 10^3$–$7.2 \times 10^4$ ($2.5 \times 10^4$) | $5.0 \times 10^4$–$6.0 \times 10^5$ ($9.1 \times 10^4$) |
| 0 | 0 | $3.2 \times 10^3$–$3.0 \times 10^4$ ($1.2 \times 10^4$) | $9.0 \times 10^4$–$1.6 \times 10^5$ ($1.2 \times 10^5$) | $3.0 \times 10^4$–$2.2 \times 10^5$ ($6.5 \times 10^4$) |
| 0 | 0 | $1.0 \times 10^3$–$1.4 \times 10^4$ ($7.8 \times 10^3$) | 0–$8.0 \times 10^3$ ($6.9 \times 10^3$) | $2.0 \times 10^2$–$2.4 \times 10^4$ ($8.7 \times 10^3$) |
| 0 | 0 | $1.0 \times 10^3$–$8.0 \times 10^3$ ($2.9 \times 10^3$) | $2.0 \times 10^2$–$1.0 \times 10^4$ ($1.1 \times 10^3$) | $3.0 \times 10^2$–$3.0 \times 10^3$ ($1.7 \times 10^3$) |
| 0 | 0 | $5.6 \times 10^2$–$4.3 \times 10^3$ ($7.9 \times 10^2$) | $2.8 \times 10^2$–$2.9 \times 10^2$ ($2.8 \times 10^2$) | $1.4 \times 10^2$–$1.9 \times 10^3$ ($1.2 \times 10^3$) |
| 0 | 0 | $2.0 \times 10^3$–$2.4 \times 10^4$ ($1.1 \times 10^4$) | 0 | $2.7 \times 10^3$–$8.0 \times 10^4$ ($3.2 \times 10^4$) |
| 0 | 0 | $8.9 \times 10^2$–$7.7 \times 10^3$ ($2.6 \times 10^3$) | $4.8 \times 10^2$–$7.0 \times 10^2$ ($5.9 \times 10^2$) | $1.8 \times 10^3$–$9.8 \times 10^3$ ($4.3 \times 10^3$) |
| 0 | 0 | $6.2 \times 10^2$–$5.8 \times 10^3$ ($2.6 \times 10^3$) | $1.6 \times 10^2$–$4.0 \times 10^3$ ($7.8 \times 10^2$) | $1.6 \times 10^3$–$4.8 \times 10^3$ ($3.1 \times 10^3$) |
| 0 | 0 | $1.0 \times 10^3$–$2.3 \times 10^4$ ($7.8 \times 10^3$) | $1.6 \times 10^3$–$1.0 \times 10^4$ ($1.5 \times 10^3$) | $4.0 \times 10^3$–$2.8 \times 10^4$ ($4.5 \times 10^3$) |
| 0 | 0 | $4.0 \times 10^2$ | $8.0 \times 10^2$ | $1.6 \times 10^3$ |
| 0 | 0 | ND[c] | ND | ND |

## MATERIALS AND METHODS

### *Media and conditions of incubation*

Faecal coliforms were enumerated on membrane filters (type HAWG 047)[a] incubated on pads saturated with 0.1% sodium lauryl sulfate broth (*13*, *14*). The medium was prepared from the dehydrated base, Membrane Enriched Teepol Broth,[b] and rehydrated with water containing 0.1% sodium lauryl sulfate instead of 0.4% Teepol (*15*). The plates were incubated at 30 °C for 4 h and then at 44 °C for 18–20 h. All yellow colonies were counted as presumptive *Escherichia coli*.

Faecal streptococci were enumerated on membranes incubated on KF streptococcal agar (*16*). The plates were incubated initially at 37 °C for 4 h and then at 44 °C for 44 h. All maroon-coloured colonies were counted as faecal streptococci. *S. bovis* was enumerated on modified membrane-Bovis agar (M-BA) (*6*). The modified medium contained exactly the same composition as the original formulation (*3*) except that the concentration of sodium azide was reduced from 0.09 to 0.05 g/l. Incubation was done anaerobically in GasPak jars[c] containing envelopes of $H_2 + O_2$ at 30 °C for 4 h and then at 39 °C for 48–72 h.

Total bifidobacteria were enumerated on YN-17 medium (*5*), a modification of the YN-6 medium of Resnick & Levin (*4*). Sorbitol-fermenting bifidobacteria were enumerated on Human Bifid Sorbitol Agar (HBSA) (*5*). All plates were incubated anaerobically in GasPak jars at 37 °C for 48 h. Colonies of bifidobacteria growing on membranes incubated on YN-17 medium were dark green with a pale periphery measuring 1–2 mm in diameter, whereas on HBSA medium the sorbitol-fermenting strains were yellow and dome-shaped.

*R. coprophilus* and associated actinomycetes (*Micromonospora* and *Streptomyces* spp.) were

[a] Millipore Corporation, Bedford, MA, USA.

