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Effect of Faecal Consistency on Virological Diagnosis

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A study was set up to investigate the effect of consistency of routine faecal specimens on the diagnostic yield by electron microscopy (EM) and virus isolation. A total of 3078 specimens were characterized as solid, semisolid, or liquid. Of 2568 specimens processed by EM a virus was demonstrated in 8.6% of liquid, 19.9% of semisolid and 25.2% of solid specimens (Chi-squared for linear trend, P value <0.0001). This observation was valid for both adenovirus (2.4% 5.0% and 6.6%) and rotavirus (5.2% 13.6% and 16.6%). Virus isolation was positive in 3.6% of liquid, 17.4% of semisolid and 18.1% of solid specimens. (Chi-squared for linear trend, P value <0.0001).

We suggest that solid faecal specimens at the end of an episode of diarrhoea will have a higher diagnostic yield than liquid specimens at the peak of symptoms. Our findings repudiate the commonly held dogma that viruses of gastroenteritis are more likely to be found in liquid than in solid faecal specimens. This finding has important implications for those establishing diagnostic algorithms for the investigation of viral gastroenteritis.

Introduction

Electron microscopy (EM) on faecal specimens is a time-consuming and expensive method for diagnosis of viral gastroenteritis. During the investigation of outbreaks of gastroenteritis it may be necessary or desirable to prioritize specimens processed. Traditionally in our laboratory this was done by assessing the consistency of the faeces received and preferentially processing those specimens which were liquid and leaving the more solid specimens to later. A more restrictive approach has been employed in the Public Health Laboratory Service of England and Wales, where the customary practice is to seek only "unformed" faeces for EM (PHLS North West Electron Microscopy Policy, August 1996). The current recommendations from Centers for Disease Control, U.S.A., are that the more formed the stool specimen the lower the diagnostic yield, and therefore liquid specimens only should be collected and examined by EM.1,2 We set up a study to investigate the validity of this approach to EM and also to look at the relationship between faecal consistency and virus isolation rates.

Materials, Methods and Patients

From 1 March 1995 to 30 June 1996 a total of 3078 faecal specimens arriving at the laboratory were examined on receipt and categorized as liquid, semisolid or solid. They were processed according to our laboratory's routine procedures for virus isolation and EM. A 10% suspension of faeces was prepared in 10 ml of Hank's salt solution containing 1000 units of penicillin, 1000 μg of streptomycin and 2.5 μg of amphotericin B per ml in a glass universal bottle containing sterile glass beads. The suspension was shaken in a mechanical floor mounted shaker for 15 min and centrifuged at 4000 g for 1 h at 4 °C. All faecal specimens were processed for virus isolation. Those specimens which from the clinical details on the request form were clearly from patients with an illness other than gastroenteritis were not tested by EM. All other specimens were tested by EM, including those with no clinical details on the request form. For the period of the study the consistency category of the faecal specimen was not used to decide whether or not to do EM.

EM was performed using a protocol based on a published methodology.3 In brief, drops of clarified 10% faecal suspension were placed on a sheet of dental wax. A glow discharged carbon-coated Formvar grid, film side downwards, was placed on each drop of specimen suspension and left at room temperature for 3 h or overnight. Grids were dried and stained with 2% methylamine tungstate pH 6.5 for 5–10 min and examined at a magnification of 34 000.

Virus isolation was carried out using a microtitre plate based system using cells inoculated in suspension.4 All specimens were inoculated into six cell lines: (HEp2 human epithelial continuous ATCC CCL23), Vero E6...
Table I. Electron microscopy results on 2568 faecal specimens categorised by consistency.

| Result                  | Liquid n=407 | Semisolid n=1384 | Solid n=777 |
|-------------------------|--------------|------------------|-------------|
| EM positive             | 35 (8.6)     | 275 (19.9)       | 196 (25.2)  |
| EM negative             | 372 (91.4)   | 1109 (80.1)      | 581 (74.8)  |
| Adenovirus              | 10 (2.4)     | 69 (5.0)         | 51 (6.6)    |
| Astrovirus              | 0            | 2 (0.14)         | 0           |
| Coronavirus             | 0            | 1 (0.07)         | 0           |
| Adeno and Rotavirus     | 0            | 4 (0.3)          | 0           |
| Rotavirus               | 21 (5.2)     | 188 (13.6)       | 129 (16.6)  |
| SRSV*                   | 2 (0.5)      | 9 (0.7)          | 7 (0.9)     |
| SRV†                    | 2 (0.5)      | 2 (0.14)         | 9 (1.2)     |

* Small round structured virus.
† Small round virus.

(African green monkey continuous ATCC CRL1586), RD (human rhabdomyosarcoma continuous ATCC CCL136), primary rhesus monkey kidney (supplied by CPHL, Porton Down) and two human fibroblast semicontinuous cell lines (established in the Regional Virus Laboratory (RVL), Belfast, U.K.).

The case notes of two inpatients who had a negative followed by a positive result for rotavirus during the same clinical episode were examined to see how the consistency of their faeces varied at the time of these results.

