

The Discovery of *Giardia*¹

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INTRODUCTION

The pear-shaped flagellate *Giardia duodenalis* is a cosmopolitan protozoan parasite of humans. It has a global distribution, and indeed is ranked by Farthing in the top ten of human parasitic diseases (1). Infection with *G. duodenalis* is normally self-limiting. Human giardiasis can be divided into two disease phases: acute and chronic. The acute phase is usually short-lived, characterised by flatulence with sometimes sulphurous belching and abdominal distension with cramps. Diarrhoea is initially frequent and watery but later becomes bulky, sometimes frothy, greasy and offensive. The gas may cause the stools to float.

In chronic giardiasis, malaise, weight loss and other features of malabsorption become prominent and stools are usually pale or yellow, frequent and of small volume. Malabsorption of vitamins and D-xylose can occur, while disaccharidase deficiencies (most commonly lactase) are frequently detected in chronic cases. In young children, 'failure to thrive' is frequently due to giardiasis, and all infants being investigated for causes of malabsorption should have a diagnosis of giardiasis excluded (2).

The parasite exists in two distinct morphological forms. The reproductive phase is a trophozoite which parasitizes the cells lining the upper small intestine. There is also a resistant, resting phase, an environmentally-resistant cyst which is voided in the faeces. This is the infective and disseminating stage. *Giardia* is the most commonly detected intestinal protozoan parasite in the world, and the prevalence of giardiasis in developing countries is approximately 20% com-

pared to about 5% in the developed world (3). Between 100,000 to 2.5 million *Giardia* infections occur annually in the United States (4).

The name of this organism has become increasingly familiar in recent years, yet it was first observed as long ago as 1681. Its discoverer was Antony van Leeuwenhoek, the pioneering microscopist of Delft, Netherlands. As Clifford Dobell (5) clearly documented, Leeuwenhoek's verbal description was accurate and unambiguous. In his watery excrement van Leeuwenhoek discovered '... small animalcules a-moving very prettily; some of 'em a bit bigger, others a bit less, than a blood globule, but all of one and the same make, ...' which Dobell in 1932 concluded were the vegetative (trophozoite) stage of the infection. But a question remained: how could Leeuwenhoek have seen such a diminutive microorganism with the single-lensed microscopes at his disposal?

It was recently proposed to me that Leeuwenhoek's observations might be reprised, and this presentation reveals how van Leeuwenhoek could have observed *G. duodenalis* some three and a quarter centuries ago.

BACKGROUND

Although Leeuwenhoek discovered *Giardia* in 1681, its pathogenicity in humans was not formally established and Koch's postulates fulfilled until less than 20 years ago (6). Currently, the progress of research into *Giardia* is marked by regular symposia that bring together research on this organism with that on *Cryptosporidium* (an organism which I discussed at Inter Micro seven years ago) (7). As keynote speaker at the International Conference on *Giardia* and *Cryptosporidium* in September 2004, I discussed Leeuwenhoek's revelation of *Giardia* (8). Although the literature on his discovery is well established, nothing had been done to investigate whether the organism could be satisfacto-

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rily observed through primitive microscopes, and this remained a crucial question. Professor Huw Smith of the Scottish Parasite Diagnostic Laboratory, Glasgow, Scotland, proposed that I should attempt to repeat the experiments from 1681, to confirm the observations reported by Leeuwenhoek. He offered to send living trophozoites for micrography. As the holder of a NESTA Fellowship (see acknowledgements) I was able to set aside time for this research project.

Antony van Leeuwenhoek (1632-1723) wrote his description of *Giardia* in 1681 as he was examining his own diarrhoeal stools under the microscope. The next description of this parasite was not until 1859 when it was observed by Vilem Dusan Lambl and described by him as *Cercomonas intestinalis*. The term *Lamblia intestinalis* was coined in 1888 by Raphael Anatole Émile Blanchard. In 1915, Charles Wardell Stiles (1867-1941) *et al* introduced the name *Giardia lamblia* to commemorate the work done by Professor A. Giard in Paris and Dr. Lambl in Prague. Today, the organisms that infect humans are variously referred to as *G. lamblia*, *G. intestinalis* or *G. duodenalis*, indicating that intense debate continues to surround this intriguing protozoan parasite.

