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Foldscope as a diagnostic tool for identification of parasites of domesticated animals

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ABSTRACT

The present study was carried out to evaluate the effectiveness of foldscope (portable paper microscope) attached with smartphone as a novel, valuable low cost tool for quick diagnosis of animal parasitic diseases under field condition so as to manage the infection at an early stage. To fulfil the objective of the study, specimens of ectoparasites, helminth parasites, haemoparasites, faecal and skin scrapings from animals/clinical cases were examined by foldscope and pictures were taken. All the parasitic slides were also examined by conventional light microscope for comparison purpose and to validate the results. From the results it was observed that foldscope (with magnification of 140×) can be used as a cheap, effective and reliable diagnostic tool to identify helminth and arthropod parasites with comparable efficacy to that of expensive light microscope. Faecal samples with heavy infection of parasitic eggs/ova/cysts and *Trypanosoma evansi* were detectable by foldscope with comparable efficacy with optical microscope. However, low grade gastrointestinal parasitic infections and intracellular haemoparasites were not detected using foldscope. This is the first report on evaluation of diagnostic efficacy of foldoscope to detect economically important parasitic infections of livestock and companion animals in India and its field application. Mobile phone enabled imaging and diagnostics technologies have the potential to bring revolutionary changes in the field of animal disease diagnosis.

Key words: Animal parasites, Diagnostics, Foldscope, Light microscope

Parasitic diseases pose a serious threat to animal health and are difficult to control and eradicate. All the three major types of parasites, viz. helminthes, protozoans and arthropods resulted in huge economic losses to animal owners in terms of mortality, reduced production (milk, meat, wool etc.) and increased treatment costs. The common parasites affecting domestic animals are helminths, arthropods and protozoans. Majority of the parasites are zoonotic (Krauss *et al* 2003) and in particular cystic hydatidosis, neurocysticircosis, cutaneous larvae migrans (CLM), visceral larvae migrans (VLM), cryptosporidiosis, toxoplasmosis and leishmaniosis have gained importance as human pathogens.

These parasitic infections are diagnosed usually by the conventional parasitological techniques based on optical light microscopy. In developing countries and low socio-economic regions of the nation, where diagnostic laboratories are not in ease access, the diagnosis of these parasites remain uncharted. Moreover, the cost and time incurred on the transportation and processing of the samples hampers the diagnosis and treatment.

Foldscope is a portable and handy microscope made of water proof paper, which magnifies the wonders of the microscopic world. It was developed in USA. Another stalwarts invention from the same laboratory is paperfuge; a paper centrifuge (Bhamla *et al.* 2017).

The main purpose of foldscope is to bring microscopy out of science laboratories and into the hands of people around the world. Basically, it is an optical microscope that can be assembled from a punched sheet of cardstock, a spherical glass lens, a light emitting diode and a diffuser panel, along with a watch battery that powers the LED (Mathews 2014). Foldscope is attachable with smart phone to view and capture images. The foldscope design accommodates different optical configurations, including spherical ball lenses, spherical micro-lens doublets and more complex assemblies of aspheric micro-lenses. Since magnification varies inversely with ball-lens diameter, commonly available ball lenses provide magnifications ranging from 140 to 2180× (Cybulski *et al* 2014).

Considering the economic importance of animal parasitic diseases especially under field conditions, low cost microscope may prove to be a beneficial aid. To our knowledge, it is the foremost comprehensive study to determine the efficiency of foldscope as compared to optical

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microscope for diagnosis of parasitic diseases of animals in India.

MATERIALS AND METHODS

Assembling of foldscope: The unassembled foldscopes were obtained from Department of Biotechnology, New Delhi. The foldscope used in present study could magnify images by 140×. The unfolded foldscope is comprised of three stages cut from paper; illumination, sample-mounting, and optics. Other primary components include a spherical ball lens (or other micro-lenses), lens-holder apertures, an LED with diffuser or condenser lens, a battery, and an electrical switch. The three stages are weaved together to form an assembled foldscope. The foldscope was attached with smartphone with help of magnetic couplers provided in the kit to view parasitic specimens/samples.

Standardization of foldscope on permanent mounts of the parasitic specimens: For preliminary investigations about the use of foldscope, the existing ectoparasite (ticks, lice, fleas, mosquitoes) and endoparasites (helminthes) specimens preserved in departmental laboratory were examined by foldscope and microscope simultaneously.

Application of foldscope on clinical samples: Tick specimens, faecal samples, skin scrapings and blood samples were processed by standard parasitological techniques before examination by foldscope. All the samples were also examined by optical light microscope.

Ticks: Ticks were collected from bovines (n=30) and dogs (35) in and around Ludhiana district and processed in the laboratory as per method of Soulsby (2005) and then examined by foldscope.

