

Usefulness of Kato-Katz and trichrome staining as diagnostic methods for parasitic infections in clinical laboratories

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فائدة وفاعلية طريقتي الكاتوكاتز وصبغة الترايكروم في تشخيص الطفيليات في المختبرات الطبية

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الملخص: الهدف: دراسة فائدة وفاعلية طريقتي الكاتوكاتز وصبغة الترايكروم في تشخيص الطفيليات ومقارنتها بالطرق التشخيصية المتبعة في مختبر الأحياء الدقيقة بمستشفى جامعة السلطان قابوس وإعطاء فكرة عن نسبة الإصابة بالطفيليات في شريحة صغيرة من العاملين في مجال تداول الأغذية بمنطقة مسقط. **الطريقة:** تم فحص 100 عينة من البراز جمعت من عمال الأغذية باستخدام خمس طرق تشخيصية مختلفة. **النتيجة:** وجد أن 53 من العينات تحتوي على طفيل أو أكثر من بين 11 نوعا مختلفا من الطفيليات. أوضحت الدراسة أن طريقة الكاتوكاتز وصبغة الترايكروم أكثر فاعلية في تشخيص الديدان والطفيليات الأولية بالتوالي مقارنة بطرق التشخيص الأخرى. كما أوضحت الدراسة أن صبغة الأبرومين هي الطريقة الوحيدة التي نجحت في تشخيص حويصلات طفيل الكريبتوسبورديوم بارفم. **الخلاصة:** أثبتت الدراسة أن الجمع بين طريقتي الكاتوكاتز وصبغة الترايكروم تغني عن استعمال بقية الطرق التشخيصية الأخرى كما أنه يمكن استعمال صبغة الأبرومين في حالة الإشتباه بالإصابة بطفيل الكريبتوسبورديوم.

ABSTRACT. Objectives: To assess the efficacy of the Kato-Katz technique and to re-evaluate other routine procedures conducted in the Microbiology Clinical Laboratory at Sultan Qaboos University Hospital (SQUH) and to throw light on the prevalence of intestinal parasitic infections among a small group of food handlers in Muscat. **Method:** Faecal samples collected from food handlers were examined using five parasitological techniques. **Results:** Out of 100 faecal samples, 53 were positive for one or more of 11 intestinal parasites. The Kato-Katz and trichrome stain methods were found superior to the other techniques in detecting helminthic and protozoan infections, respectively. The auramine stain was useful only in detecting *Cryptosporidium parvum* oocysts. **Conclusion:** A combination of trichrome stain and Kato-Katz techniques for stool examination is sufficient and recommended for busy laboratories; auramine stain should be applied only to samples with suspected cryptosporidial infections.

Key Words: Kato-Katz, wet preparation, concentration, trichrome and auramine stains, intestinal parasites, food handlers, Oman.

FAECAECAL-ORAL TRANSMISSION OF PATHOGENS, WHERE water, food and hands become contaminated with faecal material, and then come in contact with mouth, is the most common mode of gastrointestinal infections.¹ Studies indicate that parasitic infections affect the health of the population through malabsorption, diarrhoea, stunted growth in children and impaired work capacity.²⁻⁵ Morbidity caused by enteric parasites has always been a public health problem, especially in the tropics. The incidence and severity of intestinal parasitic infections may vary with place and time.⁶⁻⁹ Estimates of prevalence

of intestinal parasitic diseases are at best extremely rough, especially in regions where these diseases are endemic, due to poor reporting.¹⁰

The prevalence of intestinal parasites has been studied in many regions of Oman, especially in Dhofar.^{11,12} In a study conducted in 1994 among adults and children in Dhofar, 25% had parasites.¹³ Studies of schoolchildren in Muscat¹⁴ and in Nizwa¹⁵ and of patients from Musandam¹⁶ have shown percentage rates of 28, 33, and 42% respectively. Recent studies from Sur¹⁷ and Al-Hamra Wilayat¹⁸ also reported high prevalence. A survey among food handlers,

mostly Indians, revealed that 12.7% were infected with intestinal parasites.¹⁹

Although numerous methods for diagnosing parasitic infections by stool examination are available, no single technique is satisfactory as none is equally applicable for trophozoites and cysts of Protozoa, and eggs or larvae of helminths. For this reason, an optimum combination of two or more techniques of stool examination is desirable.^{6,10}

Accordingly, the objective of this study was to compare the sensitivity, reliability and practicability of the Kato-Katz technique in detecting intestinal parasites, with the stool tests routinely performed at the clinical microbiology Laboratory in SQUH, namely, the direct wet smear, concentration technique, trichrome stain and auramine-phenol staining, in order to recommend the most appropriate procedures. A subsidiary objective of this study was to throw light on the prevalence of enteric parasitic infections among a small group of food handlers in the capital city, Muscat, because of the potential of transmitting these infections to the general population.

