# A potential link between gastrointestinal protozoa and diarrhea in the primates of Stichting AAP examined by mircroscopic examination and multiplexPCR

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**Summary**

The purpose of this master research internship was to examine whether the primates at Stichting AAP, excrete and so have, the pathogenic gastrointestinal protozoa *Giardia* spp., *Cryptosporidium* spp. and *Entamoeba histolytica/dispar*. It also was examined if it is possible to determine how many of these protozoa they have in their feces. In this research the feces was examined for *Giardia* spp., *Cryptosporidium* spp. and *Entamoeba histolytica/dispar*, because only these protozoa are known to cause diarrhea in NHP (Non Human Primates) (Levecke, 2010). *Giardia* spp. and *Cryptosporidium* spp. are both very common in NHP and cause in general not much harm, but can have clinical importance. *Entamoeba histolytica* can cause more serious symptoms and a sudden outbreak of diarrhea. The presence and clinical impact on primates is still unknown (Levecke et al., 2007).
Additionally it was examined whether there was a link between the presence of each of these three species of protozoa and the fecal consistency. For seven weeks feces of the primates was tested for the three species of protozoa. The primates at Stichting AAP were housed both individually and in groups. There were also changes between the individuals and groups. This research included 32 primates. The animals were all housed on deep litter.
Diarrhea, mush and normal feces were collected and examined during seven weeks, two to three times a week. The samples were taken randomly out of the enclosures of the primates. Fecal samples were collected when the enclosures of the primates were cleaned. The collection and further processing of the feces samples was done as sterile as possible and, the keepers were instructed how to do this.
In this study all three species of examined pathogenic protozoa were found in the feces samples of the primates. These protozoa were found in solid, mush and diarrhea feces samples in more or less the same percentages. Also after statistical analysis, with this research could not be proven that there was a link between one of the protozoa found and the diarrhea of the primates.
By microscopic examination samples were found with *Giardia* spp., *Entamoeba* spp. and *Cryptosporidium* spp. By multiplexPCR only samples with *Entamoeba histolytica/dispar* and very few samples with *Giardia* were found. Also other influences like shifts and medication/sedation that can cause the diarrhea are mentioned, but to examine these possible influences further research is necessary.

## Introduction

During several months there are some primates, especially crab eating macaques and Barbary macaques, at Stichting AAP that have diarrhea. Stichting AAP would like to know what the cause of the diarrhea is. Probably pathogenic gastrointestinal protozoa like *Giardia* or *Entamoeba histolytica* cause the diarrhea. Stichting AAP is an European rescue center for exotic animals, specialized in primates and small mammals. The goal of Stichting AAP is to rehabilitate rescued animals, and when mentally and physically healthy to replace them in a more natural habitat (zoo or reserve).
(Gastrointestinal) protozoa are unicellular eukaryotic organisms, which are diverse. Some of them are pathogenic. They exist most of the time in a proliferative stage, the trophozoit, and in a dormant stage, the cyst. The cyst is the infective stage and the trophozoit is the stage that causes (intestinal) disorders when pathogenic, like diarrhea. It is still difficult to classify protozoa in the taxonomy, because of their different characteristics and because of the fact that not all species of protozoa have available DNA. In general, in their proliferate stage, protozoa are motile.
Research has been performed that proves that gastrointestinal protozoa (*Giardia* spp. and *Cryptosporidium* spp.) are well known in NHP, but clear evidence is never found that these protozoa are also the cause of diarrhea in these primates (Levecke et al., 2007; Levecke et al., 2010). So, as said before, this study focuses additionally to finding protozoa in the primates at Stichting AAP also on the question if there is a link between the protozoa, when found, and diarrhea. Protozoa most well known in primates are *Giardia*, *Cryptosporidium* and *Entamoeba histolytica*. These protozoa are also the only protozoa known to probably cause diarrhea in NHP, next to *Balantidium coli* (Levecke, 2010).

### *Giardia*

*Giardia* consist of six species of which the species *Giardia duodenalis* consists of seven assemblages, A to G. Other names for *Giardia duodenalis* are *Giardia lamblia* or *Giardia intestinalis*. *Giardia duodenalis* has a simple structure (Carranza, 2009). *Giardia duodenalis* is also able to infect many mammals, also humans. Only assemblage A and B are of zoonotic importance, these assemblages can cause Giardiasis in both different groups of animals and humans. The infective cyst of *Giardia* is very resistant in the environment, because the cyst has a rigid, protective cyst wall which contains the trophozoites. After oral uptake of the infective cyst of *Giardia*, this cyst forms into four motile, flagellate trophozoites in the upper small intestine. These trophozoites can reproduce by asexually binary fission and probably also by sexual reproduction. In the end the carrier is going to excrete cysts and trophozoites in the feces.
The trophozoites attach to the epithelial cells of the intestinal wall when they are in the small intestine and then proliferate. They cause gastrointestinal disorders in this way, by a loss of the epithelial barrier caused by both the parasite and the host immune response (Levecke, 2010; Carranza, 2009). This has for example malabsorption with diarrhea as a result. *Giardia duodenalis* is non-invasive and also toxin or virulence factors are not clearly found till now. (Carranza, 2009). The incubation period in NHP is not known, but probably comparable to humans: 3 to 25 days (Levecke, 2010). *Giardia* are very common in captive NHP and they might have clinical importance. There are indications that *Giardia* can cause diarrhea and failure to thrive in young animals (Levecke et al., 2007; Levecke et al., 2010).