THE ORGANISM

G. duodenalis is a binucleate flagellated protozoan with a life cycle that alternates between an actively swimming trophozoite and an infective, resistant cyst. Trophozoites measure 12 - 18 μm in length, from their broad (anterior) end to their narrow (caudal or posterior) end, up to 10 μm in breadth and 2 - 4 μm in thickness. Cysts are smaller, measuring about 10 μm x 8 μm . Cysts form as trophozoites pass through the increasingly alkaline intestinal tract. The process of encystment can be observed under the light microscope; the trophozoites cease active motility, become rounded and increasingly refractile as encystment begins. Nuclear, but not cytoplasmic, division occurs to produce the quadrinucleate, mature and infectious cyst.

As the cysts pass through the acidic pH regime of the stomach, the low pH and elevated CO_2 followed by slightly alkaline environment of the proximal small intestine induce excystation. One trophozoite emerges from each quadrinucleate cyst, which now undergoes rapid cytoplasmic, but not nuclear division, to form two binucleate trophozoites. Trophozoites attach themselves to the luminal surface of the epithelial cells (enterocytes) that line the duodenum and jejunum, then undergo further division by asexual binary fission.

The shape resembles that of a pear cut in half along its long axis (a pyriform shape). On the anterior half of the flattened ventral surface is located a distinctive concave disc with a raised ridge at its anterior end. This ventral disc is used to attach the trophozoite to the epithelium of host enterocytes, so that the parasite is not swept away with digested food. Once the trophozoite loses hold and leaves this preferred environment to pass out of the body of the host, it is unable to survive. Attachment onto the surface of the enterocyte is therefore crucial. This tiny 'sucker' holds the cell fast to its host, and the outline of the ventral disc can be seen impressed on the surface of affected enterocytes.

Trophozoites possess four pairs of flagella arranged in bilateral symmetry. These are the organs of locomotion and trophozoites can attach and detach from the microvillous surface of enterocytes. Two curiously "claw-hammer" shaped median bodies, composed of microtubules, lie transversely in the mid-portion of the organism, though the function of these remains unknown.

Motile trophozoites exhibit forward movement during which the organism tends to rotate around its longitudinal axis displaying both a tumbling movement resembling that of a falling leaf and an up and down movement referred to as 'skipping'. Trophozoites can become detached and can subsequently re-attach themselves to the surface of another enterocyte. The cycle of attachment, detachment and subsequent re-attachment may be necessary to compensate for rapid enterocyte turnover and the ensuing sloughing of host cells into the intestinal lumen.

Giardia was once thought to be a primitive cell, lacking identifiable glycosomes, peroxisomes or mitochondria. That's not the case, and the discovery of the *Giardia* mitosome has changed this view. It now seems that the amitochondrial state is not primitive, but is the result of reductive evolution. Under electron microscopy we can observe numerous cytoplasmic cisternae and tubular elements that appear to be a transitional condition between a formal endoplasmic reticulum and the true Golgi as seen in more specialized cells (10). *Giardia* is itself parasitized by other organisms, and has been found to contain bacteria or *Mycoplasma*. There is also a 32 nm double-stranded ribonucleic acid virus which is characteristic of the genus. This, known as the *Giardia lamblia* virus (GLV), has been identified in many isolates of the host.

Transmission of *Giardia* to humans can occur through any mechanism by which material contaminated with faeces containing infectious cysts from infected human beings or animals is ingested by a sus-

ceptible host. Transmission routes include person to person, waterborne, foodborne and zoonotic transmission. Waterborne transmission of *Giardia* associated with community water systems, drinking untreated water whilst backpacking and immersion watersports is well documented, and epidemic giardiasis, associated with contaminated potable water, has been frequently reported. *Giardia* is the most commonly identified agent of waterborne disease in the USA with over 120 waterborne outbreaks affecting more than 25,000 persons, since 1965 (11). Until purified water became generally available in the United States since World War Two, it was normal for members of the public to become infected at an early age. Foodborne transmission of giardiasis was suggested in the 1920s and eight foodborne outbreaks have been documented.

