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Infecting dose and severity of typhoid: analysis of volunteer data and examination of the influence of the definition of illness used

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SUMMARY

Data from volunteers challenged with *Salmonella typhi* were reanalysed to explore the relationship between challenge dose and severity of disease. Among 120 ill volunteers who received between 10^5 and 10^9 organisms, dose was weakly correlated with peak temperature ($r = 0{\cdot}22$, 95% CI 0·04–0·39), duration of temperature above 103 °F (39·4 °C: $r = 0{\cdot}13$, 95% CI −0·03 to 0·55) and symptom score ($r = 0{\cdot}27$, 95% CI 0·09–0·43). The association with symptom score was lost after adjusting for year, and the findings depended on the definition of illness used: with stricter definitions the associations with temperature were also lost. The study shows the need for caution in interpreting the relationship between dose and severity of disease.

INTRODUCTION

If the infecting dose of *Salmonella typhi* influences severity of disease, an intervention, such as an improvement in sanitation, which lowers the inoculum would be expected to have a greater effect on severe than on total disease [1]. This has implications both for implementing and assessing public health programmes. Among volunteers challenged with typhoid, Hornick and colleagues [2] found that larger inocula produced higher attack rates and shorter incubation periods. They noted that 'once illness occurred, the clinical courses were comparable regardless of the dose of the infectious inoculum'. A review of natural typhoid outbreaks using indirect markers of dose such as incubation period or type of vehicle, and case fatality rates to assess severity, also found no evidence of an association between dose and severity, in contrast to the situation with other salmonelloses [3].

The focus of the challenge studies lay elsewhere, thus further details on dose and severity were not given. The definition of illness used included an oral temperature of at least 103 °F (39·4 °C) for at least 24–36 h; this would exclude mild cases and leave only a small range of severity available for assessment. We have returned to the original files and reanalysed the data on all unvaccinated volunteers challenged

orally with varying inoculum sizes of wild type *S. typhi* to examine the dose–severity relationship in greater detail.

METHODS

Charts were reviewed from experimental challenge studies carried out between 1959 and 1970 by investigators at the University of Maryland, working with volunteers consisting of healthy male inmates at the Maryland House of Correction, Jessup, MD. We have excluded those who received previous vaccines or *S. typhi* challenges. Various inocula of Quailes strain *S. typhi* (phase type D-1) were administered in milk and gargled before swallowing [2]. Antibiotics (usually chloramphenicol) were started when the temperature was at least 103 °F (39·4 °C) for 24–36 h [2]. From 1965, most volunteers were seen as outpatients unless and until they became ill. Inpatients had their temperatures recorded at 4-h intervals during the day. Symptoms and signs were recorded daily on checklists. Laboratory results were not used in this analysis.

We used different definitions of illness to assess the influence of the definition on the results obtained:

Definition (I): temperature $\geqslant$ 100 °F (37·8 °C) for $\geqslant$ 12 h continuously and a peak $\geqslant$ 101 °F (38·3 °C).
Definition (II): illness that resulted in antibiotic therapy.
Definition (III): temperature $\geqslant$ 101 °F (38·3 °C) for $\geqslant$ 12 h continuously.
Definition (IV): temperature spiking to $\geqslant$ 103 °F (39·4 °C) for $\geqslant$ 36 h.

Symptoms were not included in any definitions because none are specific to typhoid (so would not help to exclude other diseases). Since normal temperatures vary between individuals and diurnally [4], and occasional wrong recordings may occur, a sustained fever may be more reliable than a single peak. However, in some studies patients were only admitted when temperatures over 100 °F (37·8 °C) or 101 °F (38·3 °C) were recorded, and temperatures were recorded only once a day. Outpatient temperatures would have been recorded during working hours, whereas temperatures are expected to be at their highest in the evening [4]. Definition (I) incorporates both the peaks and duration. For the 10^5 dose, attack rates were compared between inpatients, outpatients seen once a day and outpatients seen twice a day to see if patients with mild disease would be missed if outpatients were included in the analysis.

Antibiotic treatment was only given to those regarded as ill at the time, and some patients were treated with fevers less than 103 °F (39·4 °C) depending on their other symptoms [2]. Using treatment as the definition of illness (definition (II)) should minimize the false positives but will miss cases of mild disease and so will bias the results towards a failure to find a dose effect. With this definition the few patients who only had rectal temperatures recorded were included, the peak temperature being estimated as the recorded temperature minus 0·7 °F (0·4 °C) [5].

Although volunteers who were vaccinated during the studies were excluded, many of the volunteers had served in the armed forces and so would have received

typhoid vaccination previously. This was treated as a possible confounder. Other potential confounders were age, race and year.