***Cryptosporidium***

*Cryptosporidium* consists of about 22 species and more than 40 genotypes have been described. *Cryptosporidium* exist in many animals and has a complex asexual and sexual life cycle which consists of oocysts, sporozoites, trophozoites, meronts and merozoites. The ingested oocysts releases four sporozoites which attach to the epithelial cells of the intestinal wall of the small intestine. These sporozoites develop into trophozoites, which undergo sexual proliferation after which meronts arise. There are two types of meronts. Type 1 meronts can develop into type 2 meronts through merozoites. Type 1 meronts have eight merozoites. These merozoites also invade other epithelial cells. Type 2 meronts have four merozoites, which form micro- and macro-gamonts and these micro- and macro-gamonts form micro- and macro-gametes. The micro-gametes invade the epithelial cells of the small intestine in which the macro-gametes exist. After fertilization again four sporozoites are released from the zygote. So, much of the life cycle of *Cryptosporidium* takes place in the intestinal epithelial cells and this damages these cells. This causes for example maldigestion and malabsorption with diarrhea as a result (Levecke, 2010).

*Cryptosporidium* are very common in NHP and can have, just as *Giardia*, clinical importance, but cause most of the time not much harm. On the other hand, they are able to cause diarrhea, and failure to thrive in young animals. Levecke et al., 2007; Gomez et al., found in 1992 that *Cryptosporidium* did not cause much clinical symptoms in infected animals and they also did not find *Cryptosporidium* in infant primates in their research.

***Entamoeba histolytica***

For a long time it was unclear whether E. histolytica and E. dispar were different species because they look morphologically the same. After long debate nowadays *Entamoeba histolytica* and *Entamoeba dispar* are accepted as two different species. They only differ in their pathogenicity, because *E. histolytica* is pathogenic and *E. dispar* is not pathogenic. Many hosts shed *Entamoeba* cysts or trophozoites without having clinical symptoms. Currently research is performed if humans and NHP have their own variants of *E. histolytica*. The life cycle of *E. histolytica* is simple and asexual. The infectious cyst of *E. histolytica* is very resistant in the environment and contains four nuclei. When ingested this cyst enters the lumen of the large intestine. After excystation of the cyst, eight trophozoites arise. These trophozoites then multiply through binary fision, encyst or invade the host. When these trophozoites encyst, this induces a commensal infection. The host is going to shed both cysts and trophozoites. Because of the life cycle of *E. histolytica* the colonic barrier disrupts, the mucosa is invaded and the host epithelial and immune cells are killed. *E. histolytica* avoids the hosts immune response and in this way causes also pathologies in extra-intestinal organs, like the liver.

Probably *E. histolytica* infections are common in NHP, especially in Old World primates, but the extent of the reservoir is unclear (Levecke et al., 2010). New World primates further show more distinct lesions. Most of the time clinical symptoms are shown 1 to 4 weeks after infection (Levecke, 2010). In general *E. histolytica* causes not to many signs. But there is a possibility that *E. histolytica* might also cause serious symptoms in NHP, like watery diarrhea, hemorrhagic dysentery and pathologies in other organs like liver abscesses. Sometimes *E. histolytica* enters the lymphatics, but normally they do not pass the lymph nodes. After this invasive intestinal and extra-intestinal amebiasis in NHP, it can finally lead to death (Levecke et al., 2007; Verweij et al., 2003). Asymptomatic animals have less pronounced lesions which are most of the time limited to the large intestine (Levecke, 2010). Factors like the species and nutritional status of the host, the enteric bacterial flora and environmental factors can contribute more or less to pathogenicity.

Because *E. histolytica* can also be infectious and pathogenic to humans, it is important to know if there is a risk of zoonotic transmission from NHP to humans and from humans to NHP. This is unclear. Probably there is a human variant and a NHP variant of *E. histolytica*, but it is unclear if both variants are coming from the same ancestor and if they can reproduce. Only with molecular methods, like PCR, it is possible to make a difference between the *Entamoeba*. With only microscopic examination of cysts or trophozoites, on morphological features, it is impossible to make this difference (Levecke et al., 2010).
NHP can get infected by oral uptake of the cysts of *E. histolytica*, *Giardia* or by oral intake of the oocysts of *Cryptosporidium*. The oral-fecal transmission route can be direct or indirect. The direct route consists of coprofagy or contact with infected animals, the indirect route consists of transmission form contaminated food or water. Probably transmission from NHP to NHP is most common, but because these protozoa exist in many species outside NHP it is also possible that transmission from other species then NHP to NHP exist. Also transmission by arthropods or the equipment of the animal caretakers, mechanical transmission, is possible. The transmission of protozoa is facilitated because the (oo)cysts can survive well in the environment for months and are quite resistant, they are shed quite quickly after infection and they are immediately infectious once shed.
In captive NHP *Giardia* is found most in Old World primates (and prosimians, a taxon of primate that include lemurs, lorises, bushbabies, and tarsiers, but not simians: primates, apes or humans), *E. histolytica* and *Cryptosporidium* are found less in OW primates, in contrast to New World primates and apes (and prosimians). Furthermore young primates are more susceptible to have *Giardia* and *Cryptosporidium* then older primates (Levecke, 2010).