Faecal samples: Faecal samples (110: 35 dogs, 45 bovines, 30 small ruminants) from clinical cases of diarrhoea or suspected for gastrointestinal parasitic infections were collected in and around Ludhiana district. Samples were processed by faecal flotation and sedimentation techniques (Soulsby 2005). Paper slides with slits were used for viewing through foldscope. One side of slit was covered with cello tape. In case of sedimentation, 2–3 drops of sediment was put on slit and slit was closed using cello tape. In case of flotation, cover slip used for examination was placed on the slit. Cello tape was applied to close the slit. Alternatively parasitic eggs/ova adhered to cover slip placed on glass slide were examined after sealing with cello tape. Three paper slides were used jointly to examine by foldscope attached to smartphone.

Skin scrapings: Skin scrapings of dogs (n=25) were collected with clinical symptoms of dermatitis and alopecia from in and around Ludhiana. Skin scrapings were processed as per method of Soulsby (2005) and were examined by foldscope. Paper slides as described above were used for examination by foldscope.

Blood smears: Leishman stained thin blood smears slides (20) positive for trypanosomiasis, theileriosis, babesiosis and anaplasmosis were examined using foldscope attached to smartphone.

Comparative efficacy of foldscope and optical microscope: The results of foldscope were compared with conventional microscope considering the efficacy of optical microscope as 100%.

RESULTS AND DISCUSSION

Standardization of foldscope: This preliminary study was intended to customise foldscope as a diagnostic tool particularly for field condition and to explore its relative efficacy with conventional microscope. The foldscope was at first standardized on permanent mounts of parasite specimens and its photographs of the same are given in Fig. 1. It was deduced that foldscope with magnification of 140× useful to identify the key characteristic features of the microscopic specimens of arthropods and helminth parasites.

Application of foldscope on clinical samples: The standardised protocol of foldscope was employed on the samples (faecal, skin, and blood) collected from animals of field and clinical laboratories.

Ticks collected from bovines showed predominance of *Rhipicephalus* (*Boophilus*) *microplus* (27) followed by *Hyalomma anatolicum anatolicum* (3) and in case of dogs ticks (35) *Rhipicephalus sanguineus* was only seen (Table 1). The relative efficacy of foldscope for detection of the ticks was 100% as compared to conventional light microscope.

A total of 110 faecal samples (35 dogs, 45 bovines, 30 small ruminants) were examined using foldscope and light microscope for identification of parasitic ova/cysts. A total of 14 and 29 faecal samples were found positive by foldscope and microscope, respectively (Table 1). The positive samples include eggs of strongyle, hookworm, *Moniezia* and oocysts of coccidia. The foldscope was able to diagnose precisely the faecal samples with high grade level of gastrointestinal parasitic infections (++++)

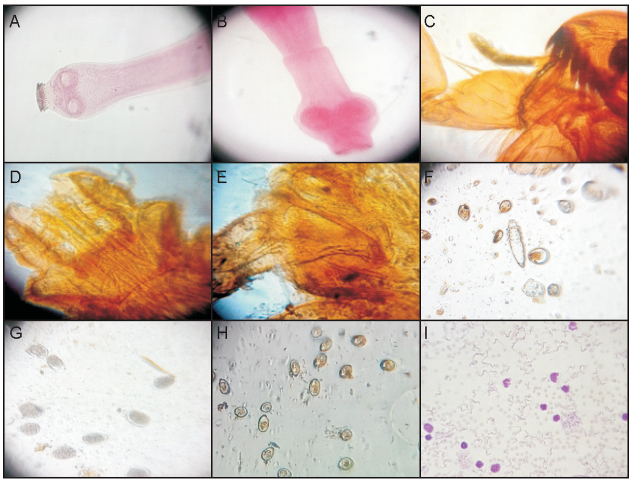

Fig. 1. Developing parasitic stages captured through foldscope (magnification 140×) attached with smart phone. A: *Dipylidium caninum*, B: *Echinococcus granulosus,* C: *Ctenocephalides canis*, D: Hexagonal basis capituli of *Rhipicephalus sanguineus*, E: Bifid coxa of *Rhipicephalus sanguineus*, F: *Demodex canis*, G: Hookworm (*Ancylostoma* spp.) eggs, H: *Coccidian oocyst* and I: *Trypanosoma evansi.*

Table 1. Comparative efficacy of foldscope and light microscope for identification of ectoparasites and endoparasites.

Parasitic sample	Species	Number of samples examined	Number of Samples positive /parasites identified by foldscope	Number of samples positive/ parasites identified by microscope	Relative efficacy of foldscope as compared to light microscope
Ticks	Bovines	30	30 (*Rhipicephalus* (*Boophilus*) *microplus*; 27, *Hyalomma anatolicum;*3)	30 (*Rhipicephalus* (*Boophilus*) *microplus*; 27, *Hyalomma anatolicum;3*)	100%
	Dog	35	35 (*Rhipicephalus sanguineus*)	35 (*Rhipicephalus sanguineus*)	100%
Skin scrapings	Dog	25	3 (*Sarcoptes* sp.;1, *Demodex* sp.;2)	5 (*Sarcoptes* sp.; 1, *Demodex* sp.;4)	60%
Blood slides	Cattle	20	1 (*Trypanosoma evansi*)	20 (*Babesia bigemina;* 2, *Theileria annulata;* 10, *Anaplasma marginale;* 7,*Trypanosoma evansi;*1)	5%
Faecal sample	Bovines	45	4* (Strongyle)	11 (Strongyle; 10, coccidian oocysts,1)	36.36%
	Small Ruminants	30	5* (Strongyle+ Coccida oocysts;4, Coccidia oocysts;1)	10 (Strongyle+ Coccida oocysts;8, Strongyle+, Moniezia;1 and coccidia oocysts;1)	50%
	Dog	35	5* (Hook worm;4, Coccidia oocysts;1)	8 (Hookworms, Coccidia oocysts,)	62.5%

