Validity of Polymerase Chain Reaction (PCR) Versus Coproscopic Examination for Diagnosing Infection with *Schistosoma mansoni* in Low Intensity Endemic Area in Egypt.

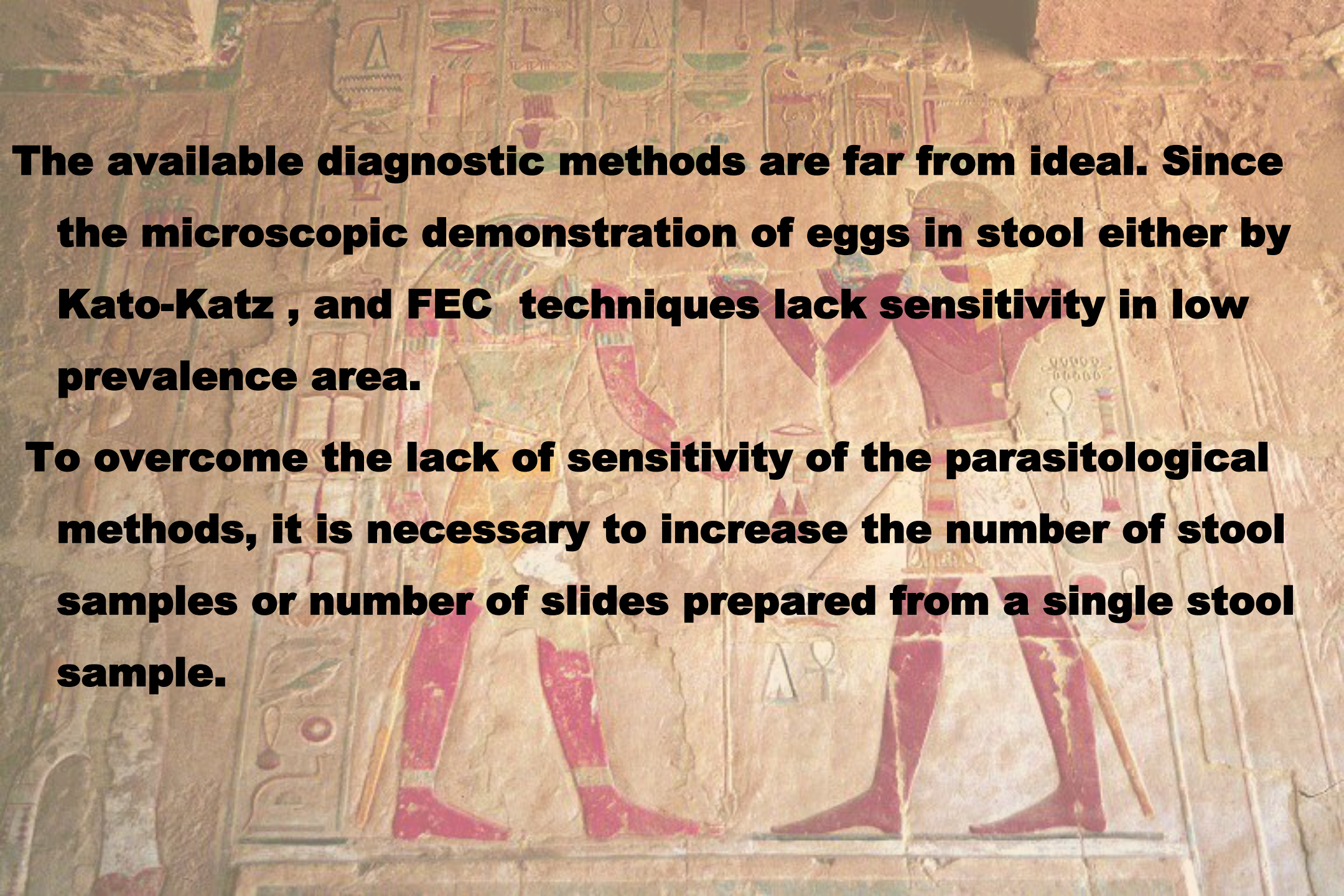
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Introduction

Schistosomiasis has been endemic in Egypt since the time of the ancient pharaohs as indicated by the presence of calcified ova in the Egyptian mummies.