## Aim of the study

The aim of this study is to find out whether the primates at Stichting AAP have pathogenic protozoa and to find out if there is a link between each of these protozoa, when present, and the diarrhea of the examined primates. If there is no clear link, another aim of this study is to evaluate other influences that can cause the diarrhea in the primates. These other influences can be the food-related, management-related (the primates included in this research were all housed on deep litter, but a research after primates on different housing could say more about this influence), or influenced by medication/sedation and changes in group structure that have occurred during this research internship.

## Materials and methods

The primates observed in this study were crab eating macaques (*Macaca fascicularis*) and Barbary macaques (*Macaca sylvanus*), both Old World primates. Crab eating macaques naturally live in Southeast Asia. They live in wooded areas, close to rivers or in coastal areas and can deal well with people. Their diet consists mainly of fruits. The crab eating macaque is one of the primate species most used in laboratories.
Barbary macaques are the only macaques that live outside Asia. They live in North Africa and in Spain on the rock of Gibraltar. The population on the rock of Gibraltar contains about 30 to 40 animals and this population is maintained artificially. They are diurnal and omnivores. This species is kept often as a pet in North Africa, but is unsuitable for this purpose because of its breaking behavior and aggression. As result Stichting AAP takes in many Barbary macaques. It is unknown if one of these two species of primates is more predisposed to get diarrhea caused by one or more than one of the three species of examined protozoa. So in this research is assumed that all three species of examined protozoa are equal pathogenic to the two species of primates.
At Stichting AAP the primates are caged individually or in groups with their conspecifics. The age and gender of most of the primates is unknown. Individual sampling for the primates in the groups is impossible in this research, so there were taken as many samples as individuals in each of the examined groups (Levecke et al., 2007; Levecke, 2010). The samples were taken at random out of the enclosures of the primates. The collection of the samples was done for seven weeks in a row, two to three times a week. This because protozoa are often shed intermittently, so repeated sampling increased the sensitivity (Levecke et al., 2010). The collection and further processing of the samples was done as sterile as possible, to prevent contamination from the environment. The keepers were instructed how to do this as sterile as possible. They wore gloves when collecting the samples and tried to take feces samples only, not in touch with deep litter. Only when primates were kept individually it was possible to take an individual sample. This was not included in this research because it was exceptional that one animal was kept individually during the research period. During this research many individuals switched to other groups or between the groups for socialization. This makes it even more difficult to determine, which sample belongs to which individual. It is only known which sample belongs to which group.
In the first place the samples that contain SAF and feces were examined microscopically after applying the direct centrifuge sedimentation flotation method with sucrose. The feces was first microscopically examined without and afterwards examined with iodine staining.
In the second place the feces was send to the UMC were it was examined by multiplexPCR, because especially for detecting Entamoeba histolytica molecular methods like multiplexPCR

are necessary (Levecke, Dorny, Geurden, Vercammen, & Vercruysse, 2007; Levecke et al., 2010; Verweij et al., 2003; Levecke, 2010). Both methods were also compared.
After collection of the feces at Stichting AAP, during cleaning of the cages, every sample was split in two samples. To one of the two samples SAF (Sodium acetate acid formaldehyde) is added. The ratio on which SAF is added to the feces is three parts SAF and one part feces. The other sample, to which nothing was added, was called the fresh sample. Both samples get a label on which the number of the sample, the date/time, the freshness and consistency of the sample was noted. The aim of this research was to get the samples as fresh as possible. Also was noted which SAF sample belongs to which fresh sample, so that no confusion occurs. The samples then were send by post, in the afternoon of the same day they are collected in duplicate, to the VMDC at the veterinary faculty of Utrecht University. At Stichting AAP, before the samples are send to Utrecht University, and at Utrecht University the fresh samples were preserved in a fridge for maximal seven days, and the SAF samples were preserved at room temperature.
The samples are numbered as followed: the number behind the # is the number of the week the sample is collected in. The second letter and number are of the group of which the sample is collected. The number following is the version number of the sample. The last number is the number of the subsample (if two samples are taken from one version sample in the cage). So for example sample #3B142 is the fourth sample and second subsample of group Berani, taken in the third week of this research.
The groups were numbered as follows. *Macaca fascularis:* group Jakob: J1 (at the beginning of the research five individuals in this group), group Roland: R (at the beginning of the research two individuals in this group), group Besar: B2 (at the beginning of the research two individuals in this group), group Berani: B1 (at the beginning of the research four individuals in this group), group Joelie: J2 (at the beginning of the research two individuals in this group). *Macaca sylvanus:* group Fugitive: F (at the beginning of the research three individuals in this group), group Nepal: N (at the beginning of the research three individuals in this group), group Sjeik: S (at the beginning of the research three individuals in this group), group Anou: A (at the beginning of the research three individuals in this group), group Jonk: J3 (subgroup Joek: J4) (at the beginning of the research two individuals in this group), group Lùlu: L (at the beginning of the research one individual).