The severity of illness was assessed by several measures: the peak temperature reached; the duration of fever continuously above 103 °F (39·4 °C, assessed from the temperature charts as the number of 4-h periods); the occurrence of individual symptoms and signs; the total number of symptoms and signs recorded (the symptom score); and the occurrence of relapses. Relapses were defined as a recurrence of fever and symptoms occurring after completing a course of treatment.

Since incubation period has been considered as an indirect marker of dose in other studies, the analyses were repeated using incubation period as the exposure variable.

Means or geometric means of the continuous variables in the different exposure groups were compared, and linear regression for trends with increasing dose or decreasing incubation period were carried out, adjusting for confounding using multiple linear regression. For the binary outcome variables, associations were assessed using χ^2 trend (with continuity correction), adjusting for confounding using stratification and multiple logistic regression.

RESULTS

Information was available on 278 volunteers who received between 10^3 and 10^9 organisms. Ages ranged from 20 to 54 years (median 28), 116 were black and 153 white (unrecorded for 9); 102 had been in the military (unknown for 70). Military service had finished a mean of 12 (S.D. 5·9) years previously, where known. All those challenged after 1965 received 10^5 organisms, compared to only 12 earlier.

Attack rate

Attack rates using the different definitions of illness are shown in Table 1. Outpatients with insufficient charts to know whether they fulfilled the criteria, were counted as not ill. Attack rates increased with dose, independent of age, race and military service. Further analysis of attack rates used definition (I). Attack rates were higher among those under 30 than those 30 years and over (82/145 compared to 37/117, RR 1·79, 95% CI 1·32–2·42); among those without military service than those with (57/105 compared to 30/96, RR 1·74, 95% CI 1.23–2·45); and among blacks than whites (59/114 compared to 56/142, RR 1·31, 95% CI 1·00–1·72), and these associations were independent of each other.

The attack rates for those who received 10^5 organisms were similar among inpatients, outpatients seen twice a day and outpatients seen once a day. For the subsequent results only patients meeting the definitions of illness are considered.

Incubation period

The incubation period was taken as the time to the first temperature of at least 100 °F (37·8 °C). The incubation period was log normally distributed and was inversely related to dose (Table 2), and the results were similar using definition (II). The correlation persisted when outpatients were excluded and was similar if

Table 1. *Attack rates (%) by challenge dose using different definitions of illness among volunteers after oral challenge with* S. typhi, *Quailes strain*

Dose (no. of organisms)	Definition (I): temperature ⩾ 100 °F (37·8 °C) for 12 h + peak ⩾ 101 °F (38·3 °C)	Definition (II): antibiotics given	Definition (III): temperature ⩾ 101 °F (38·3 °C) for 12 h	Definition (IV): temperature spiking over 103 °F (39·4 °C) for 36 h
10^3	0/13 (0·0)	0/13 (0·0)	0/13 (0·0)	0/13 (0·0)
10^5	83/200 (41·5)	72/204 (35·3)	77/200 (38·5)	52/200 (26·0)
10^7	13/27 (48·1)	13/27 (48·1)	13/27 (48·1)	8/27 (29·6)
10^8–10^9	24/25 (96·0)	30/34 (88·2)	24/25 (96·0)	18/25 (72·0)

Table 2. *Outcome by challenge dose using definition (I), i.e. temperature ⩾ 100 °F (37·8 °C) for 12 h and peak ⩾ 101 °F (38·3 °C), among volunteers after oral challenge with* S. typhi, *Quailes strain. Incubation period and measures of severity are given only for those volunteers fulfilling the definition of illness*

Dose (no. of organisms)	No. ill	Geometric mean incubation period in days (95% CI)	Mean peak temperature reached (°F) (95% CI)	Geometric mean time above 103 °F (39·4 °C), i.e. no. of 4-h periods + 1 (95% CI)	Mean symptom score (95% CI)
10^5	83	9·3 (8·4–10·4)	103·8 (103·6–104·0)	3·2 (2·5–4·0)	7·7 (7·2–8·2)
10^7	13	7·4 (4·9–11·2)	103·6 (103·1–104·1)	2·6 (1·4–5·0)	8·9 (7·8–10·0)
10^8–10^9	24	4·7 (4·1–5·4)	104·4 (104·1–104·7)	4·8 (3·4–6·7)	9·2 (8·3–10·0)

the first temperature of 101 °F (38·3 °C) was used rather than 100 °F (37·8 °C). There were no associations between age, race or previous military service and the incubation period, and they did not confound the relationship with dose.

Severity of illness

The peak temperature and symptom score were approximately normally distributed. The duration of temperatures over 103 °F (39·4 °C) approximated to a log normal distribution. One hundred and twenty cases fulfilled definition (I). With this definition, the peak temperature was positively correlated with log dose (Tables 2, 3). When outpatients were excluded (leaving only 12 patients in the 10^5 dose group, 49 in all) the correlation was stronger: $r = 0{\cdot}44$ (0·18–0·64), $b = 0{\cdot}28$ (0·16–0·45). The regression coefficient was hardly altered by adjusting for age, race or military service, and increased after adjusting for year.

