

Mitt. Österr. Ges. Tropenmed. Parasitol. 9 (1987) 121 - 128

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## Hein's Coprologic Method in the Light of New Diagnostic Possibilities

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Hein's method in searching stool specimens for parasitic ova celebrates 60 years of its existence (6). It was based on the then newly discovered property of cedar oil in clarifying dried thick smears of stool on microscopic slide. This method using cedar oil and later also other clarifying agents (2, 3) had become a basic coprologic method in microscopic diagnosis of parasitic ova. Its advantage rests in its simplicity (thick smear of stool and water spread over a slide with wooden stick and allow to dry at room temperature), economy (a drop of cedar or paraffin oil spread over a dry smear with a square of soft paper), possibility to transport the specimens and stability (slides can be kept unchanged for months even years).

With a growing number of new concentration methods in parasitologic microscopic diagnosis (5, 8, 13, 19) the Hein's method was subject to more and more criticism in respect of its relative shortcomings, namely destruction of thin-capsulated ova (7, 3) and low potential of the method for diagnosis of cysts of intestinal protozoa. The latter point was ascribed high significance, and thus, before introduction of Faust's flotation method to our routine parasitologic diagnosis (12), the cysts in our country were diagnosed only rarely or not at all (15).

Our laboratory started ovoscopic diagnosis in 1966 using Hein's method, and a year later, Faust's method was added to it as a second routine examination method, supplemented by the examination of sediment (16).

Since late 1970s we have focused our attention on parasitologic examination of foreign apprentices coming from the tropics and subtropics. Since 1981 we have been comparing the yield of positivity of Hein's and Faust's method for the wide scale of helminths and protozoa. Testing the diagnostic value of Hein's method from the new so far unpublished aspects, we have arrived at following conclusions: Hein's thick smear of stool has shown the widest diagnostic scope and in some species it has rendered also the highest positivity rate.

### **Examined persons and methods of examination**

Of the total 2581 persons of the tropics and subtropics we report on 890 with complete documentation on the results from both examination methods — Hein's thick smear (6) and Faust's concentration method with examination of supernatant and sediment (15, 16).

We divided our study into two stages — 1981-1983 and 1984-1985 — because, after having gathered some experience in the first stage of the study, in the second one we focused our attention specifically on Hein's method, even as far as the diagnosis of intestinal protozoa and morphologically changed thin-capsulated ova of some helminths were concerned.

Before examination all specimens were stored at refrigerator temperature to prevent maturing and hatching of some ova (2, 17). From both examination techniques (Hein, Faust) microphotographs were taken from selected findings. In both used methods percentages of positivity were calculated separately for both stages of the study and for particular species of intestinal parasites. Differences were tested by  $\chi^2$ -test (4, 10).

### Results

Of 890 examined persons 558 were positive for intestinal helminths (62.7%) and 209 for intestinal protozoa (23.5%) (Tab. 1).

TABLE 1  
Intestinal parasites in foreign apprentices  
(1981-1983, n = 693; 1984-1985, n = 197)

YEARS	No. OF EXAMINED	POSITIVE			
		HELMINTHS		PROTOZOA	
		No.	%	No.	%
1981-1983	693	421	60,8	151	21,8
1984-1985	197	137	69,5	58	29,4
TOTAL	890	558	62,7	209	23,5

The positive rate of intestinal helminths in the first stage of the study (Tab. 2) was in Hein's method 94.6% while in Faust's method 75% (diff. = 19.3%;  $p < 0.0005$ ). Only by Hein's method were detected 25.0%, only by Faust's method 5.4% of all the positives. The positivity rate of all the species of detected helminths was in Hein's method higher than in Faust's method except *Clonorchis sinensis* and *Hymenolepis nana*, where the results in both methods were identical or almost identical. The differences of positivity rate in favour of Hein's method were in all other species statistically significant. (The findings of *Enterobius vermicularis* in stool specimens are accidental, therefore excluded from testing.)