METHODS

During January to March 2001, stool samples of 100 food handlers, mostly Indians, were collected in labelled plastic containers with covers at the Communicable Diseases Centre in Muscat. The samples were investigated in the Clinical Microbiology Laboratory, SQUH. Each sample was subjected to the following five different techniques.

1. Direct saline and iodine mount (wet preparation)

An applicator stick was used to mix about 50 mg of faeces with one or two drops of normal saline or iodine placed on a clean slide. A uniform thin suspension was made and covered with a 22 mm square cover slip. The entire film was screened systematically for the presence of helminth ova and larvae or protozoan cysts and trophozoites.

2. Formalin-ether concentration technique (FEC)

Using an applicator stick, about 1 g of faeces was placed in a clean 15 ml conical centrifuge tube containing 7 ml formalin saline. The sample was dissolved and mixed thoroughly with a vortex mixer. The resulting suspension was filtered through a sieve into a beaker and the filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. After adding 3 ml of diethyl ether to the formalized solution, the contents were centrifuged at 3,200 rpm for 3 minutes. The supernatant was poured away and the tube was replaced in its rack. Iodine stain preparation was made. The entire area under the cover slip

was systematically examined using $\times 10$ and $\times 40$ objective lenses.

3. Trichrome staining of faeces (Gomori's trichrome stain)

About 1 g of faeces was placed into sodium acetate formalin (SAF), mixed well and left overnight. The contents were centrifuged at 3,200 rpm for 10 minutes. The supernatant SAF was poured off, the deposit re-suspended and replaced with normal saline, and then centrifuged for 5 minutes. A smear was prepared using an orange stick and a small drop of Myer's albumin to bind a drop of concentrated faeces to the slide. The smear was dried for 10–15 minutes.

The prepared smear was then washed in 95% ethanol for 10 minutes and then in 70% ethanol for 3 minutes. Thereafter the smear was stained with Gomori's trichrome for 10 minutes, differentiated in 90% ethanol for 1–2 seconds, and rinsed in 100% ethanol for 1 second. It was dehydrated in two changes of ethanol, each lasting 2 minutes. The slide was cleaned twice in xylene, each time for 2 minutes, and mounted in DPX mounting medium with a large 22 \times 60 mm cover slip. The slide was left overnight before examining with the $\times 100$ oil immersion objective lens.

4. Kato-Katz technique, cellophane faecal thick smear

A small amount of faecal material was placed on newspaper or scrap paper and a piece of nylon screen was pressed on top so that some of the faeces sieved through the screen and accumulated on top. A flat-sided spatula was scraped across the upper surface of the screen to collect the sieved faeces. A template was placed on the slide and the sieved faeces was added with the spatula so that the hole in the template was completely filled. The spatula was passed over the filled template to remove excess faeces from the edge of the hole. The template was removed carefully so that a cylinder of faeces was left on the slide. The faecal material was covered with a pre-soaked cellophane strip.

The slide was inverted and the faecal sample was pressed firmly against the hydrophilic cellophane strip to spread evenly. The slide was placed on the bench with cellophane upwards to enable the evaporation of water while glycerol cleared the faeces. For all helminths, except hookworm eggs, the slide was kept for one or more hours at room temperature to clear the faecal material, prior to microscopic examination.

5. Auramine-phenol staining

The air-dried faecal smear was fixed for 3 minutes in absolute methanol and the slide was flooded with auramine for 15 minutes, washed in tap water and decolorized in 1% acid alcohol for a minimum of 3 minutes. The slide was again

washed in tap water, stained with potassium permanganate solution for 3 minutes, washed and dried. The slide was then examined under ultra/violet microscope to detect *Cryptosporidium* oocysts that fluoresce brightly against a dark red background.

RESULTS

Out of the 100 stool samples, 53% were positive for one or more species of 11 intestinal parasites (3 helminths and 8 Protozoa). The prevalence and percentages of parasites among food handlers obtained by using the five procedures are shown in Table 1. *Ascaris lumbricoides* showed the highest overall prevalence (24%), followed by *Blastocystis hominis* (17%), *Trichuris trichiura* (9%), hookworm (7%), *Giardia lamblia* and *Entamoeba coli* (6% each), *Endolimax nana* (4%), *Cryptosporidium parvum*, *Dientamoeba fragilis* and *Iodamoeba butschlii* (2%) each and *Entamoeba histolytica* (1%). Single, double and multiple (3–7 species of parasites) infections showed positive rates of 34%, 13%, and 6%, respectively.