One hundred and fifteen cases fulfilled definition (II) including six patients who only had rectal temperatures recorded who all received 10^9 organisms. Using this definition, there was no correlation between log dose and peak temperature. including or excluding those with rectal temperatures, with or without adjusting for the confounders: $r = 0{\cdot}08$ ($-0{\cdot}10$ to 0·26).

Under definition (I), the duration of fever above 103 °F (39·4 °C) was longer in the highest dose group but the correlation (with log duration) was weak (Tables 2, 3). After excluding outpatients the trend was stronger: $r = 0{\cdot}31$ (0·03–0·55). Under definition (II) no correlation between log dose and log duration of fever

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Table 3. *Correlations between the three main outcomes and log dose among ill volunteers after oral challenge with* S. typhi, *Quailes strain under three different definitions of illness*

Outcome	Definition (I): temperature ⩾ 100 °F (37·8 °C) for 12 h + peak ⩾ 101 °F (38·3 °C) (n = 120)	Definition (III): temperature ⩾ 101 °F (38·3 °C) for 12 h (n = 112)	Definition (IV): temperature spiking over 103 °F (39·4 °C) for 36 h (n = 78)
Peak temperature			
r (95% CI)	0·22 (0·04–0·39)	0·18 (0·00–0·35)	0·17 (−0·05–0·38)
b (95% CI)	0·13 (0·03–0·24)	0·09 (0·00–0·19)	0·07 (−0·02–0·16)
Log time over 103 °F (39·4 °C)*			
r (95% CI)	0·13 (−0·05–0·31)	0·09 (−0·09–0·28)	0·03 (−0·20–0·25)
b (95% CI)	0·04 (−0·01–0·09)	0·03 (−0·03–0·08)	0·01 (−0·05–0·06)
Symptom score			
r (95% CI)	0·27 (0·09–0·43)	0·24 (0·06–0·41)	0·22 (0·00–0·42)
b (95% CI)	0·39 (0·14–0·64)	0·33 (0·08–0·57)	0·26 (0·00–0·52)
b' (95% CI)†	0·27 (−0·12–0·65)	0·09 (−0·29–0·46)	−0·12 (−0·56–0·32)

* I.e. log (no. of 4-h periods when temperature ⩾ 103 °F (39·4 °C) continuously + 1).
† Partial regression coefficient, adjusted for year of experiment.

over 103 °F (39·4 °C) was seen: $r = 0{\cdot}06$ (−0·13 to 0·25). There was no evidence of confounding in any of these associations.

Under both definitions the symptom score increased with dose (Tables 2, 3), but the association was lost after adjusting for year, and when the outpatients were excluded. There was no confounding by age, race or military service.

Eighteen individual symptoms and signs were considered (anorexia, nausea, vomiting, abdominal pain, diarrhoea, constipation, hepatomegaly, splenomegaly, jaundice, cough, sore throat, headache, sweats, malaise, rigors, photophobia, rose spots, and lymphadenopathy). Only lymphadenopathy showed a significant increase with dose under either definition, but the proportion was highest in the middle dose group and the association was lost after adjusting for year in a logistic regression analysis. The results were little altered by stratifying by age group, race or military service, or by excluding outpatients.

Relapse rates were similar in all dose groups. Under definition (I) treatment was given to 87% of the 10^5 group, 92% of the 10^7 group and 100% of the 10^8–10^9 group. This trend was more marked after excluding the outpatients and after adjusting for year. There was no other evidence of confounding in these relationships, and adjusting for drug type had no influence on the results.

There were no associations between age, race, previous military service, or time since service and any of the major outcomes: peak temperature, duration of temperature over 103 °F (39·4 °C) or symptom score. Using definition (I), the year of the experiment was weakly positively associated with peak temperature, after adjusting for dose. There was no correlation with duration of high temperatures. Among those who became ill after 10^5 organisms, the mean symptom scores showed no significant differences or trends between the different years or studies. Among all those who were ill, an inverse correlation between year and symptom score was lost after adjusting for dose. The signs (hepatomegaly, splenomegaly,

Table 4. *Outcome by incubation period using definition (I), i.e. temperature ≥ 100 °F (37·8 °C) for 12 h and peak ≥ 101 °F (38·3 °C), among volunteers after oral challenge with* S. typhi, *Quailes strain*