Egypt is endemic country with localized transmission foci. Although control programs are ongoing, and have reduced the infection rates, re-infection is continuing problem in rural areas due to poor sanitary conditions.



The available diagnostic methods are far from ideal. Since the microscopic demonstration of eggs in stool either by Kato-Katz , and FEC techniques lack sensitivity in low prevalence area.

To overcome the lack of sensitivity of the parasitological methods, it is necessary to increase the number of stool samples or number of slides prepared from a single stool sample.



Other diagnostic alternatives include immunologic methods such as detection of parasite-specific antibodies and circulating antigens. These techniques have been shown to be more sensitive than the parasitological examination but generally lack specificity.

In an attempt to surpass these diagnostic limitation, Pontes et al. 2002 Described PCR for the detecting *S.mansoni* DNA in human faecal samples.

The amplification reaction is capable of detecting as little as 1fg of a highly repetitive *S.mansoni* DNA sequence, shows no cross-reactivity with other related helminthes and is 10 times more sensitive than Kato-Katz technique.

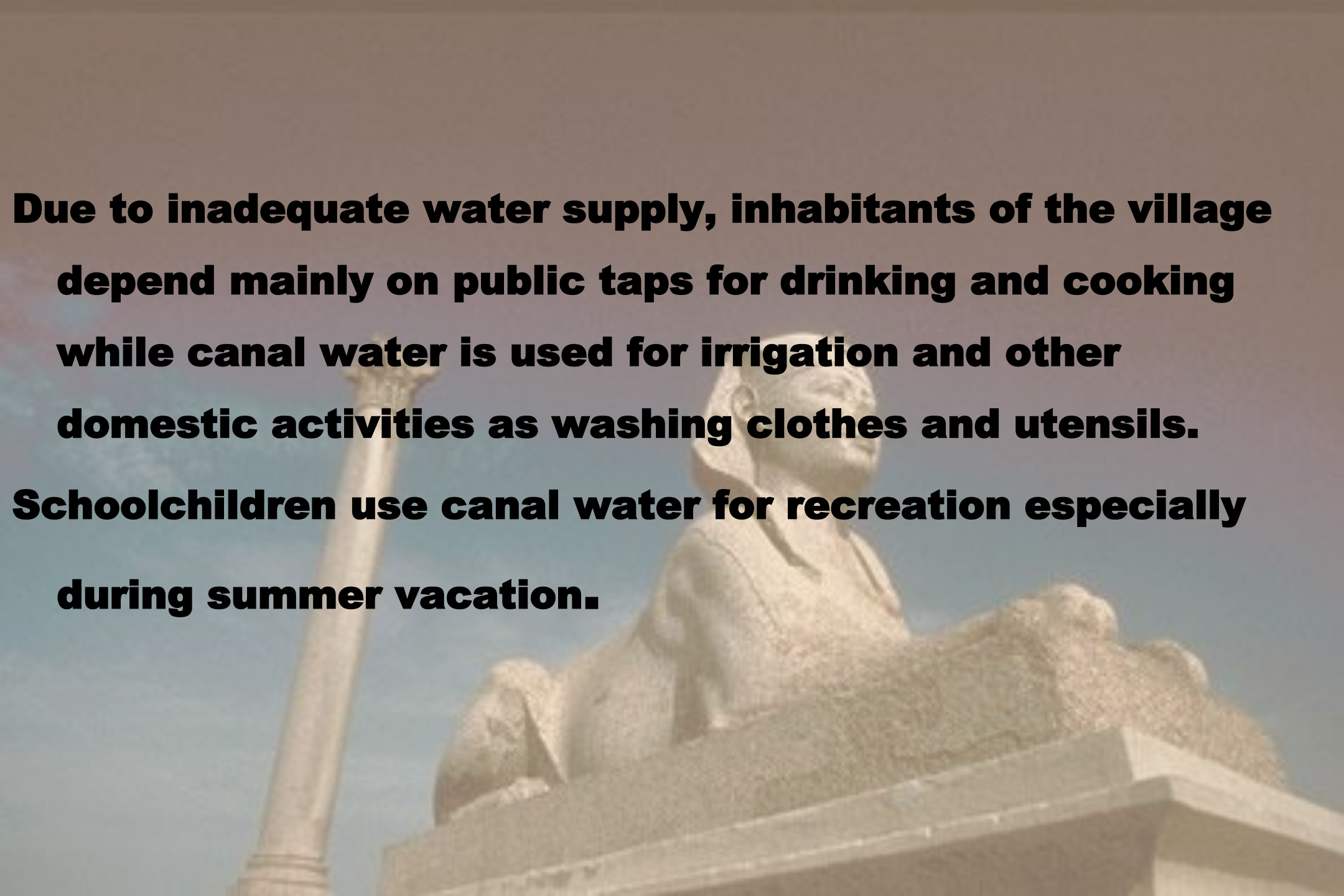
Aim of the Work

The aim of this study was to evaluate the validity of conventional PCR (cPCR) versus coproscopic examination for the diagnosis of *S.mansoni* infection in low intensity endemic area in Egypt.