Out of the 24 cases of ascariasis recovered by Kato-Katz during this study, direct smear and FEC recovered 9 and 15 cases, respectively. Out of 9 cases of trichiuriasis recovered by Kato-Katz, 3 and 6 cases were recovered by direct smear and FEC, respectively. Out of 7 hookworm cases recovered by each of Kato-Katz and FEC, one case was recovered by

Table 1. Number of parasites recovered from 100 faecal samples using five different techniques

Parasite	Techniques used for parasite identification					
	Total cases	WP	FEC	TRICH	AURAM	KATO
<i>A. lumbricoides</i>	24	9	15	-	-	24
<i>T. trichiura</i>	9	3	6	-	-	9
Hookworm	7	1	7	-	-	7
<i>G. lamblia</i>	6	1	2	4	-	-
<i>E. histolytica</i>	1	0	0	1	-	-
<i>E. coli</i>	6	4	4	6	-	-
<i>I. butschlii</i>	2	0	0	2	-	-
<i>B. hominis</i>	17	1	0	17	-	-
<i>D. fragilis</i>	2	1	1	2	-	-
<i>E. nana</i>	6	0	1	6	-	-
<i>C. parvum</i>	2	-	-	-	2	-

WP: Direct saline and iodine mount (wet preparation), FEC: Formalin-ether concentration technique, TRICH: Trichrome staining of faeces, AURAM: Auramine-phenol staining, KATO: Kato-Katz technique

direct smear. The two cases of *Cryptosporidium* were recovered only by auramine stain. The Kato-Katz technique and auramine stain did not recover any other protozoan species, which were all partly recovered by trichrome stain, direct smear and FEC methods.

Table 1 shows that the total number of helminthic infections was 40. All of them were detected by Kato-Katz followed by the FEC (70%) and direct smear (32.5%). Out of the 40 protozoan infections, 38 (95%) were recovered by trichrome stain, 8 (20%) by FEC and 7 (17.5%) by smear wet preparation.

DISCUSSION

Many pathogenic intestinal parasites are transmitted through the faecal-oral route. Since unhygienic preparation, storage and handling of food by infected individuals are a major cause for food-borne diseases, food handlers need to be screened before they are allowed to work in food establishments such as restaurants, hotels, food stores, factories or as helpers and cooks in private houses. Unfortunately, such screening is not simple, since no single technique of stool examination detects cysts and trophic forms of Protozoa and helminth eggs or larvae equally well. As our results indicate, a combination of two or more techniques is more likely to be effective.

Our results indicate that the Kato-Katz technique is more sensitive for detecting helminthic parasites, followed by the FEC technique and the smear wet preparation. These findings agree with those of previous studies that showed the Kato-Katz technique to be an efficient means of diagnosing intestinal schistosomiasis and intestinal helminths.²⁰ Auramine stain was useful only in the detection of *Cryptosporidium* oocysts. The trichrome stain was superior to all other procedures in the detection of cysts and trophozoites of Protozoa. Trichrome stain detected comparatively high numbers of seven different species of Protozoa while FEC and direct smear could detect only small numbers of four protozoan species [Table 1].

Trophozoites can be difficult to detect in faeces particularly when they are no longer motile and the cysts may be confused with pus cells or macrophages. The use of Gomori's trichrome stain helps to overcome these problems and greatly assists in distinguishing between pathogenic and non-pathogenic *Entamoeba*. Furthermore, some parasites such as *D. fragilis* may only be seen using a staining procedure.²⁰

This study generally revealed low prevalence for all intestinal parasitic infections, especially for ascariasis, hook worm and trichiuriasis which are considered to be the most prevalent in man, especially in developing countries.^{6,21-23}

However, the small sample inspected in this study may not represent the whole community of food handlers in Oman. It is also possible that some of our subjects had been inspected and treated in their home countries before travelling to Oman, or in Oman before they came for screening, which may account for the low infection rates.

Inspection and treatment of food handlers are necessary to reduce food borne infections and should be an integral part of public health education. We recommend that whenever possible more than one parasitological technique should be used in screening faecal samples before an individual can be considered free from intestinal infection. We also suggest that all food handlers should particularly be screened using multiple parasitological techniques and, if found infected, should be effectively treated before they are licensed, and re-examined annually.

CONCLUSION

The current findings show that the Kato-Katz and trichrome stain are superior to other stool examination techniques for detecting helminthic and protozoan infections, respectively, and the authors recommend them for the clinical laboratory. The auramine stain method should be used only when *Cryptosporidium* is suspected. The study also stresses the need to screen individuals, and particularly food handlers, using a combination of parasitological techniques before they can be considered free from enteric parasites.

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