*Samples with high grade infection (++++/6-8 per field of light microscope at 10×)

qualitatively with an average 6–8 eggs/oocysts per field of light microscope 10× (Soulsby 2005). The low grade infections were not diagnosed using foldscope, may be due to problem in focusing wet slides in contrast to conventional microscopy, where slides are in stationary phase. Direct examination of parasites in clinical samples especially faecal and skin scrapings showed problem in focussing of slides because these slides were not stationary. Hence these samples were examined using paper slides (as given in materials and methods). Patience and gentle handling is must for the proper examination. Richard *et al.* (2015) used a foldscope attached with smartphone for diagnosis of *Schistosoma haematobium* infection in Ghana. Typical eggs of *S. haematobium* were detected in urine samples of infected people and observed a sensitivity of 55.9% as compare to 67.6% by conventional microscopy.

Relative efficacy of foldscope for detection of helminthic ova/cysts was found to be 36.36%, 50% and 62.5% in bovines, small ruminants and dogs, respectively. From 25 skin scraping samples, mites were observed in three by foldscope and in five samples by microscope (Table 1). The relative efficacy of foldscope was evaluated to be 60% in detecting parasitic mites in skin scrapings. The detection of haemoprotozoan parasite (20) by foldscope was limited only to *Trypanosoma evansi,* however *Babesia bigemina*, *Theileria annulata*, *Anaplasma marginale* were not observed under foldscope. Comparative analysis showed that foldscope could detect only the infection. However, *Babesia bigemina, Theileria annulata* and *Anaplasma marginale* were not observed. This is due to the large size of *T. evansi* (15–34 μm) in contrast to other haemoparasites that range 0.5 μm to 5 μm and being intracellular. So the relative efficacy of foldscope in detecting haemoparasites was only 5% as compared to light microscope. Use of the foldscope lens of higher magnification (>400×) will expand the usefulness of this technology even furthermore, especially for diagnosis of haemoparasites. Cybulski *et al.* (2014) successfully examined the thin smears of *Trypanosoma cruzi, Giardia lamblia, Leishmania donovani, Dirofilaria immitis* and *Schistosoma haematobium*. Briefly, the samples were fixed in methanol and stained in freshly prepared Giemsa solution using standard protocols before imaging. High magnification bright field images of *G. lamblia*, *L. donovani*, *T. cruzi* and low-magnification bright field images of *S. haematobium* and *D. immitis* were captured using foldscope with magnifications ranging from 140× to 2,180× and none require immersion oil.

Mobile phone based microscopes (Bogoch *et al* 2013) and handheld microscopes (Bogoch *et al.* 2014) were used by researchers for the diagnosis of soil-transmitted helminthiasis. These devices have the benefit of being portable and easy to use. Other recent innovations that support inexpensive diagnostic testing in underserviced locations include low-cost filters for the diagnosis of *Schistosoma haematobium* infections (Ephraim *et al*. 2014) and paperfuge (Bhamla *et al.* 2017). The use of optical microscope in high level laboratories as a diagnostic tool for the diagnosis of parasitic diseases has been practised hitherto but due to its high cost and sophistication, it is not the tool of every hand. In contrast, the manufacturing cost of foldscope is around 50 US cents (Mathews 2014). It is proposed that foldscope will be a valuable diagnostic tool in the field areas which lack high cost technology. Disease-specific foldscope designs are an important vision for future development (Baron *et al.* 2013, Garcia 2007).

Smartphone is an advanced form of mobile phone, with efficient computing ability and user-friendly features such as digital cameras, internet access etc. Smartphones are setting their roots into the medical field as an alternative to expensive laboratory instruments for various diagnostic purposes (Kwon *et al.* 2016), especially in regions with limited resources (Tuijn *et al.* 2011). However, the use of smartphones and mobile devices in the diagnosis of parasitic diseases is still in infancy.

It can be concluded that the foldscope (magnification 140×) attached with smart phone can be utilised as a cheap and reliable diagnostic tool to identify helminth and arthropod parasites with comparable efficacy to that of expensive light microscope. However, low grade parasitic infections and intracellular haemoparasites by foldscope needs higher magnification. Mobile phone enabled imaging and diagnostic technologies have the potential to bring diagnostic testing to areas under field conditions and pen site applicability.

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