Intra-specimen and day-to-day variations of *Fasciola* egg counts in human stools

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التباين في نفس العينة وبين يوم وآخر في أعداد بيض الدودة المتورّقة في براز الإنسان

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الخلاصة: تم استقصاء التباين بين يوم وآخر والتباين في نفس العينة في أعداد بيض الدودة المتورّقة *Fasciola* في براز الإنسان في ست عشرة حالة تأكدت إصابتها بداء المتورّقات، وذلك بأن تؤخذ من كل حالة عينة واحدة من الـبراز، وتُقسّم على ست من الشرائح المستخدَمة في اختبار كاتو، وتفحص كل يوم لمدة خمسة أيام متتالية. وقد دلّت نتائج الاختبار على وجود تباين ملحوظ في نتائج فحص نفس العينة في أكثر من ثلث العينات، بينما يكاد التباين لا يذكر بين عينة وأخرى. تتراوح دقة اختبار كاتو – كاتز في تشخيص الإصابة بداء المتورّقات باستخدام ثلاثة من الشرائح المستخدَمة في اختبار كاتو من نفس العينة أو بفحصها على عدة أيام بين 96.0٪ و99.1٪. إن ما يتيسر إجراؤه في الدراسات الميدانية من فحص ثلاث لطاخات من نفس عينة البراز، يعطي تشخيصاً دقيقاً لداء المتورّقات. وباتّباع هذه الطريقة تُعدُّ طريقة كاتو – كاتز ذات حساسية عالية في تشخيص داء المتورّقات.

ABSTRACT Intra-specimen and day-to-day variations of *Fasciola* egg counts in stools were investigated for 16 cases of established fascioliasis. For each case six Kato slides from a single stool sample were examined daily for 5 consecutive days. The results indicated the presence of significant intra-specimen variations in more than one-third of the examined series, while the inter-specimen variation was almost negligible. The sensitivity of the Kato–Katz test for diagnosing *Fasciola* infection with three Kato slides from the same specimen or on different days ranged from 96.0%–99.1%. The examination of three Kato smears from a single stool specimen, which is more feasible in field studies, would give an accurate diagnosis of fascioliasis. Used as such, the Kato–Katz technique is highly sensitive in the diagnosis of fascioliasis.

Variations intra-échantillon et journalière du nombre d'œufs de *Fasciola* dans les selles humaines
RESUME Les variations intra-échantillon et journalière du nombre d'œufs de *Fasciola* dans les selles humaines ont été examinées pour 16 cas de fasciolase établie. Pour chaque cas, six lames réalisées à partir d'un seul échantillon de selles selon la technique de Kato ont été examinées quotidiennement pendant cinq jours consécutifs. Les résultats indiquaient la présence de variations intra-échantillon significatives dans plus d'un tiers des séries examinées, tandis que la variation inter-échantillon était quasiment négligeable. La sensibilité du test basé sur la technique de Kato-Katz pour le diagnostic de l'infection à *Fasciola* avec trois lames réalisées à partir du même échantillon ou à différents jours se situait entre 96,0 % et 99,1 %. L'examen des trois frottis réalisés selon la technique de Kato à partir d'un seul échantillon de selles, qui est plus praticable dans les études sur le terrain, permettrait un diagnostic précis de la fasciolase. Utilisée en tant que telle, la technique de Kato-Katz est très sensible pour le diagnostic de la fasciolase.

Introduction

Studying the variability of egg excretion patterns is essential for the diagnosis of individual cases of parasite infection. At the community level, it is also necessary for formulating an epidemiological model that can maximize the efficacy and cost-effectiveness of control measures. In humans, the egg output and dynamics of egg excretion of the liver fluke (*Fasciola hepatica*) are unknown [1].

The rapid, low cost and reproducible cellophane thick-smear technique (Kato–Katz) [2] has been used for diagnosis in most studies on fascioliasis [3–5], but opinions differ about its sensitivity [6]. This method is considered the best for the diagnosis of intestinal schistosomiasis [7,8]; however, the accuracy of diagnosis and of individual intensity measurements may be influenced by intra- and inter-specimen egg count variability [9].

