CHAPTER 6

CONCLUSION
6.1 Conclusion

The study for the first time have shown the prevalence of *D.fragilis* in two main groups namely the Orang Asli and school children. This parasite has been shown to be found mainly mixed with *Blastocystis* sp. The study therefore has provided some easy techniques to differentiate both these parasites. The study also reports for the first time a new life cycle stage demonstrating mode of reproduction other than binary fission. The study also confirms the existence of a cyst-like structure. In general the findings of the study can be e summarized as follows:-

1. The study clearly provided evidence that *D.fragilis* do exist in the Malaysian population especially in the Orang Asli population. This is the first study to document this finding. The fact that rural school children have been found positive shows that active transmission of this parasite still goes on.

2. The study for the first time has shown that participants whose ages were less than 18 years, showed association with live stock, did not wash hands after handling animals, showed gastrointestinal symptoms and showed greater frequency of visits to the toilet per day were identified as significant (p<0.05) risk factors for acquiring *D.fragilis* in these communities.

3. This is the first finding to show that Potassium dichromate can be used as a preservative. The study recommends that future stool surveys should be carried out with this preservative substituting preservatives that have mercuric chloride.
4. Jones’ medium which is easier to prepare and have been successfully used previously for the detection of *Blastocystis* sp. should be used to isolate *D. fragilis* from fecal samples. The study showed that the growth of the parasite cannot exceed for more than two days. The study recommends that this culture medium be used for the initial detection especially in stool surveys and then positive cultures be transferred to Loeffler’s medium for longer maintenance.

5. The study for the first time identifies the 18\textsuperscript{th} hour of culture as the best time point to harvest time the parasite as the parasite count appears to be at the optimum. When harvested parasites are sub-cultured the parasite could be kept *in vitro* as long as 8 days is break-through finding enables the right time point to sub culture in order to propagate continuous culture for longer *in-vitro* maintenance.

6. The study demonstrates another mode of reproduction other than binary fission. The organism elongates to release a nucleated progeny leaving an empty space at the far end in the original mother cell. This proves that binary fission is not the only mode of reproduction that *D. fragilis* has.

7. The study shows that cyst-like structures do exist which show a thickened membrane that provides a robustness to resist distil water. These forms for the first time have been shown to cause experimental infections in Spraque dawley rats.
1.2 Future studies

1. The present study reported prevalence study carried out on *D. fragilis* in only two vulnerable group which are the orang asli and school children. There is a need to carry out more prevalence studies in other groups such as patients suffering from Irritable bowel syndrome colorectal cancer, HIV, people who work in the zoo and farms, rural communities, day care centers housing children. There is a need to correlate the presence of the parasite and gastrointestinal symptoms to ascertain further its pathogenic role.

2. All hospital laboratories should include screening of *D. fragilis* as this parasite could go unidentified in mixed cultures with *Blastocystis* sp. It is important to differentiate both these parasites.

3. The cyst-like structures obtained from the fecal samples of infected rats could not excyst in cultures and therefore more excystation studies need to be carried out.

4. More experimental animal infection studies should be carried out with better molecular approaches to identify the parasites in stool samples of infected rats.