Curiously, it may be that our ultra-hygienic modern environment serves to make outbreaks of giardiasis more distressing than they once were. In an era when we all contracted the organism as little children, immunity to *Giardia* was inevitably more widespread than it is now, and outbreaks under such conditions would be much milder.

Metronidazole (Flagyl®) has been the drug of first choice for giardiasis treatment for over 40 years and a combination of metronidazole and quinacrine has been used to treat refractory cases. Other nitroimidazoles, such as Tinidazole, are also effective and widely used around the world (12). Nitazoxanide (NTZ) also appears to be equally effective as metronidazole and NTZ has recently received FDA approval for the treatment of giardiasis in children. Albendazole has been reported to be as effective as metronidazole with fewer side effects among children aged 2 -12 years (13).

In Leeuwenhoek's time parasites like *Giardia* and *Cryptosporidium* were universally widespread and we can conclude that the general distribution of these organisms would have conferred a greater degree of immunity on the population.

LEEUVENHOEK'S ACCOUNT

Giardia is an attractive organism (14) and has long been a favourite of microscopists. The first observation of *Giardia* was set out clearly by Leeuwenhoek in 1681 (15). In his letter, he set out a detailed account with an unambiguous description. It was written in his native early modern Dutch and dated November 4 1681. The letter was translated for the Royal Society and read in English to the Fellows at their meeting of November 9 1681. In it he described what he observed in a stool sample:

"I have sometimes also seen tiny creatures moving very prettily; some of them a bit bigger, others a bit less, than a blood-globule but all of one and the same make. Their bodies were somewhat longer than broad, and their belly, which was flattish, furnished with sundry little paws, wherewith they made such a stir in the clear medium and among the globules, that you might even fancy you saw a woodlouse running up against a wall; and albeit they made a quick motion with their paws, yet for all that they made but slow progress."

This description is remarkable. The activity of the flagella does indeed cause *Giardia* to display rapid movement though its forward motion is – just as Leeuwenhoek recorded – far less than the activity of the flagella would lead an observer to expect. When Clifford Dobell published this account (5) he com-

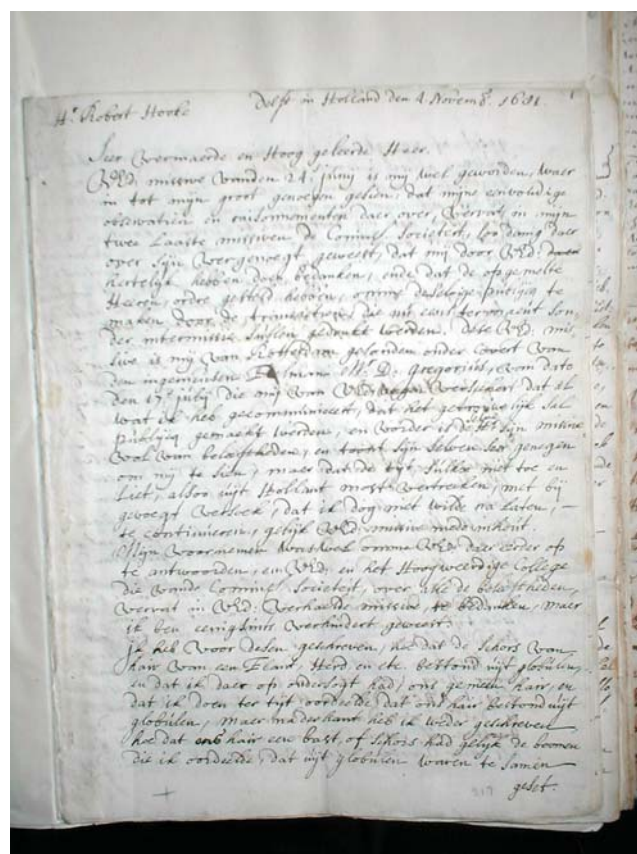


Figure 1. The opening section of Leeuwenhoek's letter describing the discovery of *Giardia*. This is letter No 66 addressed to Robert Hooke dated November 4 1681, in Book L1 in the Leeuwenhoek archive of the Royal Society of London. I am grateful to Mr Harry Leechburch of Leiden for bibliographical advice and to the President and Council of the Royal Society for their assisting my access to the original documentation.