Incubation period (days)	*n*	Mean peak temperature reached (°F) (95% CI)	Geometric mean time above 103 °F (39·4 °C), i.e. no. of 4-h periods + 1 (95% CI)	Mean symptom score (95% CI)	Antibiotics given (%)*	Relapse (%)†
< 7	41	104·2 (103·9–104·4)	4·2 (3·2–5·5)	9·0 (8·4–9·6)	40 (97·6)	13 (32·5)‡
7–9	47	103·9 (103·6–104·2)	3·2 (2·3–4·5)	8·2 (7·5–8·9)	43 (91·5)	10 (21·3)
≥ 10	31	103·6 (103·3–103·9)	2·6 (1·8–3·8)	6·9 (6·2–7·7)	24 (77·4)	2 (6·5)

* χ^2 trend = 6·5, $P = 0·01$.
† χ^2 trend = 6·2, $P = 0·01$.
‡ Information missing for one patient.

jaundice, lymphadenopathy and rose spots) were considered separately as a more sensitive measure of how thoroughly the patients were assessed over time. The proportion of patients with at least one sign decreased with the year of experiment, even after allowing for dose.

Table 3 shows the associations of log dose with each of the three main measures of severity using the definitions based on temperature. As the criteria became more stringent the associations between dose and severity were lost.

The analyses were repeated using incubation period as an indirect measure of dose. Approximate tertiles were used to form groups for analysis, but since nearly 40% of subjects had incubation periods between 7 and 9 days the groups were unequal. For regression analyses log incubation period was used. Using definition (I), longer incubation periods were associated with lower peak temperatures ($r = -0·22$, $-0·38$ to $-0·04$), less time with fever over 103 °F (39·4 °C) ($r = -0·16$, $-0·33$ to $0·02$) and fewer symptoms ($r = -0·31$, $-0·47$ to $-0·14$). Those with long incubation periods were less likely to receive treatment and to relapse (Table 4). These associations were little changed by adjusting for age, race, military service or year. Among the individual symptoms, constipation, sore throat and malaise were more common in those with short incubation periods, in the crude analysis and after adjusting for confounders. Abdominal pain and lymphadenopathy were also associated with shorter incubation periods, but these associations were lost after adjusting for the year of the challenge. Using stricter definitions of illness the associations with the three main outcome measures, peak temperature, duration of temperatures over 103 °F (39·4 °C), and symptom score, were lost (although only after adjusting for year in the association with symptom score).

DISCUSSION

The relationships between dose, attack rate and incubation period were confirmed in this analysis. No evidence of an association was found between dose and severity using stringent definitions of illness, as before [2]. However, when the criteria were relaxed, some association between dose and severity was noted.

At high doses almost all patients fulfil a strict definition of disease, leaving few who could be misclassified. At lower doses fewer patients fulfil the strict criteria leaving many who might genuinely have mild disease or who might be misclassified. Misclassification of non-typhoid as typhoid will give a greater proportion of less severe disease at low doses, and may produce a spurious dose–severity effect. Misclassification of mild typhoid as not typhoid will bias the results in the other direction, and a true dose–severity effect may be lost.

Table 3 shows that the biases are acting in the direction predicted. Only the correlation with number of symptoms persists in all definitions, but could be due to changes in assessing patients over time. Year has been treated as a confounder, but because it is so closely correlated with dose it is difficult to disentangle the two effects. There was little correlation between dose and individual symptoms; symptoms which might in themselves indicate severe disease, photophobia, hepatomegaly and splenomegaly, were no more frequent after higher doses.

The correlation with peak temperature was less robust under different illness definitions. The duration of temperatures over 103 °F (39·4 °C) was only weakly correlated with dose, but this might be expected since patients were treated after prolonged high fever. Relapse rates were similar at all doses.

It might be expected that incubation period should be more closely correlated with severity than is dose, since variations in individual susceptibility could confound the relationship. However, using incubation period as the exposure, the results were very similar to those obtained using dose. This suggests that, for typhoid, any influence of individual susceptibility on the incubation period is not also manifest in an effect on severity of disease. Much stronger correlations between incubation period and disease severity have been found for food-poisoning salmonellae [6].

The changes in results seen between the definitions depend on the classification of only a few patients. Twelve patients included in definition (I) were not treated. Of these, nine had peak temperatures of at least 102 °F (38·9 °C). The three others had symptom scores of 2, 5 and 6. The patient with the lowest score had a temperature over 100 °F (37·8 °C) for more than 24 h. It seems likely that the majority if not all of these patients had typhoid. In fact some typhoid cases may be missed even by definition (I). One treated case did not fulfil the criteria, and nine additional patients had peak temperatures of at least 101 °F (38·3 °C).

The first definition seems to provide a reasonable compromise between sensitivity and specificity, suggesting a weak correlation between dose and severity. By concentrating on disease definition, this analysis illustrates the difficulties of separating the known influence of dose on attack rate from the unknown influence of dose on severity of disease. The relationship between severity and inoculum size needs to be interpreted with caution. In natural outbreaks the chances of misclassification of disease are much larger.

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