**Microscopic examination**
For the microscopic examination the scale of protozoa seen in the microscope at a magnification of 400 times is: 0 protozoa: -, 1 till 4 protozoa: +/-, 5 till 10 protozoa: +, 10 or more protozoa: ++ (Gomez et al., 1992).
For the microscopic examination for cysts and trophozoites of each sample is written down on which date and time it was taken and what its freshness and consistence were like, see attachment 1. As said before, the samples that contain SAF and feces were examined microscopically after applying the direct centrifuge sedimentation flotation method with sucrose. Further the feces was first examined microscopically without and afterwards with iodine staining, this because microscopic examination with iodine staining can make the detection of *Cryptosporidium* easier (Gomez et al., 1992; Levecke, 2010).
There are different methods for examining the feces microscopically, next to microscopic examination after applying the direct centrifuge sedimentation flotation method with sucrose. These other methods are for instance examining the feces immediately after adding SAF, sucrose or saline, or after applying the direct centrifuge sedimentation flotation method with saline, or after applying the Ridley method. It was not well known with which method the primate feces could be examined best under a microscope for protozoa, although the Ridley

method results in a sediment deficient in fat. This can be useful, because stool samples of NHP contain often a great amount of fat, which can complicates the detection of protozoa in these samples (Levecke, 2010). In this research was decided to examine the samples that contain SAF and feces after applying the direct centrifuge sedimentation flotation method with sucrose, because this works out the best in the laboratory that is used in this research. The samples could not be examined directly at Stichting AAP, because their laboratory facilities were not sufficient.

**MultiplexPCR**

Microscopic examination is most used for the detection of protozoa, but there was also searched for other methods to detect protozoa, like IFA (indirect fluorescent antibody(test) ), ELISA (Enzyme-Linked Immuno Sorbent Assay) and multiplexPCR (multiplex Polymerase Chain Reaction) (Levecke, 2010). For the examination through multiplexPCR the CT value was written down, the higher this value the less DNA of the examined protozoa was found in the feces, see attachment 2. The manual of the PCR used says that the polymerase chain reaction consists of three distinct stages: denaturation, annealing and elongation. Further primers are necessary. A primer is a short single-stranded DNA, which is used as the starting point of the PCR. Always two primers are necessary, for both sides of the DNA strain. The temperature at the denaturation stage was 95 degrees. At this stage, the double helix of the DNA splits. At the annealing stage, when the double helix is still split, primers get stuck in the DNA. The temperature at the annealing stage was 60 degrees. At the elongation stage the protein polymerase is released, so that the primers at the DNA only multiply a specific piece of DNA of the protozoa species. This specific piece of DNA can then be amplified and further researched. At the elongation stage the temperature was 72 degrees and this stage was done 45 times to get more DNA. This takes 1,5 to 2 hours. The robot of the PCR used in this research was the Rosch and till a CT value of 40 the result can be seen as positive for DNA of the species of protozoa. Before examination by PCR the samples were preserved in a freezer at a temperature of -80 Celsius. Then, the samples were boiled at a temperature of 95 degrees for 10 minutes. This was done in order to ensure that the DNA will mix in the whole buffer. After the boiling, the monsters were put in the so called MagNA Pure 96. In the MagNA Pure 96 the DNA was isolated for 50 minutes. The multiplexPCR samples were taken with a 10 µL loop and collected in a Eppendorf cup containing 200 µL of Cobas buffer. Control samples of saline were also added at random in the multiplexPCR. In the first week samples were examined in duplicate, to see if the outcomes were the same or differ. This was done to examine if the multiplexPCR was reliable for primate feces, because this was unknown. Second the outcomes of the examination by microcope and the examination through multiplexPCR were compared, see attachment 3. 0 means that there were no protozoa found of the type mentioned at the top of the table by microscope and/or multiplexPCR in the sample. 1 means that there were protozoa found of the type mentioned at the top of the table by microscope and/or multiplexPCR in the sample. The black numbers mean that the outcome was the same between examination by microscope and by multiplexPCR. The yellow numbers mean that the outcome was different between examination by microscope and by multiplexPCR.