Figure 2. One of the best public-domain light micrographs of *Giardia*, published by the National Institute of Infectious Diseases (formerly the National Institute of Health) of Tokyo, Japan. The binucleate structure of the cell and its appendages are clearly visible. This image has been processed using Adobe Photoshop CS® from the original published on the following web-site: www.nih.go.jp/niid/para/atlas/japanese/lambl.html.

mented that Leeuwenhoek's description was unmistakable.

In his later great book (16), Dobell took to task the other writers who had misinterpreted Leeuwenhoek's findings. Dobell often did this kind of thing. He had a penchant for including scarcely-concealed insults towards writers who raised questions that Dobell considered improper or ill-founded. In later years I came to know Clifford Dobell's widow, Monica, whose step-father (the eminent bacteriologist William S. Bulloch) once worked with Louis Pasteur. She described her husband to me as pedantic and a difficult man to know, and one of his research colleagues Professor Walter Perry (later Lord Perry of Walton) often spoke to me of Dobell's brittle and uncompromising character.

Although Dobell's single-mindedness made him an uncompromising individual, it also served to fuel his determination to ensure Leeuwenhoek was given his due. He learnt the early modern Dutch in which Leeuwenhoek communicated, painstakingly worked through the descriptions which (as in the case of *Giardia*) made identifications unmistakable, and he also systematically demolished the claims of Leeuwenhoek's detractors.

RECREATING THE OBSERVATIONS

One central aspect of Leeuwenhoek's work that Dobell failed to address was the microscopy. This is hard to understand, for Dobell himself was a master of the art. His early works on amitotic figures within protist cells produced results of peerless exactness, and he was able to culture pathogenic protozoan species with greater success than his contemporaries. Dobell was a superb microscopical observer and a skilled technician. He could culture organisms that others could not, and observe details that eluded most microscopists. If there is a microscopical equivalent in the laboratory to having a 'green thumb' in the garden, then Dobell had that facility. Thus it seems to me very surprising that he failed to pay attention to Leeuwenhoek's microscopical methods.

In order to gain insights into the microscopy of *Giardia*, I resolved to obtain digital images of the organism with a single-lensed microscope. Horace Dall, a remarkable optical experimenter, had bequeathed to me a fine spinel lens that Dall himself calibrated to have a magnification of 295x, similar to the best of Leeuwenhoek's home-made microscopes. It was made and mounted in 1950. My first experiments in imaging living cells had been with chlorophyte algae and I had

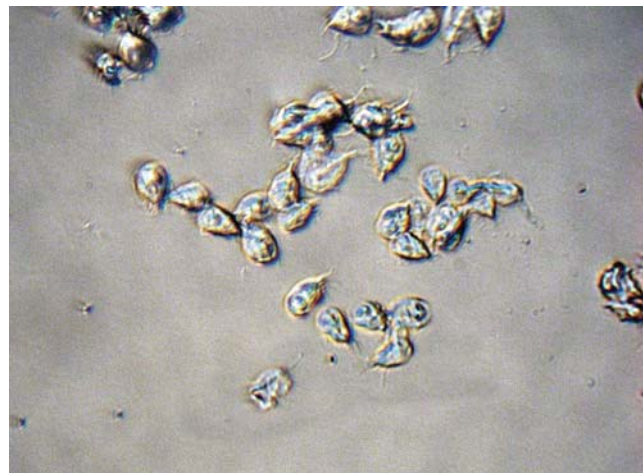


Figure 3. Living and unstained *Giardia* trophozoites imaged through the single lensed microscope made by the late Horace Dall of Luton. He ground this lens from spinel, which has dispersion lower than that of soda glass, and thus exemplifies the best image quality that we could obtain from an uncorrected lens. In order to maximise relief, the light source is slightly off-center and this image has been normalized with Photoshop (as in Figure 2) to give a clear impression of the image generated by a simple microscope at its best. Flagella and nuclear structures are discernible.

