

PRELIMINARY STUDY TO USE FOLDSCOPE FOR IMAGING AND IDENTIFICATION OF RUMEN PROTOZOA

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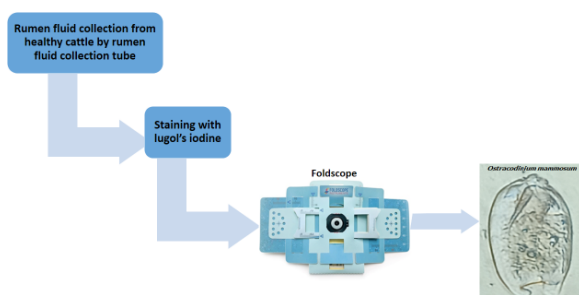
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Abstract–The present study was undertaken to use Foldscope for imaging and identification of rumen protozoa. Samples of rumen fluid were collected from healthy crossbred cows and stained with Lugol's iodine and examined using Foldscope connected to a mobile. Based on morphology the protozoa were identified. Protozoa belonging to the genera *Epidinium*, *Diplodinium*, *Enoploplastron*, *Ostracodinium*, *Eudiplodinium*, *Dasytricha*, *Blepharoconus*, *Metadinium*, *Isotricha* were identified in healthy cattle using foldscope. This is the first report of imaging and identification of rumen protozoa using Foldscope. Foldscope is a paper microscope which can be used for imaging and identification of rumen protozoa at the field level by Veterinarians as it is handy and portable.

GRAPHICAL ABSTRACT



INTRODUCTION

Manu Prakash and Jim Cybulski created the Foldscope. The creation took place in Manu's Stanford University lab while Jim was a Ph.D. candidate there. Visits to field stations where they frequently encountered heavy, damaged, or nonexistent microscopes served as the inspiration for the Foldscope.

Foldscope is made to be very portable, and strong, and to provide optical quality comparable to that of traditional research microscopes. It has a resolution of 2 μm and a magnification of up to 2000. The goal of Foldscope Instruments is to create affordable scientific equipment that will make

research more exciting. The foldscope is easily purchased from microcosmos (<https://microcosmos.foldscope.com>) and is commercially available online. It is practical and helpful for field study because the foldscope uses either natural light (during the day) or battery-operated artificial light sources (at night) to view the specimen. For more clear observation of specimens, it can be also coupled with the camera lens of mobile phones and pictures can be easily captured (Kissi and Dreesmann, 2018). Foldscope is small, light, and very helpful for field researchers to make quick observations even in remote locations (Mukunth, 2014).

A toolset of accessories, including paper slides, plastic coverslips, and other items, is included with the Foldscope (Devi *et al.*, 2019). A watch battery with an electrical switch that can be attached to both the microscope (Mathews, 2014) and the rear cover of a smartphone using magnetic strip couplings powers an LED with a diffuser or condenser lens that gives light.

Foldscope can be put together in less than a minute, and adjustments may be made along all three axes, i.e., X, Y, and Z. With a weight of roughly 8.8 g, it is trivially small enough to fit in a pocket and is suitable for on-site field studies (Cybulski *et*

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al., 2014). Additionally, the foldscope image that was shown on the smartphone screen may be watched using an HDMI cable and a TV module.

Identification of rumen protozoa in ruminants is significant for species identification, ecological population structure survey, and protozoa behavior analysis. Hence the present study has been undertaken to evaluate whether Foldscope can be used for the identification of rumen protozoa at the field level by veterinarians

MATERIALS AND METHODS

Rumen fluid samples were collected from a healthy crossbred cow using a rumen fluid collection tube in a sterile thermally insulated air-tight container. The samples were taken immediately to the lab. Under the sterile condition, the rumen fluid sample was placed on a sterile clean slide and stained with Lugol's iodine. The stained slides were examined using Foldscope connected to a mobile and photographed. Based on the morphology the protozoa were identified (Dehorty, 1993). In the present study, the following criteria were taken into consideration for the identification of protozoa. Presence and location of ciliary areas on the whole

body or a specific area of the body; Shape of the cell, Presence of skeletal plates; Number and location of the contractile vacuole.

RESULTS

Protozoa belonging to the genus *Epidinium*, *Diplodinium*, *Enoploplastron*, *Ostracodinium*, *Eudiplodinium*, *Dasytricha*, *Blepharoconus*, *Metadinium*, *Isotricha* were identified in healthy cattle using foldscope are given in Fig. 1. This is the first report of imaging rumen protozoa using foldscope.

DISCUSSION

Identification of Protozoa under field conditions is very difficult as it needs a good microscope. Foldscope, a paper microscope can be taken anywhere. This is the first report of imaging rumen protozoa using a foldscope. Lugol's iodine staining is very useful for the identification of protozoa. Iodine stains protozoan nuclei and intracytoplasmic organelles brown making them easier to identify. The images taken using a foldscope were compared with images taken with an ordinary microscope by other authors (Soichi Imai, 1998; Sanghai and