[b] Oxoid Ltd, Basingstoke, England.

[c] Becton Dickinson, Cockeysville, MD, USA.

Table 2. Ranges and geometric mean counts of indicator bacteria per 100 ml of water samples from various sources in the two study areas in Nigeria and Zimbabwe

| Samples | | | | |
|---|---|---|---|---|
| Source | No. examined | *Escherichia coli* | Faecal streptococci | *Streptococcus bovis* |
| *River water:* | | | | |
| LZ[a,b] | 3 | $1.0 \times 10^3$–$4.1 \times 10^3$ ($2.5 \times 10^3$) | $6.1 \times 10^4$–$6.5 \times 10^4$ ($6.3 \times 10^4$) | $6.1 \times 10^2$–$7.8 \times 10^3$ ($3.0 \times 10^3$) |
| KZ[a,b] | 3 | $5.0 \times 10^2$–$6.2 \times 10^2$ ($5.8 \times 10^2$) | $1.7 \times 10^3$–$1.8 \times 10^3$ ($1.7 \times 10^3$) | $8.0 \times 10^2$–$1.0 \times 10^3$ ($9.5 \times 10^2$) |
| EN[a,d] | 3 | $4.8 \times 10^3$–$2.6 \times 10^4$ ($7.2 \times 10^3$) | $6.9 \times 10^2$–$6.1 \times 10^3$ ($1.4 \times 10^3$) | $1.0 \times 10^2$–$3.2 \times 10^2$ ($2.1 \times 10^2$) |
| *Stream water:* | | | | |
| ON[a,d] | 3 | $2.6 \times 10^3$–$5.0 \times 10^3$ ($3.5 \times 10^3$) | $4.0 \times 10^3$–$6.0 \times 10^3$ ($4.6 \times 10^3$) | $1.4 \times 10^2$–$1.8 \times 10^2$ ($1.4 \times 10^2$) |
| Oba Nta, Okposi, ON[d] | 3 | $8.8 \times 10^3$–$1.2 \times 10^4$ ($1.0 \times 10^4$) | $1.9 \times 10^3$–$2.5 \times 10^3$ ($2.2 \times 10^3$) | $1.7 \times 10^3$ |
| *Pond:* | | | | |
| Dom Pan, HZ[a,b] | 3 | $1.8 \times 10^3$–$7.7 \times 10^3$ ($4.5 \times 10^3$) | $2.7 \times 10^3$–$6.9 \times 10^3$ ($5.0 \times 10^3$) | $5.0 \times 10^2$–$7.0 \times 10^3$ ($1.5 \times 10^3$) |
| Guvalala Pan, HZ[b] | 3 | $3.5 \times 10^2$–$6.2 \times 10^2$ ($4.7 \times 10^2$) | $1.4 \times 10^3$–$2.3 \times 10^3$ ($1.8 \times 10^3$) | 0 |
| Nyamandhlovu Pan, HZ[b] | 3 | $1.6 \times 10^3$–$2.2 \times 10^3$ ($1.8 \times 10^3$) | $8.2 \times 10^3$–$2.2 \times 10^4$ ($1.3 \times 10^4$) | $1.4 \times 10^3$–$4.3 \times 10^3$ ($2.8 \times 10^3$) |
| Anyuro, ON[d] | 3 | $1.4 \times 10^3$–$1.3 \times 10^4$ ($5.2 \times 10^3$ | $9.8 \times 10^2$–$4.4 \times 10^3$ ($1.4 \times 10^3$) | 0 |
| Ama-Nkanu, Ogbu, ON[d] | 1 | $1.4 \times 10^3$ | $7.0 \times 10^2$ | 0 |
| *Borehole:* | | | | |
| TBH2 Okposi, ON | 2 | 3[e] | 0 | 0 |
| TBH3 Umuka, ON | 2 | 0 | 0 | 0 |
| TBH4 Okposi, ON | 2 | 0 | 0 | 0 |
| TBH5 Mebiowa, ON | 2 | 0 | 0 | 0 |

[a] LZ = Lukozi, Zimbabwe; KZ = Kaputi, Zimbabwe; EN = Esu, Nigeria; ON = Ohaozara, Nigeria; HZ = Hwange National Park, Zimbabwe.
[b] Water sources polluted with animal faeces.
[c] ND = not done.
[d] Water sources polluted by both human and animal faeces.
[e] Isolates were identified as *Klebsiella* spp.

enumerated on modified M3 (MM3) agar (*2*) by the surface spread-plate technique. The plates were incubated at 30 °C for 10–14 days, followed by exposure to sunlight for 3–4 days. *R. coprophilus* appeared as stellate colonies with bright orange central papillae.