At last was examined how many samples, percentages, of diarrhea, mush and normal feces contain one or more of the three species of protozoa, see table 1 and 2. This is done to say something about a possible link between the one or more species protozoa and diarrhea. The examination only included samples of which the consistence was known and samples of which the multiplexPCR succeeded. By multiplex PCR are the feces samples not only examined for *Giardia*, *Cryptosporidium* and *Entamoeba histolytica/dispar*, but for more

species of protozoa such as *Dientamoeba fragilis*. Because these other species of protozoa were not examined by microscopic examination, there was done nothing with these outcomes by multiplexPCR. Also does this multiplexPCR make no difference between *Entamoeba histolytica* and *Entamoeba dispar*, see attachment 2.
For statistical analysis, assumed that there was no difference in pathogenicity between the three species of examined protozoa, a regression analysis based on the microscopic counting of the number of samples with diarrhea (100% diarrhea), mush (50% diarrhea and 50% non diarrhea) and solid (0% diarrhea) and the concentration of the three types of protozoa was made.
For statistical analysis, assumed that there was a difference in pathogenicity between the three species of examined protozoa, the chi-squared test was used for the two most extreme values found, from *Entamoebe* spp and *Giardia* spp.

## Results

### Comparing microscopic examination and multiplexPCR

By both microscopic examination and multiplexPCR examination protozoa were found. But between these two examination methods quite some differences exist in what type of protozoa were found. By microscopic examination many more *Cryptosporidium* and *Giardia* were found and by multiplexPCR examination many more *Entamoeba histolytica/dispar* were found. By microscopic examination all three species of examined protozoa were found and also all combinations of all three species of protozoa were found. By multiplexPCR next to samples that only contain *Entamoeba histolytica/dispar*, only two samples containing *Giardia* and *Entamoeba histolytica/dispar* were found, see table 1 and 2 for the percentages of protozoa found. The percentages of samples in which *Entamoeba histolytica/dispar* was found, were much higher by examination by multiplexPCR then by microscopic examination.

Examined was how many of the outcomes for the detection of these three species of protozoa were the same between the microscopic and the multiplexPCR examination. The result was a percentage of 60,8% samples that contain the same species of protozoa, see attachment 3. Black numbers mean that outcomes were the same and yellow numbers mean that the outcomes differ, as said before.

Overall was unknown if the three examined protozoa species cause diarrhea. Also was nothing known about the age, gender et cetera of the primates to which the feces samples belong. The only thing that was known was to which group the feces samples belong. So for finding a possible link between having diarrhea and having protozoa no distinction was made between the different species of protozoa, assumed was that all three species of examined protozoa were equal pathogenic in the first place. So the only possible link to examine was a link between having diarrhea and having (one or more) of the three examined species of protozoa.

### MultiplexPCR

For the prevalence of one protozoa species or more than one species in samples of diarrhea, mush and normal feces it was examined how many of the different samples, percentages, examined by multiplexPCR and microscopic examination, contain protozoa. These percentages were almost similar for *E. histolytica/dispar* in the samples examined by multiplexPCR of diarrhea, mush and normal feces. The found percentage of *E. histolytica/dispar* in diarrhea by multiplexPCR is even 10% less than the percentages of *E. histolytica/dispar* in mush and normal faeces. Only two samples contain more than one protozoa species. This are too few samples to say something about the appearance of more than two protozoa species in the examined feces samples. Without any further statistical analysis no positive link between having diarrhea and having protozoa (*E. histolytica/dispar*) was found by examination the feces for protozoa species by multiplexPCR in this research. See the tables below and attachments 1 and 2.

**Table 1 and 2. Prevalence of protozoa by examination by multiplexPCR and by microscopic examination.**

| **Multiplex PCR** | | | |
|---|---|---|---|
| Protozoa | Prevalence | | |
| | Solid | Mush | Diarrhea |
| *Giardia* | 2 (1,4%) | 2 (5,3%) | |
| *Cryptosporidium* | | | |
| *Entamoeba histolytica/dispar* | 122 (85,3%) | 28 (73,8%) | 13 (72,2%) |
| *Giardia + Cryptosporidium* | | | |
| *Cryptosporidium + Entamoeba histolytica/dispar* | | | |
| *Giardia + Entamoeba histolytica/dispar* | 2 (1,4%) | 2 (5,3%) | |
| *Giardia + Cryptosporidium + Entamoeba histolytica/dispar* | | | |
| Number of diarrhea samples: 18 (9,0%)<br>Number of mush samples: 38 (19,1%)<br>Number of solid samples: 143 (71,9%) Total: 199 | | | |

| **Microscope** | | | |
|---|---|---|---|
| Protozoa | Prevalence | | |
| | Solid | Mush | Diarrhea |
| *Giardia* | 58 (40,6%) | 20 (52,6%) | 7 (38,9%) |
| *Cryptosporidium* | 35 (24,5%) | 7 (18,4%) | 5 (27,7%) |
| *Entamoeba* | 43 (30,1%) | 9 (23,7%) | 8 (44,4%) |
| *Giardia + Cryptosporidium* | 18 (12,6%) | 6 (15,8%) | 3 (16,7%) |
| *Cryptosporidium + Entamoeba histolytica/dispar* | 8 (5,6%) | 1 (2,6%) | 1 (5,6%) |
| *Giardia + Entamoeba histolytica/dispar* | 24 (16,8%) | 5 (13,1%) | 4 (22,2%) |
| *Giardia + Cryptosporidium + Entamoeba histolytica/dispar* | 6 (4,2%) | 1 (2,6%) | 1 (5,6%) |
| Number of diarrhea samples: 18 (9,0%)<br>Number of mush samples: 38 (19,1%)<br>Number of solid samples: 143 (71,9%) Total: 199 | | | |