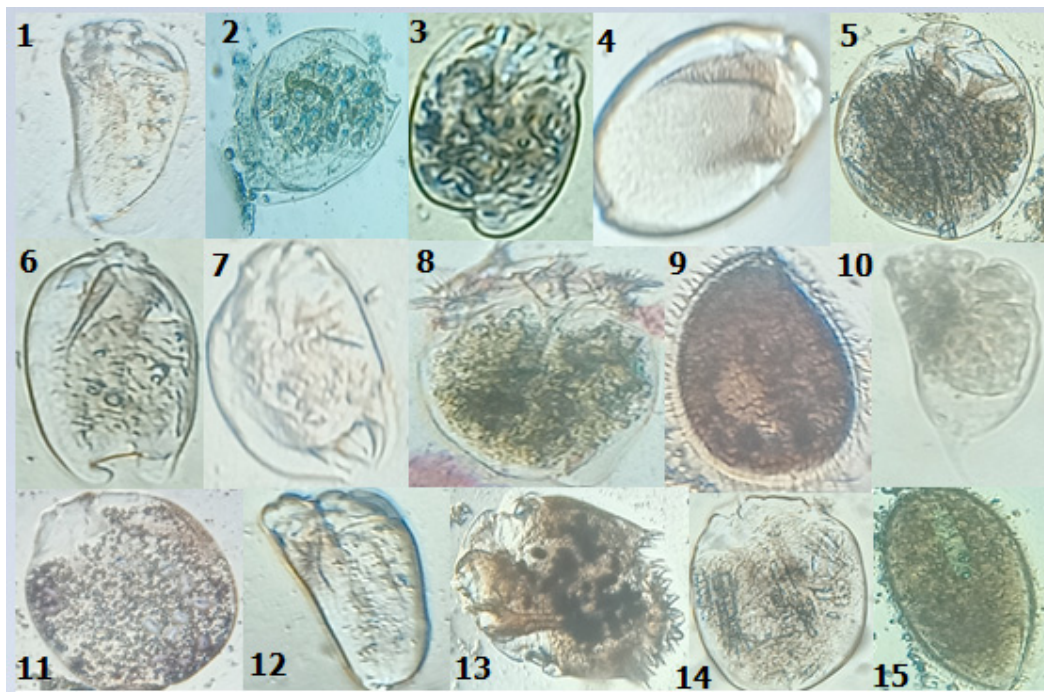


Fig. 1. Photograph of rumen protozoa taken using Foldscope connected with mobile

1. *Epidinium* 2. *Diplodinium* 3. *Entodinium yunnense* 4. *Enoploplastron* 5. *Ostracodinium nucleolobum* 6. *Ostracodinium mammosum* 7. *Diplodinium dentatum* 8. *Eudiplodinium maggii* 9. *Dasytricha* 10. *Epidinium lunatus* 11. *Blepharoconus krugerensis* 12. *Epidinium caudatum* 13. *Diplodinium flabellum* 14. *Metadinium medium* 15. *Isotricha prostoma*

Kshirsagar, 2015; Jayasree *et al.*, 2018; Svetlana Kišidayová *et al.*, 2021). The clarity and images taken using a foldscope are comparable to images taken using a light microscope. Here the advantage of using a foldscope is that it does not require any oil immersion. Images can be shared very easily online through a mobile network.

Foldscope can be used for imaging and identification of rumen protozoa at the field level by veterinarians.

Conflict of interest.

There is no conflict of interest

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REFERENCES

- Cybulski, J.S., Clements, J. and Prakash, M. 2014. Foldscope: Origami-based paper microscope. *PLoS One*. 9(6): e98781. <https://doi.org/10.1371/journal.pone.0098781>
- Dehority, B.A. 1993. *Laboratory Manual for Classification and Morphology of Rumen Ciliate Protozoa*. United Kingdom: CRC-Press. 128 pages
- Devi, N.S., Singh, T.S., Senjam, J.S., Joybi, S., Singh, T.M., Singh, L.S., Devi, M.S., Chanu, O.P., Singh, R.I. and Singh, T.J. 2019. Under the foldscope: Relatively inexpensive tool for understanding down-to-earth applications in plant sciences. *Journal of Pharmacognosy and Phytochemistry*. 8(6): 30–34.
- Jayashree Gogoi., Rajamanickam, K. and Leela, V. 2018. Research and Reviews: Journal of Veterinary Science and Technology Morphological Identification of Rumen Protozoal Population in Domestic Ruminants of Chennai. *Research and Reviews Journal of Veterinary Science and Technology*. 7: 12-15.
- Kissi, L. and Dreesmann, D. 2018. Plant visibility through mobile learning? Implementation and evaluation of an interactive flower hunt in a botanic garden. *Journal of Biological Education*. 52(4): 344–363.
- Mathews, L. 2014. Foldscope is a 50 percent paper microscope that magnifies up to 2000 times. *Geek Com*. 13.
- Mukunth, V.A. 2014. Disposable microscope for as little as \$1 (p. 13). *The Hindu*.
- Ogimoto, K. and Imai, S. 1981. *Atlas of Rumen Microbiology*. Japan Scientific Societies, Tokyo.
- Sanghai, P.K. and Kshirsagar, H.S. 2015. Two New Species of Protozoan Ciliates from the Subfamily Ophryoscolecinae, *Epidinium trilobatum* (N.Sp.) and *Ophryoscolex ecaudatum* (N.Sp.) from the Rumen of Indian Cattle *Bos Indicus*. *Cibtech Journal of Zoology*. ISSN: 2319–3883 (Online). 4 (2): 53-58.
- Soichi Imai, 1998. Phylogenetic Taxonomy of Rumen Ciliate Protozoa Based on Their Morphology and Distribution. *Journal of Applied Animal Research*. 13: 17-36
- Svetlana Kišidayová, Dominik Durkaj, Katarína Mihaliková, Zora Váradyová, Julia Puchalska, Ma³gorzata Szumacher-Strabel, Adam Cieslak and Zygmunt Gizejewski, 2021. Rumen Ciliated Protozoa of the Free-Living European Bison (*Bison bonasus*, Linnaeus). *Frontiers in Microbiology*. 12: 1-11.