Positivity rate of intestinal helminths in the second stage of the study (Tab. 3) was in Hein's method 90.8% while in Faust's method 62.5% (diff. = 28.3%;  $p < 0.0005$ ). Only Hein's method detected 37.5%, only Faust's method 9.2% of all the positives. Significant differences of positivity rate in favour of Hein's method were in *Trichuris trichiura*, *Ascaris lumbricoides*, *Ancylostoma duodenale*/*Necator americanus*. The findings of the rest helminths are excluded from statistical evaluation due to generally small numbers.

TABLE 2  
Comparison of positivity rate of intestinal helminths by Hein's and Faust's method  
(1981-1983, n = 693)

SPECIES	POSITIVE								TOTAL OF POSITIVES	
	HEIN'S METHOD		FAUST'S METHOD		ONLY HEIN'S METHOD		ONLY FAUST'S METHOD			
	No.	%	No.	%	No.	%	No.	%	No.	%
Ascaris lumbricoides	211	96,3	179	81,7	40	18,3	8	3,7	219	31,6
Trichuris trichiura	179	92,7	144	74,6	49	25,4	14	7.3	193	27,8
Ancylostoma duodenale	182	95,3	140	73,3	51	26,7	9	4,7	191	27,6
Trichostrongylus species	27	100,0	20	74,1	7	26,0	0	0	27	3,9
Heterophyes heterophyes	24	96,0	18	72,0	7	18,0	1	4,0	25	3,6
Clonorchis sinensis	17	81,0	16	76,2	5	23,8	4	19,0	21	3,0
Metagonimus yokogawai	19	100,0	9	47,4	10	52,6	0	0	19	2,7
Enterobius vermicularis	7	87,5	1	12,5	7	87,5	1	12,5	8	1,2
Hymenolepis nana	4	80,0	4	80,0	1	20,0	1	20,0	5	0,7
TOTAL	670	94,6	531	75,0	177	25,0	38	5,4	708	.

The positivity rate of intestinal protozoa in the first stage of the study was in Faust's method 90.7% while in Hein's method 28.2% (diff. = 65.2%  $p < 0.0005$ ). Only in Faust's method were detected 71.8%, only in Hein's method 9.3%. Significant differences in favour of Faust's method were in all the detected species of intestinal protozoa. The positivity rate of intestinal protozoa in the second stage of the study was in Faust's method 66.3% and in Hein's method 61.3%; the difference is only 5% not reaching statistical significance. Only Faust's method detected 33.8%, only Hein's method 31.3%. The positivity rate for small cysts (*Endolimax nana*) in Hein's method was by 12% higher.

### Discussion and conclusions

In the first stage of our study (1981-1983) we based our results, namely in case of protozoan cysts, on the findings in Faust's method, and to the Hein's method as traditionally nonspecific for the cysts, we devoted far less attention. In the second stage (1984-1985) we were able to distinguish the protozoan cysts, even if morphologically altered (Fig. 2, 3) also in Hein's thick smear, though we always confronted the findings with those yielded by Faust's method. The findings were mostly corresponding, and after some experience, in 31.3% we found protozoan cysts only in Hein's method.

TABLE 3  
**Comparison of positivity rate of intestinal helminths by Hein's and Faust's method**  
 (1984-1985, n = 197)

SPECIES	POSITIVE								TOTAL OF POSITIVES	
	HEIN'S METHOD		FAUST'S METHOD		ONLY HEIN'S METHOD		ONLY FAUST'S METHOD			
	No.	%	No.	%	No.	%	No.	%	No.	%
Trichuris trichiura	99	95,2	57	54,8	47	45,2	5	4,8	104	52,8
Ascaris lumbricoides	63	95,5	46	69,7	20	20,3	3	4,5	66	33,5
Ancylostoma duodenale	52	81,3	43	67,2	21	32,8	12	18,8	64	32,5
Trichostrongylus species	10	83,3	7	58,3	5	41,7	2	16,7	12	6,1
Metagonimus yokogawai	2	100,0	1	50,0	1	50,0	0	0	2	1,0
Hymenolepis nana	0	0	1	100,0	0	0	1	100,0	1	0,5
Clonorchis sinensis	1	100,0	1	100,0	0	0	1	100,0	1	0,5
Fasciolopsis buski	1	100,0	1	100,0	0	0	0	0	1	0,5
TOTAL	228	90,8	157	62,5	94	37,5	23	9,2	251	.