## Microscopic examination

A quantitative statistical analysis of any link between found protozoa and feces samples was only possible for the microscopic part of the research, because only by the microscopic part of the research was it possible to make a quantitative classification of the found protozoa. This was done by regression analysis. This quantitative statistical analysis was made for the three species of protozoa counted together.

199 feces samples are examined by microscope. The samples were, visually, classified in three categories:

- 100 % diarrhea. The primates have diarrhea. There were 18 samples in this category.
- 50% diarrhea and 50 % non diarrhea, or it is unclear if the primates have diarrhea. These samples were called the mush samples in this research. There were 38 samples in this category.
- 0 % diarrhea. The primates had no diarrhea. These samples were called solid samples in this research. There were 143 samples in this category.

Per sample, the numbers of *Giardia* (called type A here), *Cryptosporidium* (called type B here) en *Entamoeba* (called type C here) were count together. This on the basis of four frequency distributions as follows:

- A minus being 0 protozoa corresponding to a weighting factor 0.
- A plus-minus being 0 to a maximum of 5 protozoa per category A, B and C, corresponding to an average weighting factor of 2.5 protozoa.
- A plus sign being between 5 and 10 protozoa, corresponding to an average weighting factor of 7.5 protozoa.
- And two plus signs being more than 10 protozoa, corresponding to a rated average weighting factor 15.

Table 3 shows the measured output data including the calculated concentration of protozoa in the successive samples examined by means of the weighting factors.

**Table 3. Calculation protozoa concentration in the samples examined.**

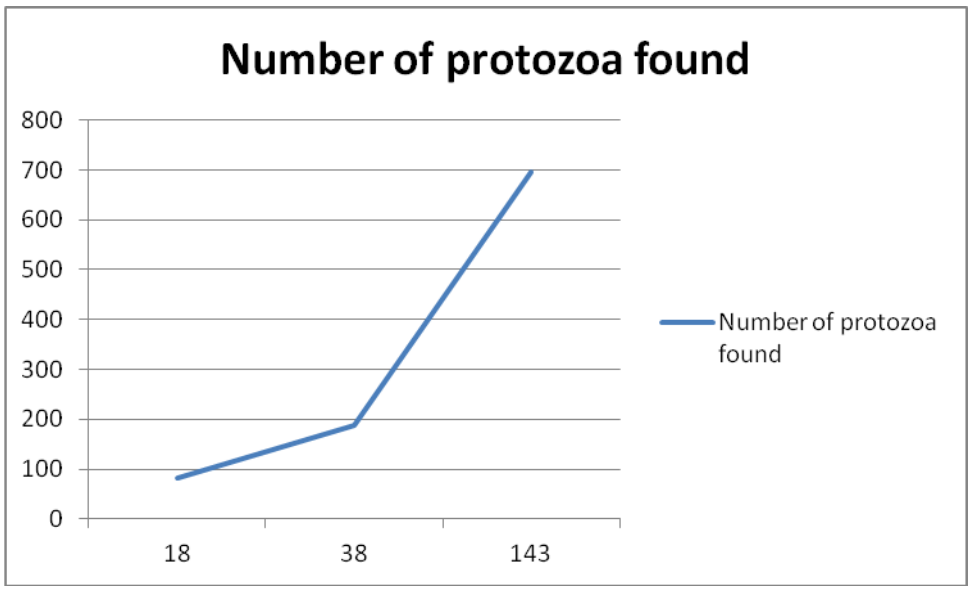
| | **Type A** ***Giardia*** | | | **Type B** ***Cryptosporidium*** | | | **Type C** ***Entamoeba histolytica*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Diarrhea | 11 | - | 0 | 13 | - | 0 | 10 | - | 0 | |
| 18 samples. | 5 | -+ | 2,5 | 5 | -+ | 2,5 | 5 | -+ | 2,5 | |
| | 2 | + | 7,5 | 0 | + | 7,5 | 2 | + | 7,5 | |
| | 0 | ++ | 15 | 0 | ++ | 15 | 1 | ++ | 15 | |
| **Weighted summation** | | | **27,5** | | | **12,5** | | | **42,5** | |
| **A+B+C=** | | | | | | | | | | **82,5** |
| Mush | 18 | - | 0 | 30 | - | 0 | 29 | - | 0 | |
| 38 samples. | 12 | -+ | 2,5 | 18 | -+ | 2,5 | 6 | -+ | 2,5 | |
| | 6 | + | 7,5 | 0 | + | 7,5 | 3 | + | 7,5 | |
| | 2 | ++ | 15 | 0 | ++ | 15 | 0 | ++ | 15 | |
| **Weighted summation** | | | **105** | | | **45** | | | **37,5** | |
| **A+B+C=** | | | | | | | | | | **187,5** |
| Solid | 86 | - | 0 | 109 | - | 0 | 99 | - | 0 | |
| 143 samples. | 32 | -+ | 2,5 | 29 | -+ | 2,5 | 22 | -+ | 2,5 | |
| | 18 | + | 7,5 | 4 | + | 7,5 | 17 | + | 7,5 | |
| | 7 | ++ | 15 | 1 | ++ | 15 | 5 | ++ | 15 | |
| **Weighted summation** | | | **320** | | | **117,5** | | | **257,5** | |
| **A+B+C=** | | | | | | | | | | **695** |