In the current study, intra and inter-specimen variability of *Fasciola* egg counts was investigated with the Kato–Katz technique. The sensitivity of the technique in the diagnosis of human fascioliasis was also established.

Methods

The Ethical Review Committee at the Medical Research Institute of the University of Alexandria approved the study. Adults volunteered for the study and for children consent was obtained from their parents.

Patients presenting to the Parasitology Department with established fascioliasis were enrolled in the study. Fascioliasis was diagnosed after one or more preliminary stool examinations undertaken routinely by the technical staff. For the study, patients were asked to provide a stool sample daily for 5 consecutive days, after which treat-

ment was initiated. Any patient who did not supply specimens regularly was excluded from analysis: 16 patients were included in the final analysis.

Each day 6 Kato slides from a standard template holding 41.7 mg of stool were prepared from 6 randomly chosen sites in each specimen. To exclude inter-observer variation bias, the slide series were examined microscopically by the same observer. Meanwhile, to minimize personal bias, all slide series were read blind without knowledge of the results of the other corresponding series. *Fasciola* eggs, if present, were counted and the number of eggs per gram of stool were calculated.

Intra-specimen variability was studied with the 6 slides prepared from each daily specimen. Assuming homogeneity of *Fasciola* eggs in stools, intra-specimen variability would correspond to a Poisson distribution, where the variance is smaller or equal to the mean. The dispersion index (D_i = variance/mean) should therefore be smaller or equal to 1.0. Variance exceeding the mean would indicate a heterogeneous distribution with aggregation of eggs.

The mean egg count for each day was calculated and the total mean over the 5 days was determined. The dispersion index of the mean egg counts on different days was then calculated for each case [10].

As all cases were known positives, the probability of detecting a positive case (sensitivity), by one or more than one slide examination, was calculated as:

Sensitivity (A) = no. of positive slides/ all examined slides, where A is the sensitivity of one slide examined.

To adjust for the possible impact of day-to-day fluctuation in egg counts on the probability to detect positive cases, the sensitivity of one or more than one slide examination in each specific day was calculated:

Sensitivity (A_d) = no. of positive slides

on a specific day/all slides examined that day.

To calculate the overall probability of diagnosis using one or more slide examination, the addition rule for the occurrence of either two or more events not mutually exclusive was adopted [10].

Results

Figure 1 shows the mean egg count per slide for the 6 slides examined for every patient. Figure 2 shows the dispersion index for each of the 80 series of egg counts. In more than one-third (36%) of the series,
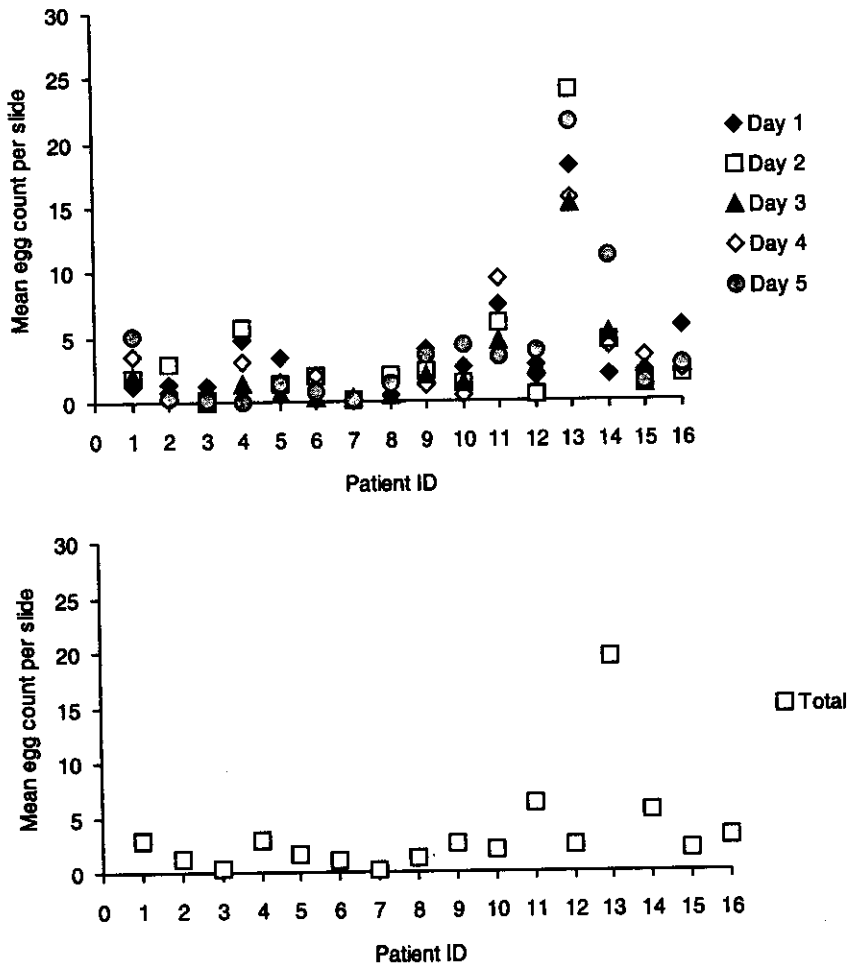