## *Sample collection*

Samples of water were collected from boreholes, pans, ponds, rivers, springs, and streams in sterile 500-ml flasks. Faecal specimens from animals in the wild were collected in sterile universal bottles between 05–07h00 and 17–19h00 from droppings deposited around the pans after the animals had been there to drink. These samples were always collected in the presence of an experienced game scout who identified the homologous animal species from the appearance of the droppings collected. Faecal specimens from cattle and sheep were collected from the Afikpo abattoir (Nigeria). All samples were examined within 4 hours of collection.

## *Test procedure*

Sterile quarter-strength Ringer's solution was used

Table 2: *continued from previous page*

| Bifidobacteria | | | | |
|---|---|---|---|---|
| Total | Sorbitol +ve | *Rhodococcus coprophilus* | *Micromonospora* | *Streptomyces* |
| 0 | 0 | $3.8 \times 10^4$–$7.2 \times 10^4$ ($4.8 \times 10^4$) | $4.0 \times 10^4$–$5.1 \times 10^4$ ($4.6 \times 10^4$) | $1.2 \times 10^6$–$8.0 \times 10^6$ ($3.7 \times 10^6$) |
| 0 | 0 | ND[c] | ND | ND |
| $4.0 \times 10^2$–$5.0 \times 10^3$ ($1.2 \times 10^3$) | $1.0 \times 10^2$–$1.8 \times 10^3$ ($3.2 \times 10^2$) | 0 | $3.0 \times 10^2$–$6.0 \times 10^2$ ($4.5 \times 10^2$) | $9.0 \times 10^2$–$1.2 \times 10^3$ ($1.0 \times 10^3$) |
| $9.9 \times 10^2$–$5.3 \times 10^3$ ($1.8 \times 10^3$) | $4.8 \times 10^2$–$9.0 \times 10^2$ ($6.0 \times 10^2$) | 0 | $5.2 \times 10^2$–$8.0 \times 10^2$ ($6.9 \times 10^2$) | $1.5 \times 10^3$–$1.7 \times 10_3$ ($1.5 \times 10^3$) |
| $4.8 \times 10^2$–$7.0 \times 10^2$ ($5.9 \times 10^2$) | $2.9 \times 10^2$–$4.0 \times 10^2$ ($3.4 \times 10^2$) | 0 | $3.0 \times 10^2$–$7.0 \times 10^2$ ($4.7 \times 10^2$) | $5.0 \times 10^2$–$1.0 \times 10^3$ ($7.2 \times 10^2$) |
| 0 | 0 | $3.3 \times 10^4$–$1.4 \times 10^5$ ($7.8 \times 10^4$) | $5.8 \times 10^4$–$4.0 \times 10^5$ ($1.1 \times 10^5$) | $2.6 \times 10^5$–$3.5 \times 10^5$ ($3.0 \times 10^5$) |
| 0 | 0 | $5.1 \times 10^3$–$6.2 \times 10^3$ ($5.8 \times 10^3$) | 0 | $5.0 \times 10^4$–$5.8 \times 10^4$ ($5.5 \times 10^4$) |
| 0 | 0 | $5.0 \times 10^5$–$6.0 \times 10^5$ ($5.5 \times 10^5$) | $2.0 \times 10^4$–$4.0 \times 10^4$ ($2.8 \times 10^4$) | $8.0 \times 10^4$–$1.0 \times 10^5$ ($8.9 \times 10^4$) |
| $2.6 \times 10^2$–$2.8 \times 10^3$ ($1.2 \times 10^3$) | $1.3 \times 10^2$–$1.8 \times 10^3$ ($7.6 \times 10^2$) | 0 | 0 | $1.9 \times 10^3$–$4.6 \times 10^3$ ($3.2 \times 10^3$) |
| $2.6 \times 10^2$ | $1.3 \times 10^2$ | 0 | 0 | $3.9 \times 10^3$ |
| 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 |

to prepare dilutions (1:10) of the water samples. Faecal specimens were first thoroughly mixed with a sterile glass rod and then 1 g was emulsified in 9 ml of Ringer's solution. Dilutions (1:10) were then made and appropriate volumes were filtered through membranes or spread on the surface of media with a sterile L-shaped glass rod.

### *Characterization of isolates*

Purified cultures were first obtained from the primary isolates growing on the various selective media by plating on non-selective media to give single, well-isolated colonies. These cultures were then characterized according to recommended methods (*15*) as well as those used by Rowbotham & Cross (*1*), Scardovi et al. (*17*), and Oragui & Mara (*3*,*6*).

