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Feconomics®; a new and more convenient method, *the routine diagnosis* of *intestinal parasitic* infections

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Abstract Direct wet mount examination and concentration are the most commonly used methods for detecting intestinal parasites from fecal samples. Concentration methods are used when there are fewer protozoan cyst, coccidian oocyst, microsporidial spore, helminth egg, and larvae in the fecal samples. Early detection of the causative intestinal parasites plays a significant role in implementing timely and correct treatment, which relieves the patients' symptoms and also prevents recurrences. Formalin-ethyl acetate concentration (FEAC) is believed to be a gold standard method to detect most intestinal parasites. Thus, in this study, we evaluated the diagnostic value of Feconomics® [manufactured by Salubris Inc, Boston, USA. Patent application number (TR): 2010/07549] which is a simple, new, and rapid fecal concentration method for the detection of the intestinal parasites in human beings. We also compared the FEAC with Feconomics® and direct wet mount examination. A total of 918 fecal samples were collected from the patients suspected to have intestinal parasitic infection. Samples were examined with the direct wet mount, FEAC, and Feconomics® methods. Different parasite species 15.9 % (146/918) with Feconomics®, 13.3 % (122/918) with FEAC, and 9.8 % (90/918) with direct wet mount examination, Feconomics®>FEAC>direct wet mount examinations were detected. They were statistically compared considering FEAC as the gold standard for parasitological diagnosis; the sensitivity and specificity of Feconomics® were calculated as 96 and 97 %, respectively. *Blastocystis hominis* was found to be the most common parasite, followed by *Giardia lamblia* with direct wet mount examination, FEAC, and Feconomics® methods. Feconomics® proved to be better than not only FEAC in concentrating parasite egg and cyst forms as well as in maintaining characteristic morphology but it is also better in direct wet mount examination. Feconomics® eliminates the need for centrifugation by using absorbent beads that help the homogenization and concentration of the sample. Feconomics® in this study was considerably better than FEAC in detecting the trophozoites of *Giardia lamblia*. We suggest that Feconomics® be used for the routine diagnosis of intestinal parasitic infection in rural areas of developing countries due to the fact that a centrifuge is not required and it eliminates large stool particles.

Keywords Intestinal parasites · Fecal samples · Concentration techniques · Turkey

Introduction

Gastrointestinal parasitic infections are among the most important health problems throughout the world. They pose a greater threat to developing countries owing to the lower standard of hygiene and poor sanitary habits. The symptoms of gastrointestinal parasitic infections, such as severe diarrhea, abdominal pain, and malnutrition leading to severe anemia, vary depending on the type of the parasite (Markell et al. 1999; Kucik et al. 2004; Harp 2003).

Examination of direct wet mounts under light microscope is the standard technique used for detecting intestinal parasites in most of the laboratories. This technique is user-friendly and good for the detection of motile protozoon trophozoites. Motility can be very helpful in identifying species, especially in the case of flagellates. These parasites

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can not be detected via direct microscopy in the event of fewer parasites in the specimens. If cysts or structures resembling cysts are present, then, they should be examined with the iodine mount so that more details can be detected (Garcia and Bruckner 1999; Sing 1990; Ok and Yereli 1996). In addition, the concentration of fecal samples can provide a lower number detection of parasites in the specimens, which may be missed by using only a wet mount examination. There are two types of concentration method as follows: flotation and sedimentation (Faust et al. 1938; Ritchie 1948). The most commonly used procedure is the formalin-ethyl acetate concentration (FEAC) method (Ritchie 1948) because all types of helminth egg, larvae, and protozoan cyst can be observed with this method. It also provides certain advantages including less alteration to organism. In this method, the supernatant is discarded, and one drop of resultant fecal sediment is mixed with one drop of Lugol's iodine solution on a clean glass of slide, and light microscopic examination is used for the preparation (Perry et al. 1990; Mendoza et al. 2003; Katagiri and Oliveira-Sequeira 2010; Ritchie 1948; Cheesbrough 1998). FEAC requires a long time (approximately 15 min) and necessitates centrifugation. Protozoan trophozoites are not seen as they are usually destroyed during centrifugation. It has been considered to be still disadvantageous since the use of ethyl acetate may be hazardous for health to laboratory personnel (Erdman 1981; Young et al. 1979; Coplu et al. 2007).