After having examined a large material with massive positivity for intestinal parasites we can conclude that a skilled examiner using Hein's method can:

- a) detect small cysts of protozoa (of *Endolimax nana* size); however, their specific diagnosis requires further specific examination methods where staining is possible
- b) diagnose the cysts of *Giardi intestinalis* on the basis of their rather constant size and shape; in a thinner marginal part of the smear they appear as regular blank spots (Fig. 2)
- c) diagnose larger, round, ovoidal cysts of *Entamoeba coli*, exceeding 25 µm in size, appearing in a thinner marginal part of the smear as round blank spots with a curtain-like remains of the ruptured membrane on their circumference (Fig. 3)
- d) detect, mostly in the thinner marginal part of the smear, regular, blank, round cysts, smaller than 25 µm, which allow to assume the suspect diagnosis of *Entamoeba histolytica*. Hein's method here should be considered only a screening method and the final diagnosis requires further specific examination methods with staining and morphological classification of the structures of intact cysts.



Fig. 1: *Ancylostoma duodenale*/*Necator americanus* in Hein's method.

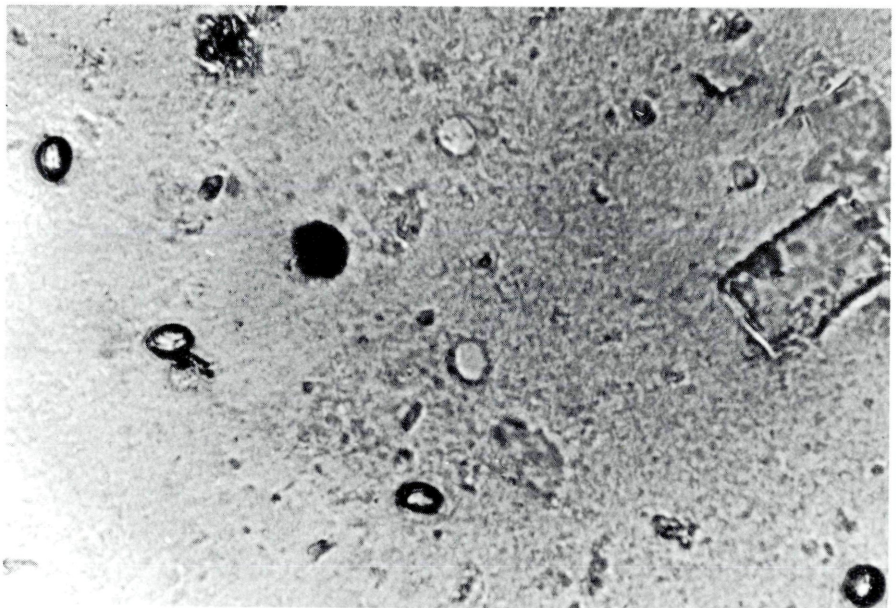


Fig. 2: *Giardia intestinalis* cysts in Hein's method.