Figure 1 **Mean egg count per slide for 16** *Fasciola* **patients on each of 5 days (top) and total days combined (bottom)**
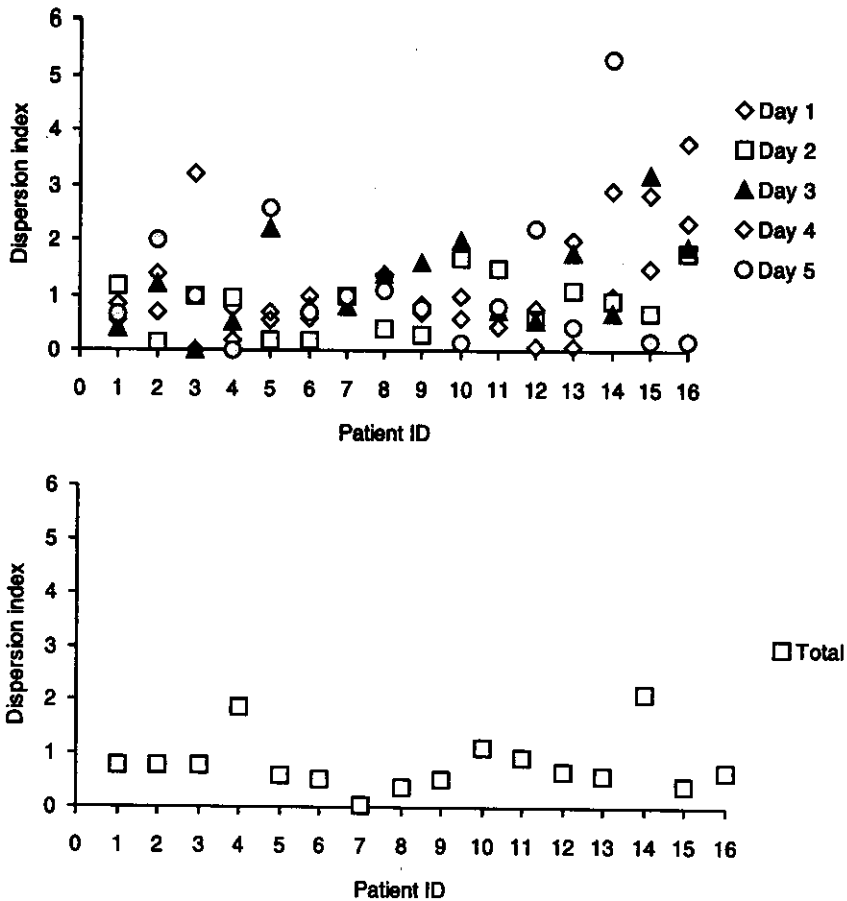
Figure 2 Dispersion index for egg counts in 16 *Fasciola* patients on each of 5 days (top) and total days combined (bottom). Shaded area shows counts with dispersion index less than 1

the dispersion index was greater than 1.0, indicating important intra-specimen variability.