The concentration methods are labor-intensive requiring a relatively long time and necessitate a specific device for centrifugation. A new, simple, and fast concentration method Feconomics® is an alternative for novel fecal concentration in routine laboratory practice. Feconomics® is ready-to-use and contains SAF solution (sodium acetate, acetic acid, and formaldehyde) and small-sized specially-designed absorbent beads which eliminate the need for centrifugation and floatation. The beads absorb most of the liquid of the homogenized sample leaving a concentrated sample behind. The pores of the beads are smaller than the parasites preventing them to penetrate into the beads during the absorption of the liquid. It is highly effective in the identification of intestinal parasites in fecal samples with lower counts. It takes only 5 min to make the sample ready for microscopic examination and does not necessitate centrifugation. It is less hazardous for the laboratory staff compared to routine FEAC method (Kurt et al. 2012).

Thus, the aim of the present study was to evaluate the diagnostic value of this new concentration method Feconomics® depending on absorption compared to FEAC centrifuge-based concentration commonly performed in laboratories for the detection of protozoa and helminths in fecal samples belonging to patients suspected of having intestinal parasites.

Materials and methods

Fecal samples

Nine hundred and eighteen fresh fecal samples were sent to the Parasitology Laboratory of School of Medicine, Cukurova University in Adana, Turkey, for routine parasitological examination between March 2010 and December 2013.

Direct wet mount examination

Initially, fecal specimens were examined with the direct wet mount. A double-blind procedure was administered in the fecal samples for routine parasite examination. All of the fecal samples were concentrated with both FEAC and Feconomics® methods using the same amount of feces (2 cm^3). The morphology and the appearance of recovered parasite species were determined.

FEAC

The routine FEAC was applied as described by Ritchie (1948); Young et al. (1979); and Markell et al. (1999). A total of 2 cm^3 of fecal sample was put in tube with a 10-ml amount of 10 % formalin and thoroughly mixed with the sediment. A 4-ml amount of ethyl acetate was added to the tube. The tube was stoppered, shaken in an inverted position for 30 s, and then centrifuged for 2 min at 450×g to 500×g (1,800 to 2,000 rpm). The usual four layers resulted as follows: solvent, a plug of debris, formalin, and sediment. The plug of debris was loosened by ringing with an applicator stick, and the top three layers were decanted, one drop of sediment was mixed with one drop of Lugol's iodine solution on a clean glass slide, a coverslip was placed over the slide, and this final preparation was examined using the light microscope at both×100 and×400 magnifications (Young et al. 1979).

Feconomics®

Feconomics® is manufactured by Salubris Inc, Boston, USA, and a patent-pending product patent application number: 2010/07549. Kocagoz T, who developed Feconomics®, is a consultant to Salubris Inc., R&D department. Feconomics® is comprised of a plastic cup containing SAF solution and a small plastic bag containing absorbent beads of 1 to 3 mm in diameter. For application, 2 cm^3 of fecal sample was put into the cup containing SAF solution. After the lid of cup was closed, the container was shaken manually for 10 s or vortexed to homogenize the mixture. Then, the absorbent beads were added to this mixture, and the lid was closed again. The container was shaken for 10 s to homogenize the mixture. The homogenization was kept with the beads for 3 min to allow the absorption of the excess of the solution. One drop of

the concentration was mixed with one drop of Lugol's iodine solution on a clean glass of slide, a coverslip was placed over the slide, and this final preparation was examined using the light microscope at both×100 and×400 magnifications, as in FEAC method.

At least, one fecal sample of each parasite and randomly selected 30 liquid fecal samples were stained with trichrome, and all of the samples were stained with Kinyoun's acid-fast method, respectively, to assess the visual clarity of parasites such as intestinal amoeba and coccidian parasite specimens processed by Feconomics®. Sensitivity and specificity values of Feconomics® were analyzed by taking FEAC as the gold standard in the account with binary (binomial) classification test.