## RESULTS

Twenty-five faecal specimens from White and Black adults of Nigerian and Zimbabwean origin were examined for various indicator bacteria (Table 1). Of 13 human faecal specimens obtained from Hwange National Park and Hwange Colliery Hospital (Zimbabwe), 4 were derived from Whites and 9 from Blacks. The results show that *E. coli*, faecal streptococci, and bifidobacteria were consistently isolated from human faecal specimens whereas *E. coli*, faecal streptococci, *S. bovis*, *R. coprophilus*, *Micromonospora* and *Streptomyces* were constantly present in the faeces of all the animals examined. In faecal specimens of human origin, the incidence of *S. bovis* was 17–31% (2 out of 12

Table 3. Percentage distribution of various coliform organisms in human and animal faeces and in water samples from the two study areas in Nigeria and Zimbabwe

| Sample and source | No. of colonies tested[a] | Types of organisms identified (%) | | | | |
|---|---|---|---|---|---|---|
| | | *E. coli* | *Klebsiella* | *Citrobacter* | *Enterobacter* | Other coliforms |
| *Faeces:* | | | | | | |
| Human, N[b] | 210 | 77.1 | 18 | 1.9 | 1.9 | 1.0 |
| Human, Z[b] | 110 | 90.9 | 0 | 9.1 | 0 | 0 |
| Cattle, N | 66 | 78.8 | 15.2 | 0 | 6.1 | 0 |
| Sheep, N | 90 | 100 | 0 | 0 | 0 | 0 |
| Buffalo, Z | 42 | 100 | 0 | 0 | 0 | 0 |
| Elephant, Z | 78 | 100 | 0 | 0 | 0 | 0 |
| Giraffe, Z | 80 | 100 | 0 | 0 | 0 | 0 |
| Impala, Z | 90 | 100 | 0 | 0 | 0 | 0 |
| Kudu, Z | 40 | 100 | 0 | 0 | 0 | 0 |
| Ostrich, Z | 27 | 100 | 0 | 0 | 0 | 0 |
| Waterbuck, Z | 24 | 100 | 0 | 0 | 0 | 0 |
| Zebra, Z | 76 | 100 | 0 | 0 | 0 | 0 |
| *Water:* | | | | | | |
| Lukozi River, Z | 106 | 96.2 | 1.9 | 1.9 | 0 | 0 |
| Kaputi River, Z | 50 | 88.0 | 12 | 0 | 0 | 0 |
| Esu River, N | 80 | 35 | 60 | 0 | 0 | 5 |
| Stream, N[c] | 50 | 28 | 56 | 8 | 8 | 0 |
| Pond, N[d] | 47 | 29.8 | 42.6 | 8.5 | 2.1 | 17 |
| Pans, Z[e] | 243 | 89.3 | 3.3 | 6 | 0 | 7.4 |

[a] Isolates were picked from membranes incubated on pads saturated with 0.1% sodium lauryl sulfate broth.
[b] N = Nigeria; Z = Zimbabwe.
[c] The streams were Ata and Oba Nta at Ohaozara, Nigeria.
[d] The ponds were Anyuro pond and Ama Nkanu at Ohaozara, Nigeria.
[e] The pans were Dom, Guvalala and Nyamandhlovu.

specimens from Nigerians and 4 out of 13 specimens from Zimbabweans). The geometric mean counts of *E. coli* and faecal streptococci generally ranged from $10^4$ to $10^8$ organisms per gram of faeces from humans and animals, except for the single specimen from a zebra. *R. coprophilus* was present in all the animal faeces examined but was absent from every human faecal sample. However, the MM3 agar used for enumerating *R. coprophilus* was found to be unsuitable when the organisms were present in small numbers; clearly, additional work is necessary to develop an improved membrane filtration medium. Significantly, bifidobacteria were not isolated from any animal faecal specimens but were present in all the human faecal samples; the numbers of total bifidobacteria ranged from $10^6$ to $10^{11}$ organisms per gram of faeces, with geometric means of $4.7 \times 10^9$ (Nigeria) and $5.6 \times 10^9$ (Zimbabwe). Lower results (from $10^5$ to $10^9$ with geometric means of $1.2 \times 10^8$ and $1.3 \times 10^9$, respectively) were associated with sorbitol-fermenting bifidobacteria.

Table 2 shows the ranges and geometric means of the various indicator organisms present in water samples from rivers, streams and ponds in Ohaozara (Nigeria) and Hwange National Park (Zimbabwe). All the water sources in Nigeria, except the boreholes, were faecally contaminated by both humans and animals. This is because these sources are used not only by the many animals that drink from them but also by humans who wash in them and defecate in the surrounding environment. On the other hand, the water sources in Zimbabwe (Kaputi and Lukozi rivers; Dom, Guvalala, and Nyamandhlovu pans) are frequented only by wild animals (in the game reserve), which drink from these sources and invariably pollute them with faecal droppings; no human contamination was evident because the reserve is a restricted area. *E. coli* and faecal streptococci were isolated

from all samples of river and stream water examined and ranged from $10^2$ to $10^4$ organisms per 100 ml water. Results from examinations of the ponds show that *S. bovis* was not isolated from the Guvalala pan but was consistently present in the samples of water from Dom and Nyamandhlovu pans; this may be explained by the high chloride concentration (1800 mg/l) in the Guvalala pan and the very low chloride concentration (13 mg/l) in the Nyamandhlovu pan (*18*). Bifidobacteria were not isolated from water samples from the Lukozi and Kaputi rivers (Zimbabwe) but were consistently isolated from the Esu river and Ata and Oba Nta streams (Nigeria), the geometric means being $1.2 \times 10^3$, $1.8 \times 10^3$, and $5.9 \times 10^2$ organisms per 100 ml, respectively. These organisms were not isolated from the water samples obtained from the three Zimbabwean pans examined. Of the eight samples of water obtained from four boreholes, only one sample yielded coliforms, the count being 3/100 ml; the three colonies were identified to genus level as *Klebsiella*.