Methodology

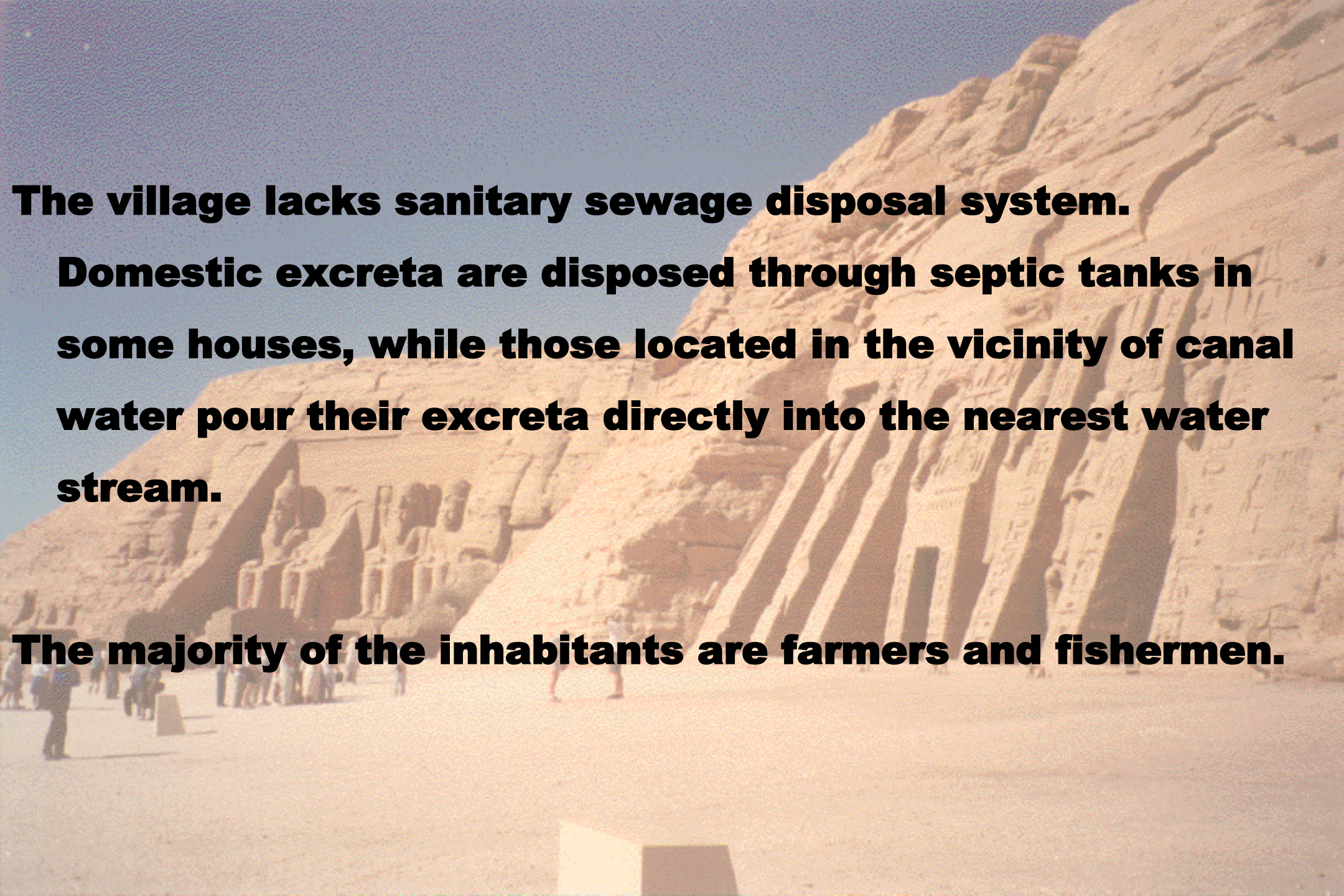
Study area

The study was carried out in Arab El- Mahdar village, Kafr El-Sheikh governorate, Egypt. The study area was selected since most of the recorded cases of *S. mansoni* infections either originated from that village or in the surrounding areas.



Due to inadequate water supply, inhabitants of the village depend mainly on public taps for drinking and cooking while canal water is used for irrigation and other domestic activities as washing clothes and utensils.

Schoolchildren use canal water for recreation especially during summer vacation.



The village lacks sanitary sewage disposal system.

Domestic excreta are disposed through septic tanks in some houses, while those located in the vicinity of canal water pour their excreta directly into the nearest water stream.