Results

A total of 918 fecal samples were collected and examined. High detection rate was observed with Feconomics® 15.9 % (146/918) and 13.3 % (122/918) with FEAC and 9.8 % (90/918) with direct wet mount examination. The detected parasites were *B. hominis* (38/918), *G. lamblia* (35/918), *Entamoeba coli* (15/918), *Ascaris lumbricoides* (12/918), *Strongyloides stercoralis* (8/918), *Fasciola hepatica* (8/918), *Trichuris trichiura* (7/918), *Enterobius vermicularis* (6/918), *Taenia* spp. (6/918), *Cryptosporidium* spp. (4/918), *Entamoeba histolytica/dispar* (3/918), *Hymenolepis nana* (2/918), *Iodamoeba butschlii* (1/918), and *Isospora* spp. (1/918) with Feconomics®. Frequency of gastrointestinal parasites detected in fecal samples from 918 patients in Adana, Turkey, with the methods such as direct wet mount examination, FEAC, and Feconomics® are shown in Table 1. In addition, the comparison of microscopic images of different parasite species with Feconomics® and FEAC methods are shown in Fig. 1. There was a statistical comparison considering FEAC as the gold standard for parasitological diagnosis; the sensitivity and specificity of Feconomics® was calculated as 96 and 97 %, respectively. It was also noted that five Feconomics®-negative samples were positive with FEAC while 24 FEAC-negative samples were positive with Feconomics®. Protozoa trophozoites and cyst or coccidian oocyst were found as 10.6 % (97/918), and helminth ova or *Strongyloides stercoralis* larvae were detected as 5.3 % (49/918). Examination of the Kinyoun-stained smears revealed coccidian parasites (*Cryptosporidium* spp. and *Isospora* spp.) and *E. histolytica/dispar*, *E. coli*, *G. lamblia*, *Blastocystis hominis*, and *I. butschlii* were confirmed with trichrome staining method.

Discussion

In copro parasitological examination, in addition to a widely used direct wet mount examination, especially when the parasites' numbers are fewer, it is necessary to perform concentration methods in order to increase the probability of detecting helminth egg, larvae, and protozoa cyst (Garcia and Bruckner 1999; Coplu et al. 2007). There have been related studies highlighting the effectiveness of concentration methods to diagnose intestinal parasitic infections. Today, there are many commercially available fecal concentration

Table 1 The frequency of gastrointestinal parasites detected in fecal samples from 918 patients in Adana, Turkey, with the methods such as direct wet mount examination, FEAC, and Feconomics®

Identified parasite	Direct wet mount examination	FEAC[a]	Feconomics®[b]
Protozoa			
Blastocystis hominis	31	32	38
Giardia lamblia	30	32	35
Entamoeba coli	12	15	15
Entamoeba histolytica/dispar	1	2	3
Iodamoeba butschlii	–	–	1
Cryptosporidium spp.	1	3	4
Isospora spp.	1	1	1
Helminths			
Ascaris lumbricoides	3	8	12
Trichuris trichiura	1	4	7
Enterobius vermicularis	2	5	6
Strongyloides stercoralis	2	7	8
Fasciola hepatica	1	5	8
Hymenolepis nana	1	2	2
Taenia spp.	4	6	6
Total	90 (9.8 %)	122 (13.3 %)	146 (15.9 %)

[a] *FEAC* formalin-ethyl acetate concentration

[b] *Feconomics®* absorbent bead concentration method that eliminates the need for centrifugation