### *Faecal coliform types*

The results of the identification of 1509 faecal coliform types isolated from faeces and water are shown in Table 3. Purified colonies from organisms growing on membranes incubated on lauryl sulfate broth were tested for indole production from tryptone water and gas from lactose peptone water at 44 °C (*15*). Organisms which produced indole, either with or without gas, at 44 °C were presumed to be *E. coli* as anaerogenic strains are known to occur (*19*). The results indicate that in general all the isolates from wild animals and sheep were *E. coli*. In human and cattle faeces and in water samples from Zimbabwe, the proportions of *E. coli* were generally above 75% with smaller proportions of *Klebsiella*, *Citrobacter* and *Enterobacter* spp. In contrast, much lower proportions of *E. coli* were associated with water samples obtained in Nigeria (28–35%). *Klebsiella* spp. were present in higher numbers and ranged from 42.6% to 60% in samples from Esu river, Anyuro pond, and Ama-Nkanu pond. *Citrobacter* and *Enterobacter* spp. comprised 10–16% of the coliforms isolated from these sources.

### *Faecal streptococcal types*

A total of 1609 colonies, picked from membranes incubated on KF and modified M-BA media, were characterized (Table 4). The results indicate that *S. bovis* is widely distributed in the faeces of animals and forms from 25% (in wildebeest faeces) to 87% (in giraffe faeces) of the total faecal streptococcal flora, as determined by membrane filtration with KF agar. *S. faecalis* and *S. faecium* appear to be the predominant faecal streptococci in the faeces of impala and wildebeest (66.7% and 62.5%, respectively). In human faeces, the numbers of *S. faecium* were 5–6 times more numerous than *S. faecalis*. The latter was notably absent in the faeces of cattle, sheep, elephant, giraffe and wildebeest. *S. durans* was not isolated from cattle, buffalo, giraffe, impala or zebra. *S. equinus* was not isolated from any of the samples of faeces or water examined. Unclassified strains were consistently isolated from human faecal samples and from most water samples. It is particularly interesting to note that *S. bovis* was isolated from some human faecal specimens. Table 4 also reveals that, with the exception of isolates from human faeces, all organisms growing on membranes incubated on M-BA medium were identified as *S. bovis*.

### *Bifidobacteria types*

Table 5 shows the type distribution of 914 colonies of bifidobacteria isolated from human faeces and water samples from Esu river and Ata stream (Nigeria). *Bifidobacterium adolescentis* was by far the most common species isolated from both human faeces and water samples on membranes incubated on YN-17 medium. This species was more numerous than *B. breve* by a factor of 15–64 in faecal and water samples. *B. liberorum*, *B. pseudolongum*, *B. suis* and *B. thermophilum* together formed less than 20% of the bifidobacteria (in human faeces and water samples) picked from membranes incubated on YN-17 medium. On HBSA medium, *B. adolescentis* was the predominant species with much smaller proportions of *B. breve* and unclassified strains. No other named species of bifidobacteria was isolated.

## DISCUSSION

There are obvious limitations inherent in distinguishing human from animal faecal pollution by bacteriological methods, for not only does the normal flora vary from individual to individual owing to dietary, physiological, immunological, and geographical factors but the resident flora is also in a constant state of adaptive change. However, given these limitations, the present study has shown that some of the indicator bacteria studied in Nigeria and Zimbabwe can be used with confidence to discriminate between human and animal faecal pollution of water. We have shown that sorbitol-fermenting bifidobacteria and *R. coprophilus* fulfil this requirement. Bifidobacteria were isolated from water sources in Nigeria but not from those in Zimbabwe. The significance of these results is that bifidobacteria are not associated with animals but are consistently isolated from human faeces and therefore, when