followed these with a series of studies of microbial cysts. These demonstrations were 'proof of concept' experiments. The results were exhibited at the Midlands Microscopical Meeting held at Penkridge, Staffordshire, England (17) and the images were awarded first prize in the meeting's micrographic competition. It was here that other microscopists raised the possibility of going further: could Leeuwenhoek have observed such living protozoan cells? How clearly could he truly have observed *Giardia*, and if he did, what features pertinent to *Giardia* morphology could he have seen through his single lens?

The specimen material that Huw Smith mailed to me after the Amsterdam meeting came in two forms. Air-dried smears of *G. duodenalis* stained with methyl violet were first to be received. Prior to microscopy, the unmounted preparations were fitted with protective strips to prevent the smears from grounding on the stage of the Dall microscope, and micrographs were obtained with an Olympus C-5000 Z digital camera resolving 5 mega-pixels. The camera was fitted with an Olympus extension tube to which the circular mount of the simple microscope could be fitted. Later



Figure 4. Selective enlargement reveals the detail that can be revealed by a single lens. Although chromatic aberration is apparent, there is no difficulty in observing the binucleate structure of each individual cell. The flagella are clearly visible; these are the 'paws' to which Leeuwenhoek alluded in his description [pooten, in the original Dutch]. Comparison with Fig 2 reveals that the single lensed microscope image compares favourably with that of the best achromatic instruments of today. Clearly, Leeuwenhoek could have observed what he claimed.

micrography was carried out using a standard Nikon Coolpix 4500 camera rated at 4 mega-pixels. In each case a frosted 60 W lamp at a distance of 1 meter was used as illuminant.

Discrete cells were immediately resolved, and flagellar structures were also discernible. Subsequently it proved possible to obtain satisfactory images of unstained *Giardia* cells, using careful control of the angle of illumination to optimize image contrast. In some images, the characteristic binucleate bodies were imaged and good micrographs were obtained.

The final stage was the observation of living cultures of actively motile trophozoites. Small drops (approximately 1/50th ml) were spread under standard circular No 1½ coverslips and directly observed. The distinctive tumbling movements of the swimming cells were immediately obvious, bringing to mind the vividly accurate description written by van Leeuwenhoek (15) 324 years earlier (*supra*). It was even possible to obtain real-time video sequences of the motile trophozoites.

CONCLUSION

Our current knowledge of the biology of *Giardia* (18) is now considerable. Microscopy has been harnessed(9) to reveal much of the complex structure (19) of this curious organism. In my extended account of Leeuwenhoek's work (20) I included examples of micrographs taken more than half a century ago by P H van Cittert and T Y Kingma Boljtes, both of whom clearly realized that Leeuwenhoek was capable of seeing more than the sceptics claimed. With these few exceptions, there has until now been little investigation of the techniques Leeuwenhoek used, and the results that he could have gained. In this case the micrographs stand as testimony to his accomplishments.

We must not overlook the capacity of the human eye and brain to restore contrast and detail to images of limited visual quality. Complex algorithms have been written for the image-restoration software in programmes like Adobe Photoshop® and these retrieve information and represent a digitized image in terms that may be closer to the way in which the human observer interprets a fleeting image. In this paper, raw data obtained from the Olympus and Nikon digital cameras was normalized through Photoshop CS® and the resulting images are presented as an indication of the power of the single lens (and the eye of the observer) to glean large amounts of data from primitive experiments. Some part of the first observations of *Giardia* in 1681 can now be experienced, and we

can now gain a fuller understanding of Leeuwenhoek and his remarkable capacity for microscopical observation.

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