The majority of the inhabitants are farmers and fishermen.

Study population

A cross sectional approach was used. Out of 165 children, who had no history of antiparasitic drugs administration in the last month prior to screening were selected for the implementation of the study, 120 children [74 (61.7%) males and 46 (38.3%) females] with mean age 10.16±0.798 years (range: 9-12 years) were able to give three stool samples on three consecutive days with a compliance rate of 72.73%.

Stool samples collection and analysis

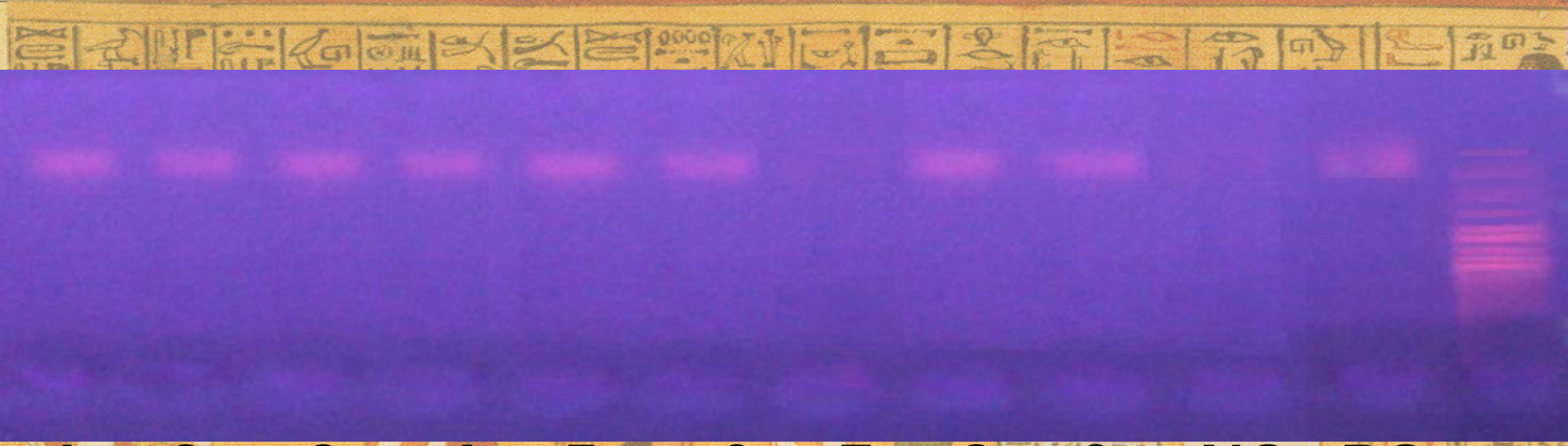
Three fresh stool samples were collected on three consecutive days from each child in the morning and examined by:

1. Kato-Katz technique (a total of 10 slides from 3 fecal samples- 6 slides on the first day of stool sample and 2 slides on 2nd and 3rd day samples respectively)
2. FEC technique (3 slides from one fecal sample of the first day).
3. One sample of the fecal specimen of the first day was examined by cPCR.

The multiple slide examination was recommended by Enk et al. 2008 to increase the sensitivity of coproscopic techniques.

DNA was extracted using Mini Kits supplied by iNtRON Biotechnology, Inc. Germany (Cat.#17451) according to the manufacturer's instructions.

The primers described by Hamburger et al. 1991 were used for the amplification of DNA sequence of *S. mansoni*. The amplified DNA & DNA ladder were separated on agarose gel electrophoresis. The bands were visualized using UV transilluminator (320nm) Fig.1



1 2 3 4 5 6 7 8 9 NC PC

Fig.1 Detection of *S. mansoni* DNA in stool via cPCR

Lanes (1-6, 8 and 9): Positive.

Lane (7): Negative

NC: Negative control PC: Positive control

Diagnostic gold standard

Since a gold-standard test is not available for the detection of parasitic infections, the combined results from the two methods, Kato-Katz (10 slides) and FEC (3 slides) techniques were used as a diagnostic gold-standard.

Results

Table 1. Distribution of *S.mansoni* infected cases according to the diagnostic techniques and GMEC (epg stool)

Diagnostic Technique	Examined sample (N= 120) Positive	Negative	% Infected	GMEC Epg stool	
FEC (3 slides)	48	72	40.0	13.35	
Kato Katz (10 slides)	83	37	69.2	33.34	
cPCR	97	23	80.8		

Table 2. Agreement between cPCR technique and gold-standard for diagnosing *S.mansoni* infection

	Diagnostic gold-standard		
	No. Negative	No. Positive	Total
cPCR			
No. Negative	23	0	23
No. Positive	11	86	97
Total	34	86	120

Cohen's Kappa Coefficient (k)=0.750 (Good agreement)

Table 3. Diagnostic parameters of cPCR versus coproscopic examination as diagnostic gold-standard

Diagnostic accuracy tests

Variable	Diagnostic efficiency %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95%CI)	NPV % (95%CI)	PLR (95%CI)	NLR (95%CI)	DA %	AUC (95%CI)
cPCR	90.8	100	67.6	88.7	100	7.82	0	88.7	0.84

PPV: Positive predictive value NPV: Negative predictive value
PLR: Likelihood ratio of positive result NPL: Likelihood ratio of negative result
DA: Discrimination ability AUC: Area under the curve

Conclusion

The present study has documented that cPCR technique is of good diagnostic performance in detecting *S. mansoni* DNA in stool samples of infected individuals with low worm burden as compared to coproscopic examination as gold-standard.

The main limitations of cPCR application are the technique necessitates the availability of expensive highly sophisticated equipment and well trained personnel. In addition to a large budget, conditions which are not available in low income communities.


Thank You