Table 4. Percentage distribution of faecal streptococcal types in human and animal faeces and in water samples from the two study areas in Nigeria and Zimbabwe

| Sample and source | Isolation media | No. of colonies tested | Types of organisms identified (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *S. faecalis* | *S. faecium* | *S. durans* | *S. bovis* | *S. equinus* | Unclassified |
| *Faeces:* | | | | | | | | |
| Human, N[a] | KF-agar | 69 | 5.8 | 34.8 | 0 | 39.0 | 0 | 20.3 |
| Human, Z[a] | KF-agar | 112 | 10.7 | 50 | 3.6 | 14.2 | 0 | 21.4 |
| Human, N/Z | M-BA | 102 | 0 | 3.8 | 0 | 92.3 | 0 | 3.8 |
| Cattle, N | KF-agar | 60 | 0 | 50 | 0 | 33.3 | 0 | 16.7 |
| | M-BA | 50 | 0 | 100 | 0 | 100 | 0 | 0 |
| Sheep, N | KF-agar | 52 | 0 | 15.4 | 3.8 | 80.8 | 0 | 0 |
| | M-BA | 50 | 0 | 0 | 0 | 100 | 0 | 0 |
| Buffalo, Z | KF-agar | 80 | 50 | 0 | 0 | 37.5 | 0 | 12.5 |
| | MB-A | ND[b] | | | | | | |
| Elephant, Z | KF-agar | 80 | 0 | 18.8 | 6.3 | 62.3 | 0 | 18.8 |
| | M-BA | 50 | 0 | 0 | 0 | 100 | 0 | 0 |
| Giraffe, Z | KF-agar | 80 | 0 | 0 | 0 | 87.5 | 0 | 12.5 |
| | M-BA | 42 | 0 | 0 | 0 | 100 | 0 | 0 |
| Impala, Z | KF-agar | 60 | 66.7 | 0 | 0 | 33.3 | 0 | 0 |
| Wildebeest, Z | KF-agar | 80 | 0 | 62.5 | 0 | 25 | 0 | 12.5 |
| | M-BA | 50 | 0 | 4 | 0 | 96 | 0 | 0 |
| Zebra, Z | KF-agar | 84 | 33.3 | 33.3 | 0 | 23.8 | 0 | 9.5 |
| | M-BA | 40 | 0 | 0 | 0 | 100 | 0 | 0 |
| *Water:* | | | | | | | | |
| Lukozi River, Z | KF-agar | 78 | 5.1 | 56.4 | 0 | 33.3 | 0 | 5.1 |
| | M-BA | 50 | | | | 100 | | |
| Kaputi River, Z | KF-agar | 40 | 25 | 25 | 0 | 50 | 0 | 0 |
| Nyamandhlovu Pan, Z | KF-agar | 77 | 18.2 | 18.2 | 0 | 45.5 | 0 | 18.2 |
| Dom Pan, Z | KF-agar | 100 | 20 | 40 | 0 | 40 | 0 | 0 |
| Oba Nta/Anyuro, N | KF-agar | 75 | 28 | 36 | 8 | 2.7 | 0 | 25.3 |
| Esu River, N | KF-agar | 48 | 25 | 4.2 | 0 | 12.5 | 0 | 58.3 |

[a] N = Nigeria; Z = Zimbabwe.
[b] ND = not done.

present in water samples, indicate that human faecal pollution is occurring or has occurred.

*Streptococcus bovis* does not appear to be a suitable indicator of animal faecal pollution in these geographical areas as it was isolated from both human and animal faeces. This organism has also been reported to be present in significant numbers in the faeces of people investigated in India (*20*). This, together with the findings from the present study, suggests that *S. bovis* may be more common in human faeces than has hitherto been assumed. It is therefore essential to screen the local population for the incidence and distribution of this organism prior to any study or investigation. Only when it has been established that this organism is absent in human faeces, can it be used to distinguish reliably between animal and human faecal pollution.

*E. coli*, faecal streptococci and *Streptomyces* were excreted by both humans and animals; therefore they cannot be used as specific indicator organisms of either human or animal pollution. On the other hand, *R. coprophilus* was recovered from the faeces of all the animals but not from human faeces; this organism can therefore be confidently used as a specific indicator of animal faecal pollution, although the currently available method for its isolation requires up to 18 days for reliable identification, which in practice limits its usefulness; further work is clearly

needed to improve the medium and method so as to reduce the incubation period.

In this study, bifidobacteria were isolated only from human faecal specimens and water samples contaminated by human faecal material but not from any animal faeces. This finding contrasts with our previous studies (*5*) and those of other workers (*4, 21*) in which this group of organisms was consistently isolated from human and pig faeces and very occasionally from the faeces of cattle, sheep, dogs, mice and rats (*4, 21*). Nevertheless, sorbitol-fermenting strains of *B. adolescentis* and *B. breve* were isolated in this study only from human faeces and not from animals. However, Resnick & Levin (*4*) and Mitsuoka (*21*) isolated *B. adolescentis* from pig faeces; a possible explanation of this is that their isolates belonged to biotypes b or d which constitute only a minor proportion of the bifidobacteria present in human faeces and are characterized by their inability to ferment sorbitol (*17*); therefore even when present in animal faeces or polluted waters, they would not be enumerated on HBSA medium.

