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Effect of Faecal Consistency on Virological Diagnosis

C. McCaughey*[1], H. J. O'Neill[1], D. E. Wyatt[1], S. N. Christie[2], P. T. Jackson[2] and P. V. Coyle[1]

[1]*Regional Virus Laboratory, Department of Microbiology and Immunobiology, Royal Hospitals Trust, Grosvenor Road, Belfast BT12 6BN and* [2]*Regional Infectious Diseases Unit, Belvoir Park Hospital, Belfast, U.K.*

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

* Address correspondence to: C. McCaughey
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Table I. Electronmiscroscopy results on 2568 faecal specimens categorised by consistency.

Result	Liquid $n=407$ n (%)	Semisolid $n=1384$ n (%)	Solid $n=777$ n (%)
Total 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 worlds. 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 $n=442$ n (%)	Semisolid $n=1640$ n (%)	Solid $n=996$ 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)
Cocksackie 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 (5.3)
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.

A	1 to 5	Liquid	Not done
	6	Liquid	Negative
	7 to 9	Liquid	Not done
	10	Semisolid and solid	Positive
B	1 to 6	Liquid	Not done
	7	Liquid	Negative
	8	Semisolid	Positive

proportion of samples with clinical details containing text strings indicating a gastroenteritis type illness did not correlate with the consistency of the faeces, but did correlate with EM positivity. This implies that the more formed faecal specimens were no more or less likely to be from cases of gastroenteritis. It has previously been demonstrated that up to 40% of children who have recovered from viral gastroenteritis will still be positive for a viral agent by EM on the day of discharge from hospital.[5]

Animal studies provide an insight. In suckling rats experimentally infected with group B rotavirus where faeces were assayed by enzyme immunoassay, 100% of the animals assayed at days 1, 4, and 5 tested positive for rotavirus. However, only 70% and 20% of the animals tested positive at days 2 and 3 postinoculation, respectively.[6] This biphasic pattern of viral excretion was confirmed by a dot hybridization assay. It was noted that the second peak coincided with the time when diarrhoea had begun to resolve and the fluid volume in the intestine had decreased. The second peak of viral excretion was shown not to be due to a second round of intestinal replication. A similar biphasic pattern has been also observed in neonatal murine rotavirus models.[7–10] It was noted that the first peak of viral excretion preceded the severe diarrhoea and that the second peak coincided with the faeces becoming solid.[8] This biphasic pattern was also noted in rotavirus infectivity titre (TCID50) in the mid small intestine of gnotobiotic piglets infected with rotavirus.[11] In this case the first peak of viral excretion was at 19–22 h and the second peak at 61–65 h. The results that we have observed would reflect a similar biphasic pattern of excretion in patients with gastroenteritis. Because the first peak precedes the peak symptomatology, diagnosis is often dependent on the second peak.

The finding that the virus isolation rate increases with faecal consistency was less surprising. As the readily cultivatable enteric viruses are not generally considered to cause gastroenteritis, one would expect them to be most efficiently isolated from solid faeces, where they are the most concentrated. In this study the virus isolation rate was low (3.6%) in liquid faeces, but there was very little difference in the isolation rate between semisolid (17.4%) and solid (18.1%) specimens.

An important lesson from this study is that it is clearly unwise to exclude fully formed faeces in a protocol for rationalizing the investigation of outbreaks, as these were the specimens with the highest yield of positive results. 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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References

1 Lew JF, LeBaron CW, Glass RI *et al.* Recommendations for collection of laboratory specimens associated with outbreaks of gastroenteritis. *MMWR* 1990; **39 (RR-14)**: 1–13.

2 LeBaron CW, Furutan NP, Lew JF *et al.* Viral agents of gastroenteritis, public health importance and outbreak management. *MMWR* 1990; **39 (RR-5)**: 1–24.

3. Johnson RPC, Gregory DW. Viruses accumulate spontaneously near droplet surfaces: a method to concentrate viruses for electron microscopy. *J Microsc* 1993; **171**: 125–136.

4. O'Neill HJ, Russell JD, Wyatt DE, McCaughey C, Coyle PV. Isolation of viruses from clinical specimens in microtitre plates with cells inoculated in suspension. *J Virol Meth* 1996; **62**: 169–178.

5. Ellis ME, Watson B, Mandal BK *et al.* Micro-organisms in gastroenteritis. *Arch Dis Child* 1984; **59**: 848–855.

6. Vonderfecht SL, Eiden J, Miskuff RL, Yolken RH. Kinetics of intestinal replication of group B rotavirus and relevance to diagnostic methods. *J Clin Microbiol* 1988; **26**: 216–221.

7. Starkey WG, Collins J, Wallis TS *et al.* Kinetics, tissue specificity and pathological changes in murine rotavirus infection in mice. *J Gen Virol* 1986; **67**: 2625–2634.

8. Osborne MP, Haddon SJ, Spencer AJ *et al.* An electron microscopic investigation of time-related changes in the intestine of neonatal mice infected with murine rotavirus. *J Pediatr Gastroenterol Nutr* 1988; **7**: 236–248.

9. Duffy LC, Zielezny MA, Riepenhoff-Talty M *et al.* Reduction of virus shredding by *B. bifidum* in experimentally induced MRV infection statistical application for ELISA. *Dig Dis Sci* 1994; **39**: 2334–2340.

10. Eydelloth RS, Vonderfecht SL, Sheridan JF, Enders LD, Yolken RH. Kinetics of viral replication and local and systemic responses in experimental rotavirus infection. *J Virol* 1984; **50**: 947–950.

11. Crouch CF, Woode GN. Serial studies of virus multiplication and intestinal damage in gnotobiotic piglets infected with rotavirus. *J Med Microbiol* 1978; **11**: 325–334.