The total mean egg count per specimen was also calculated for the 5 days, together with the total dispersion index for each patient (Figures 1 and 2). Only 3 of the 16 patients had a total dispersion index greater than 1.0. Accordingly, the day-to-day fluctuation in egg counts could be considered relatively negligible.

The probability of a positive diagnosis in relation to the number of slides examined was calculated (Tables 1 and 2). The sensitivity for one slide examination varied for different days from 65.6% to 79.2%. The overall sensitivity of one slide examination in diagnosis of fascioliasis was 73.6%. The sensitivity ranged from 88.2% to 95.7% when examining 2 Kato slides from a single stool sample. It reached 96.0% to 99.1% with 3 slides were examined (Table 1).

Table 1 Sensitivity of the Kato–Katz technique for detecting positive *Fasciola* cases according to the number of stool sample slides examined

No. of slides examined	Sensitivity (%)				
	Day 1	Day 2	Day 3	Day 4	Day 5
1	79.2	78.1	65.6	74.0	71.9
2	95.7	95.2	88.2	93.2	92.1
3	99.1	99.0	96.0	98.2	97.8

These figures were comparable with the diagnostic sensitivity of 1 slide examination per day done for 2 or 3 consecutive days (95.5% and 98.5%, respectively; Table 2).

Discussion

In the present work, the variability of *Fasciola* egg counts in the smears of five stool specimens taken on consecutive days was studied. After examination of six smears from each specimen, intra-specimen variability was calculated and day-to-day fluctuations were negligible. Therefore, examination of more specimens on the same day would give results as reliable as the same number of specimens but on different days.

Table 2 Sensitivity of the Kato–Katz technique for detecting positive *Fasciola* cases according to the frequency of examining stool sample slides

Frequency of examination	Sensitivity (%)
1 day*	79.2
2 consecutive days	95.5
3 consecutive days	98.5

*Day 1 was used as the starting day.

Fasciola worms inhabit the biliary tree and their eggs reach the intestine in its upper part, i.e. the duodenum. The whole gut may be involved in the process of peristaltic mixing, yet the caecum and colon are the principal blenders of several days' food residue [11]. Martin reported that for intestinal parasites daily differences in egg concentrations in the faeces should not be great since the worms' daily total egg output for consecutive days are blended in the colon [12].

In *Schistosoma mansoni*, eggs are deposited in the lower part of the large intestine. Intra-specimen variation of egg counts is high with significant day-to-day variation. Therefore, fewer slides examined on different days would be preferable to a greater number of slides examined from the same specimen [13]. Helminth eggs introduced into the intestinal flow above the caecum would be more thoroughly mixed than those from a parasite of the caecum and colon [14].

Concerning the sensitivity of diagnosis as related to the number of slides examined, it was found that in fascioliasis a single smear had a sensitivity rate of 73.6%, whereas sensitivity reached 98.5% after examination of three slides. The consistently high sensitivity obtained from examining different slides on the same and on different days would minimize the possible bias related to patients with established fascioliasis. In schistosomiasis, a single Kato smear had a sensitivity of 70%. After examining three smears, sensitivity reached 88.5% [15].

Examination of three smears taken from a single specimen—which is more feasible at the individual level and in field studies— would give an accurate diagnosis in around 98% of cases of fascioliasis. Accordingly, the Kato–Katz technique can be considered a very reliable method. The quantitative ca-

pacity of the Kato–Katz technique to provide true estimates of prevalence remains to be tested at community level.

Acknowledgements

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Note from the Editor

We would like to inform our readers that the next issue of EMHJ (Volume 8 No. 6) will be a Special Issue on HIV/AIDS and sexually transmitted infections.