The low incidence of *E. coli* (28–35%) in water sources contrasts with the proportion of the same organism in faeces (73–100%) from Nigerian sources and calls into question the validity of the standard *E. coli* test for tropical waters. Bifidobacteria have been suggested as a suitable alternative to the faecal coliform test (*22*),[d] and this study has shown that bifidobacteria can be used as specific indicator organisms of human faecal pollution. Its detection in water can thus be of assistance in tracing the source of contamination by determining whether the contamination is of human or animal origin. The isolation and enumeration of bifidobacteria are relatively easy using the YN-17 and HBSA media. The recognition of these organisms may, however, be difficult in some very heavily polluted waters (e.g., Esu river and Ata stream in Nigeria) that contain large numbers of faecal streptococci. Clearly, further work is needed to make the medium more selective and the method of isolation and enumeration more suitable for small laboratories.

The relationships between water, excreta and health are now reasonably well understood (*23, 24*). Many of the epidemiologically important communicable infections are related to both water and excreta and, although many excreta-related diseases, especially diarrhoeal diseases, are transmitted by a water-washed faeco-oral route, they can nevertheless also be spread by a waterborne route. While there are undoubted risks to human health from animal faeces, it is human faeces that represent a much greater risk, and thus the ability to distinguish confidently between human and animal pollution of water can be of considerable value in epidemiological investigations. The present study has shown that this distinction can be made both confidently and promptly (within 48 hours) by the detection of sorbitol-fermenting bifidobacteria.

[d] OPARA, A. A. *The role of anaerobic faecal bacteria as indicators of water pollution in hot climates.* Ph.D. thesis, University of Dundee, Scotland, 1978.

Table 5. Percentage distribution of bifidobacteria in human faeces and in water samples from the two study areas in Nigeria and Zimbabwe

| Sample and source | Isolation media | No. of colonies tested | Types of organisms identified (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *B. adolescentis* | *B. breve* | *B. liberorum* | *B. longum* | *B. pseudolongum* | *B. suis* | *B. thermophilum* | Unclassified |
| *Human faeces:* | | | | | | | | | | |
| Nigeria | YN-17 | 246 | 50 | 1.2 | 0.4 | 19.1 | 8.9 | 6.5 | 2.8 | 11 |
| | HBSA | 96 | 92.7 | 5.2 | 0 | 0 | 0 | 0 | 0 | 2.1 |
| Zimbabwe | YN-17 | 100 | 49 | 4 | 5 | 25 | 2 | 7 | 0 | 8 |
| | HBSA | 80 | 86.3 | 10 | 0 | 0 | 0 | 0 | 0 | 3.7 |
| *Water:* | | | | | | | | | | |
| Esi river, Nigeria | YN-17 | 199 | 64.3 | 1.0 | 0.5 | 15.6 | 5.0 | 3.0 | 0.5 | 10.1 |
| | HBSA | 95 | 93.6 | 4.2 | 0 | 0 | 0 | 0 | 0 | 2.1 |
| Ata stream, Nigeria | YN-17 | 50 | 52 | 10 | 8 | 16 | 4 | 6 | 0 | 4 |
| | HBSA | 48 | 83.3 | 12.5 | 0 | 0 | 0 | 0 | 0 | 4.2 |

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## RÉSUMÉ

### MÉTHODES BACTÉRIOLOGIQUES PERMETTANT DE DISTINGUER L'ORIGINE, ANIMALE OU HUMAINE, DE LA POLLUTION FÉCALE DES EAUX: RÉSULTATS DE TRAVAUX SUR LE TERRAIN AU NIGÉRIA ET AU ZIMBABWE

Pour déceler la pollution fécale de l'eau potable, on utilise traditionnellement des méthodes bactériologiques. On a récemment mis au point des techniques qui permettent de distinguer, dans les pays tempérés, la pollution fécale humaine de la pollution fécale animale. Le but de la présente étude était de voir si ces méthodes sont valables et praticables sous les tropiques. Les travaux conduits sur le terrain au Nigéria et au Zimbabwe montrent que la mise en évidence et la numération de *Rhodococcus coprophilus* sur agar M3 modifié permettent de déceler avec certitude l'existence d'une pollution fécale d'origine animale; quant à la pollution fécale d'origine humaine, elle peut être décelée par la présence de bifidobactéries fermentant le sorbitol. Chacun de ces germes a été trouvé exclusivement dans les excréments, humains ou animaux, dont il est censé être caractéristique. *Streptococcus bovi* avait déjà été utilisé, essentiellement dans les pays tempérés, pour distinguer l'origine animale ou humaine de la contamination fécale. La présente étude a montré que, sous les tropiques, ce germe n'est pas un indicateur fiable de pollution animale car il est excrété par une certaine proportion de la population humaine, au Nigéria comme au Zimbabwe. Ces divers germes indicateurs ont été recherchés dans les eaux connues pour être contaminées par les excreta humains ou animaux. Les résultats concordent avec ceux que l'on a obtenus en recherchant ces germes dans des échantillons de fèces humaines et animales. L'article examine le rôle de ces méthodes bactériologiques dans les programmes de lutte contre la pollution des eaux.