The data was extracted using an in-house developed modular and generic relational data application (PVCS95) constructed using Paradox DOS Version 4.01 (Borland), which serves as the RVL Belfast laboratory computer system. Clinical data accompanying the request was entered as free text and subsequently searched for eight indicator text strings (diarrh, vomit, watery, foul, offensiv, D+V, gastro, enteritis) to determine the number of requests specifically detailing a gastroenteritis-like illness. The text search was not case sensitive and recognized both partial and whole words. In order to assess whether group differences (positive vs. negative) were due to the consistency of the faecal sample, a Chi-squared test for linear trend was performed. All statistical analyses were performed using EPI-INFO Version 6 (CDC, Atlanta, Georgia, U.S.A.) and the conventional 5% level of significance was used throughout.

Results

Of the 3078 faecal samples received, a total of 2568 were processed for EM. A virus was seen in 506 (19.7%).

The positivity rate increased with consistency (Table I) (Chi-squared for linear trend = 33.971, P-value <0.0001). All 3078 faecal specimens were processed for virus isolation. A virus was isolated in 481 (15.6%). The positivity rate increased with consistency (Table II) (Chi-squared for linear trend = 33.963, P-value <0.0001).

Requests with text strings indicating gastroenteritis accompanying faeces processed for EM were present in 223 (54.8%) of the liquid faeces, 794 (57.4%) of the semisolid faeces and 465 (59.8%) of the solid faeces. These differences were not significant (Chi-squared for linear trend = 2.937, P-value = 0.09). In contrast, text strings indicating gastroenteritis were present in 357 (70.5%) requests accompanying EM positive faeces and in 1125 (54.5%) requests accompanying EM negative faeces (Chi-squared = 225.8, P-value <0.0001).

Case histories

Case A: a 10-month-old female was admitted to the regional infectious diseases unit on day 6 post onset of gastroenteritis, and was discharged on day 12. Faecal consistency and EM results are summarized in Table III.

Case B: a 6-month-old male was admitted to the regional infectious disease unit on day 3 post onset of gastroenteritis, and was discharged on day 9. Faecal consistency and EM results are summarized in Table III.

Discussion

The finding that the diagnostic yield from faeces examined by EM increases with consistency was unexpected. The most likely explanation for the increase in diagnostic yield with consistency is simply concentration of faeces during resolution of diarrhoea. The two illustrative case histories demonstrate that the finding of a positive EM result may occur at the time of resolution of diarrhoea. The duration of the illness in these two cases is the maximum of what would normally be expected. The
Table II. Virus isolation results on 3078 faecal specimens categorized by consistency.

| Result                  | Liquid n=442 | Semisolid n=1640 | Solid n=996 |
|-------------------------|-------------|-----------------|-----------|
| n (%)                   | n (%)       | n (%)           | n (%)     |
| Virus isolated          | 16 (3.6)    | 285 (17.4)      | 180 (18.1) |
| Virus not isolated      | 426 (96.4)  | 1355 (82.6)     | 816 (81.5) |
| Adenoviruses            | 10 (2.3)    | 113 (6.8)       | 81 (8.1)  |
| Coxsackie A viruses*    | 0           | 2 (0.1)         | 1 (0.1)   |
| Coxsackie B viruses†    | 0           | 18 (1.1)        | 7 (0.7)   |
| Echoviruses‡            | 1 (0.2)     | 49 (3.0)        | 34 (3.4)  |
| Polioviruses§           | 5 (1.1)     | 101 (6.2)       | 53 (3.3)  |
| Untypable enteroviruses | 0           | 2 (0.1)         | 4 (0.4)   |

* Only Coxsackie A9 virus was isolated during the course of the study.
† Coxsackie B2, B4, B5, were isolated during the course of the study.
‡ Echovirus: EV5, EV7, EV9, EV11, EV15, EV18, EV19, EV20, EV21, EV22, EV25, EV30, EV31 were isolated during the course of the study.
§ All polioviruses were typed as part of an ongoing program (Enteric and Respiratory laboratory CPHL, Colindale) and were all considered to be vaccine strains.

Table III. Faecal consistency and EM results for cases A and B.

| Case | Consistency | EM Result |
|------|-------------|-----------|
| A    | 1 to 5      | Liquid    |
| 6    | Liquid      | Not done  |
| 7 to 9 | Liquid | Negative |
| 10   | Semisolid and solid | Positive |
| B    | 1 to 6      | Liquid    |
| 7    | Liquid      | Not done  |
| 8    | Semisolid   | Positive  |

A negative result from faeces during the acute phase of the diarrhoea does not exclude a diagnosis of viral gastroenteritis. Our observations suggest that the optimum specimen is one taken either at the start of symptoms before the faeces have become liquid or at the time the diarrhoea is resolving. The results of this study have important implications for those establishing diagnostic algorithms for use in the investigation of viral gastroenteritis. Further studies looking at the kinetics of viral excretion in humans and the relationship to faecal consistency are required.
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