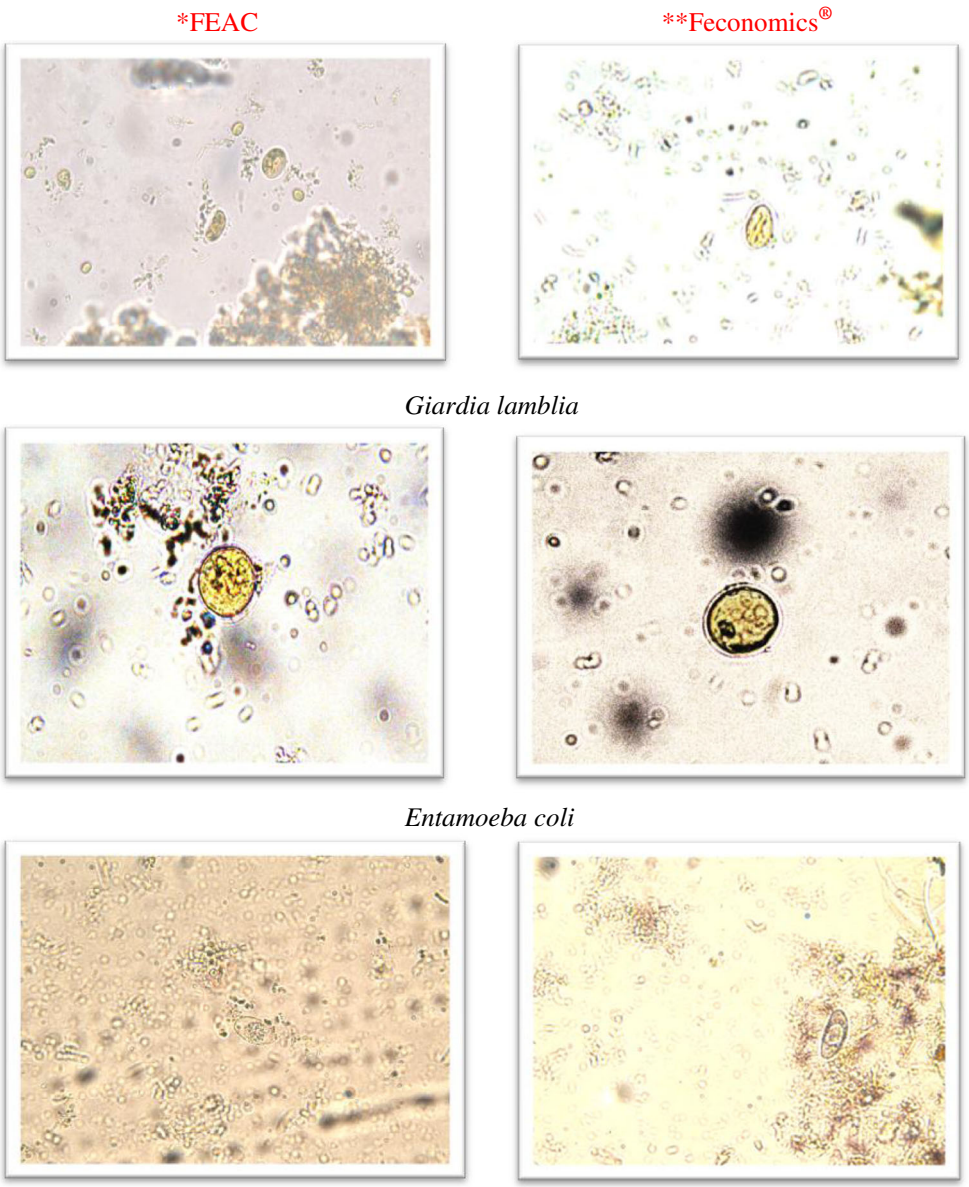

Fig. 1 Comparison of microscopic images of different parasite species with FEAC and Feconomics® methods. **FEAC* formalin-ethyl acetate concentration, ***Feconomics*® absorbent bead concentration method that eliminates the need for centrifugation

devices contributing to gaining a standardized procedure, thus improving parasite recovery (Zierdt 1984; Long et al. 1985).

In this study, being a simple, reliable, and fast concentration method, Feconomics® could increase the success of the laboratory diagnosis of intestinal parasites. Using this system, we were able to detect trophozoite and cyst of different protozoa and helminth eggs and larvae. Thus, Feconomics® is effective in improving the clarity of results in an increased accuracy of diagnosis during microscopy. Coplu et al. investigated 134 fecal specimens with different concentration methods and found that simple sedimentation techniques together with the modified $ZnSO_4$ flotation method were more useful (Coplu et al. 2007). Methanitikorn et al. found that FEAC method was successful in the detection of helminth eggs; yet, it was insufficient in detecting protozoa cysts (Methanitikorn et al. 2003). In addition, Ahmadi et al. used different concentration solutions such as gasoline to discriminate the parasites and found that 165 (35.11 %) of 470 total fecal specimens were positive with the formalin-gasoline method and 156 (33.19 %) were positive with the formalin ether concentration method (Ahmedi and Damraj 2009).

In our study, the total prevalence of intestinal parasite detected with the direct wet mount examination, FEAC, and Feconomics® techniques were 9.8, 13.3, and 15.9 %, respectively. The sensitivity and specificity of Feconomics® were found to be 96 and 97 %, respectively, with binomial