## REFERENCES

1. ROWBOTHAM, T. J. & CROSS, T. Ecology of *Rhodococcus corprophilus* and associated actinomycetes in fresh water and agricultural habitats. *Journal of general microbiology*, **100**: 231–240 (1977).
2. MARA, D. D. & ORAGUI, J. I. Occurrence of *Rhodococcus coprophilus* and associated actinomycetes in feces, sewage and freshwater. *Applied and environmental microbiology*, **42**: 1037–1042 (1981).
3. ORAGUI, J. I. & MARA, D. D. A selective medium for the enumeration of *Streptococcus bovis* by membrane filtration. *Journal of applied bacteriology*, **51**: 85–93 (1981).
4. RESNICK, I. G. & LEVIN, M. A. Assessment of bifidobacteria as indicators of human fecal pollution. *Applied and environmental microbiology*, **42**: 433–438 (1981).
5. MARA, D. D. & ORAGUI, J. I. Sorbitol-fermenting bifidobacteria as specific indicators of human faecal pollution. *Journal of applied bacteriology*, **55**: 349–357 (1983).
6. ORAGUI, J. I. & MARA, D. D. A note on a modified membrane-Bovis agar for the enumeration of *Streptococcus bovis* by membrane filtration. *Journal of applied bacteriology*, **56**: 179–181 (1984).
7. ORAGUI, J. I. & MARA, D. D. Investigations of the survival characteristics of *Rhodococcus coprophilus* and certain fecal indicator bacteria. *Applied and environmental microbiology*, **46**: 356–360 (1983).
8. COOPER, K. E. & RAMADAN, F. M. Studies in the differentiation between human and animal pollution by means of faecal streptococci. *Journal of general microbiology*, **12**: 180–190 (1955).
9. GELDREICH, E. E. & KENNER, B. A. Concepts of fecal streptococci in stream pollution. *Journal of the Water Pollution Control Federation*, **41**: R336–R351 (1969).
10. TILTON, R. C. & LITSKY, W. The characterization of fecal streptococci. An attempt to differentiate between animal and human sources of contamination. *Journal of milk and food technology*, **30**: 1–6 (1967).

11. DRASAR, B. S. Some factors associated with geographical variations in the intestinal microflora. In: Skinner, F. A. & Carr, J. G., ed., *The normal microbial flora of man*, London, Academic Press, 1974, pp. 187-196.
12. WEIR, J. S. Diversity and abundance of aquatic insects reduced by introduction of the fish *Clarias gariepinus* to pools in Central Africa. *Biological conservation*, **4**: 169-175 (1972).
13. JOINT COMMITTEE OF THE PUBLIC HEALTH LABORATORY SERVICE AND THE STANDING COMMITTEE OF ANALYSTS. Membrane filtration media for the enumeration of coliform organisms and *Escherichia coli* in water: comparison of Tergitol 7 and lauryl sulphate with Teepol 610. *Journal of hygiene*, **85**: 181-191 (1980).
14. STANFIELD, G. & IRVING, T. E. A suitable replacement for Teepol 610 in the selective isolation of coliforms from marine waters and sewage. *Water research*, **15**: 469-474 (1981).
15. *The bacteriological examination of water supplies*, Report on Medical and Public Health Subjects No.71. 4th ed., London, H.M. Stationery Office, 1969.
16. KENNER, B. A. ET AL. Fecal streptococci. 1. Cultivation and enumeration of streptococci in surface waters. *Applied microbiology*, **9**: 15-20 (1961).
17. SCARDOVI, V. ET AL. Deoxyribonucleic acid homology relationships among species of the genus *Bifidobacterium. International journal of systematic bacteriology*, **21**: 276-294 (1971).
18. WEIR, J. S. Seasonal variations in alkalinity in pans in Central Africa. *Hydrobiologia*, **32**: 69-80 (1968).
19. COWAN, S. T. *Cowan and Steel's manual for the identification of medical bacteria*, 2nd ed. Cambridge, Cambridge University Press, 1974.
20. DRASAR, B. S. & HILL, M. J. *Human intestinal flora*. London, Academic Press, 1974.
21. MITSUOKA, T. [Comparative studies on bifidobacteria isolated from the alimentary tract of man and animals.] *Zentralblatt für Bakteriologie, Parasitenkunde Infektionskrankheiten und Hygiene, 1 Abt., Originale*, **210**: 52-64 (1969) (in German).
22. EVISON, L. & JAMES, A. Bifidobacterium as an indicator of faecal pollution in water. *Progress in water technology*, **7**: 57-66 (1978)
23. WHITE, G. F. ET AL. *Drawers of water*. Chicago/London, Chicago University Press, 1972.
24. FEACHEM, R. G. ET AL. *Sanitation and disease. Health aspects of excreta and wastewater management*, Chichester, John Wiley & Sons, 1983 (World Bank Studies in Water Supply and Sanitation 3).