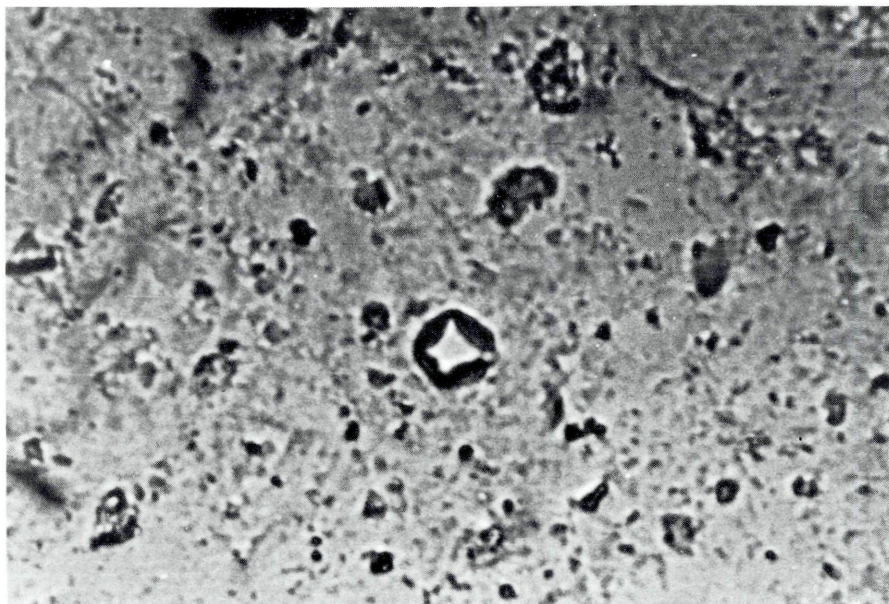


Fig. 3: *Entamoeba coli* cyst in Hein's method.

As follows from our experiences, the detail inspection of Hein's smear will take a skilled examiner 3 - 5 min., and will allow with high probability to *exclude* the presence of protozoan cysts (to conclude the finding as negative for protozoa).

Comparing the findings of the thin-capsulated helminth ova (eg. *Ancylostoma/Necator*, *Hymenolepis*) in Hein's and Faust's methods in the same positive persons, we succeeded to diagnose also these ova, despite the general opinion that Hein's method is not suitable for their diagnosis. In some parasitological atlases and monographs Hein's thick smear is even not mentioned (14, 13, 18). Thin-capsulated ova appear in this method as morphologically altered formations lacking typical inner structure with the remains of the ruptured wall (capsule) in their centre or on the margin (Fig. 1). Comparing the findings with those of Faust's method, they were always corresponding, moreover, in some cases and after some practice Hein's method yielded better diagnostic results than the Faust's method (Tab. 2, 3).

Ultimately, our study renders clear evidence that the Hein's thick smear of stool is a reliable coprologic method. Considering its simplicity, low demands for chemicals, relative stability of specimens, and, primarily — its large diagnostic scope, it deserves more attention and full justification among the existing parasitological methods also today — 60 years after its first publication.

## Summary

The authors reassessed the diagnostic yield of Hein's coprologic method in the diagnosis of intestinal parasites through the examination of 890 persons from the tropics and subtropics, comparing the result of Hein's method to those of Faust's method.

We have found that Hein's method of thick smear of stool has much broader diagnostic scope than was so far believed, involving also intestinal protozoa and hel-

minths with thin-capsulated ova, which, though morphologically altered by the technique, can be identified.

### **Key words**

Intestinal parasites, Hein's method, Faust's method.

### **Zusammenfassung**

#### **Die koprologische Untersuchungsmethode nach Hein im Licht neuer Diagnosemöglichkeiten**

Die Ausbeute der Hein- und Faust-Methoden der Parasitenbestimmung wurde durch Untersuchungen der Stühle von 890 Personen aus den Tropen und Subtropen verglichen.

Die Autoren fanden, daß die dicke Stuhlaustrichmethode von Hein eine viel umfassendere Anwendbarkeit hat. Es können neben Darmprotozoen und dickwandigen Wurmeiern auch die dünnwandigen bestimmt werden, selbst wenn sie morphologisch geschädigt sind.

### **Schlüsselwörter**

Darmparasiten, Hein-Methode, Faust-Methode.

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Jahr/Year: 1987

Band/Volume: [9](#)

Autor(en)/Author(s): Straka S., Skracikova Jela

Artikel/Article: [Hein's Coprologic Method in the Light of New Diagnostic Possibilities. 121-128](#)