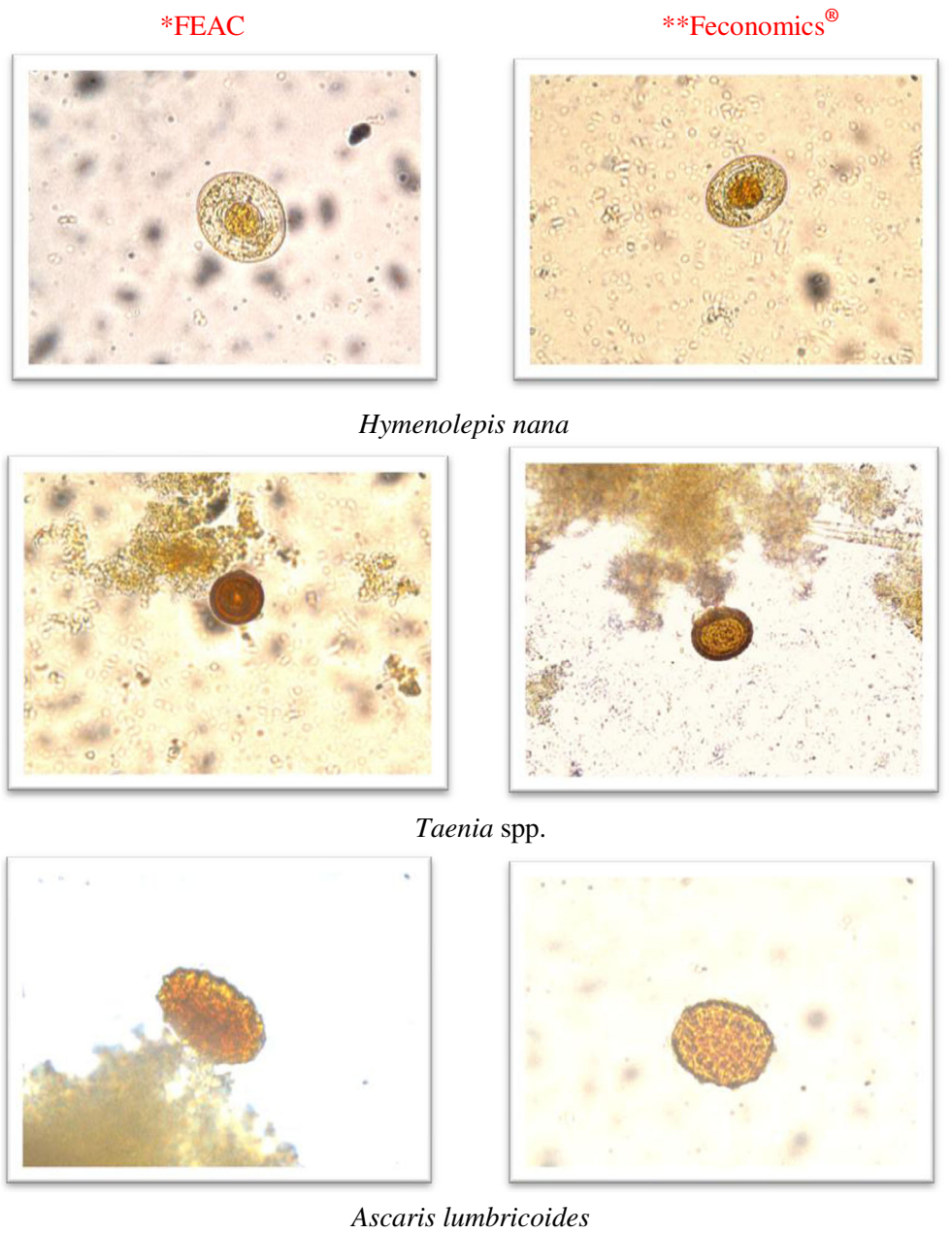

Fig. 1 (continued)

classification test. Furthermore, the direct wet mount examination exhibited the lowest performance. As shown in Table 1, *A. lumbricoides* eggs were detected with Feconomics® with more positive results (12 in column 3) compared to FEAC (8 in column 2) and direct wet mount examination (3 in column 1). Our results indicated that the concentration of stool samples with Feconomics® yielded significantly more positive results, not only *A. lumbricoides* but also other parasite species. We found no difference between both concentration methods regarding the morphological appearances of the parasites. *G. lamblia* trophozoites were well protected and preserved with Feconomics® and direct wet mount examination, but not with FEAC method. There seems to be correspondence between ours and the results reported by Kurt et al although they identified significantly more parasites than FEAC. In addition, the morphological integrity and visual appearances of the parasites were cyst or egg forms; yet, it was noticed that the vegetative forms of the parasites were only identified with Feconomics®. They also emphasized that it might be possible to conduct further molecular studies such as PCR with the fecal sample concentrated in Feconomics®, which warrants further assessments (Kurt et al. 2012).

The present study focused on the potential of Feconomics® concentration method which is proved to be easy, cost-effective, hazard-free, and reliable fecal examination for routine laboratories. In addition, Feconomics® has an advantage

for the diagnosis of fecal examination in the field because of the mesh content system in order to eliminate large fecal particles, and a centrifuge is not required.

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