On the basis of the microscopic examination as shown in table 3, the following measurement data resulted:

- 18 samples Diarrhea (100% diarrhea) with a total concentration of protozoa A plus B plus C from 82,5. 82,5 : 18 is an average protozoa concentration of 4,58.
- 38 samples Mush (50% diarrhea and 50% non diarrhea) with a total concentration of protozoa A plus B plus C from 187,5. 187,5 : 38 is an average protozoa concentration of 4,14.
- 143 samples Solid (0% diarrhea) with a total concentration of protozoa A plus B plus C from 695. 695 : 143 is an average protozoa concentration of 4,86.

By means of the method of least squares is a linear regression calculated between the concentration of protozoa in the sample, and whether or not to have diarrhea. All this is shown in graph 1 below.

**Graph 1. Linear regression between 'having diarrhea and the concentration of protozoa in the test samples'.**




**Legend graph 1.**
**x-axis of graph; the number of samples solid, mush and diarrhea. = 18, 38 and 143**
**y-axis of graph; The number of protozoa found in respectively solid, mush and diarrhea samples. = 82,5, 187,5 and 695**

**Further it is necessary for the qualitative statistical analysis below:**
**n; the number of points in the graph. = 3**
**a; the slope of the linear regression line**
**b; the intersection of the linear regression line with the y-axis**

**Comparing the system**

$$\mathbf{a.n + b\sum x = \sum y}$$
$$\mathbf{a\sum x + b\sum x^2 = \sum x.y}$$

**The solution to the system of equations will result in the linear regression line:**

**y = a x - b**
$$\mathbf{y = 4,9x - 2,0}$$

**With a correlation coefficient of:**

$$\mathbf{r = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{\left(\sum x^2 - \frac{(\sum x)^2}{n}\right)\left(\sum y^2 - \frac{(\sum y)^2}{n}\right)}} = 100\ \%}$$

The, in Table 3, found and calculated linear regression based on the microscopic counting of the number of samples with diarrhea (100% diarrhea), mush (50% diarrhea and 50% non diarrhea) and solid (0% diarrhea) and the concentration of A plus B plus C protozoa. The regression gives a slope of 4.9 per sample examined whether or not the primate has, much, little or no diarrhea. This with a correlation coefficient of 100%. This is all shown in graph 1.

Based on the calculations and the representation of the linear regression between the degree of diarrhea and the concentration of protozoa, it cannot but concluded that these protozoa were responsible for diarrhea in these primates. In conclusion, in this research there was no relation between protozoa and diarrhea in these primates. This can be explained by the protozoa concentration per sample regardless of the degree of diarrhea is 4.9 with a particularly strong correlation of 100%. Once again, it was assumed for this calculation that there was no difference in pathogenicity between the three species of examined protozoa.
Also by the average protozoa concentration calculated per sample: diarrhea: 4,58, mush: 4,14 and solid: 4,86 can be made clear without any statistical analysis that no positive link can be found between having diarrhea and having protozoa.
In case there was a difference in pathogenicity between the three species of protozoa, then the most extreme values of numbers of samples with protozoa, which could give a positive link between having diarrhea and having that type of protozoa, could also statistically be analyzed. If no positive link is found by statistical analysis for these most extreme values, it may be concluded that there is also no positive statistical link for the less extreme values found. The most extreme values between numbers of protozoa in solid, mush and diarrhea samples found by microscopic examination were those found for *Entamoeba* and *Giardia*.
So the chi-squared test was used to examine if there was a positive correlation between having diarrhea and having one of these two species of protozoa. The null hypothesis for this test is that the proportions are equal or, equivalently, so that there is no association between the factors of interest. The one hypothesis for this test is that the proportions are not equal or, equivalently, so that there is an association between the factors of interest. In this research the

two factors of interest are having a certain quantity of *Entamoeba* spp or *Giardia* spp and having solid, mush or diarrhea samples. The 3 x 4 tables for *Giardia* spp and *Entamoeba* spp used in this test are as followed:

**Table 4 and 5 Tables used for the chi-squared test; to test a positive correlation between having diarrhea and having *Entamoebe* spp or *Giardia* spp**

| Number of samples of *Entamoeba* spp | Consistency of the samples | | | Total Samples |
|---|---|---|---|---|
| | Diarrhea | Mush | Solid | |
| - | 10 | 29 | 99 | 138 |
| +/- | 5 | 6 | 22 | 33 |
| + | 2 | 3 | 17 | 22 |
| ++ | 1 | 0 | 5 | 6 |
| Total samples | 18 | 38 | 143 | 199 |

| Number of samples of *Giardia* spp | Consistency of the samples | | | Total Samples |
|---|---|---|---|---|
| | Diarrhea | Mush | Solid | |
| - | 11 | 18 | 86 | 115 |
| +/- | 5 | 12 | 32 | 49 |
| + | 2 | 6 | 18 | 26 |
| ++ | 0 | 2 | 7 | 9 |
| Total Samples | 18 | 38 | 83 | 199 |

For *Entamoeba* spp the chi-squared test gives a value of 4,33. This gives a P-value of 0,63 with 6 degrees of freedom.
For *Giardia* spp the chi-squared test gives a value of 3,19. This gives a P-value of 0,78 with 6 degrees of freedom.
We reject the null hypothesis that there is no associaton if $P < 0.05$. For both *Entamoeba* and *Giardi*a $P > 0.05$, so the null hypothesis cannot be rejected. The one hypothesis for this test is rejected. The one hypothesis is that the proportions are not equal or, equivalently, so that there is an association between the factors of interest. So for both *Entamoeba* and *Giardia* the null hypothesis is true: the proportions are equal or, equivalently, so there is no association between the factors of interest: having a certain quantity of *Entamoeba* spp or *Giardia* spp and having solid, mush or diarrhea samples.

## Discussion

This research was done because Stichting AAP had a chronic diarrhea problem, for several months, but during the study period the percentage of the collected diarrhea samples was around 10%. Furthermore the number of diarrhea samples were also not the same for every group every week, see the tables and graphs below.

**Table 6 and graph 2. Number of fecal samples collected each week and total number of samples.**

| Week | Number of samples | | | |
|---|---|---|---|---|
| | Solid | Mush | Diarrhea | Total |
| 1 | 23 | 0 | 2 | 25 |
| 2 | 19 | 0 | 6 | 25 |
| 3 | 23 | 7 | 0 | 30 |
| 4 | 17 | 16 | 0 | 33 |
| 5 | 18 | 9 | 4 | 31 |
| 6 | 19 | 6 | 6 | 31 |
| 7 | 23 | 0 | 0 | 23 |
| Total | 143 | 38 | 18 | 199 |

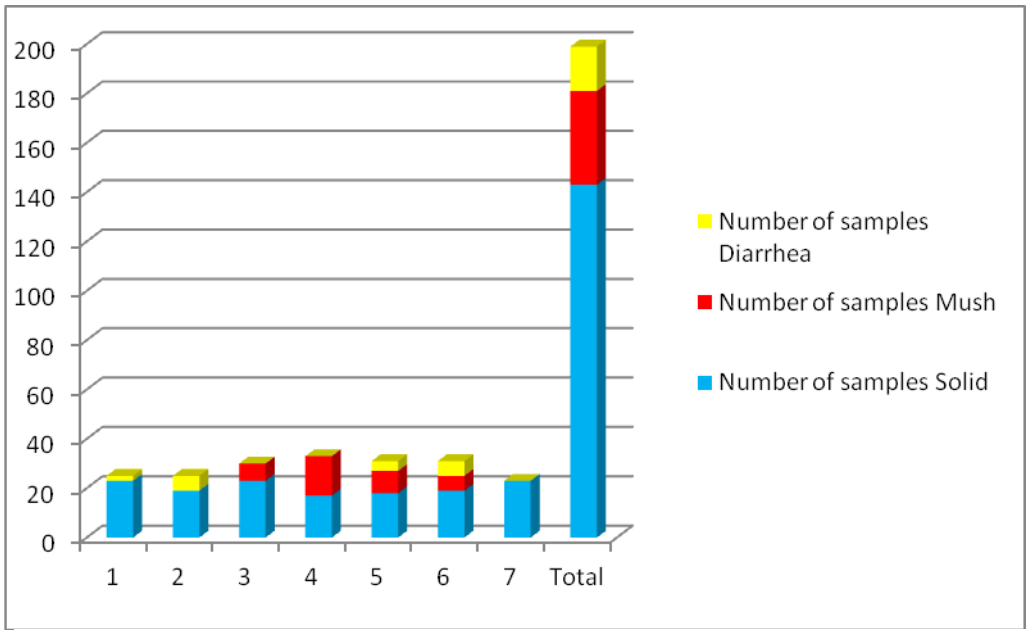
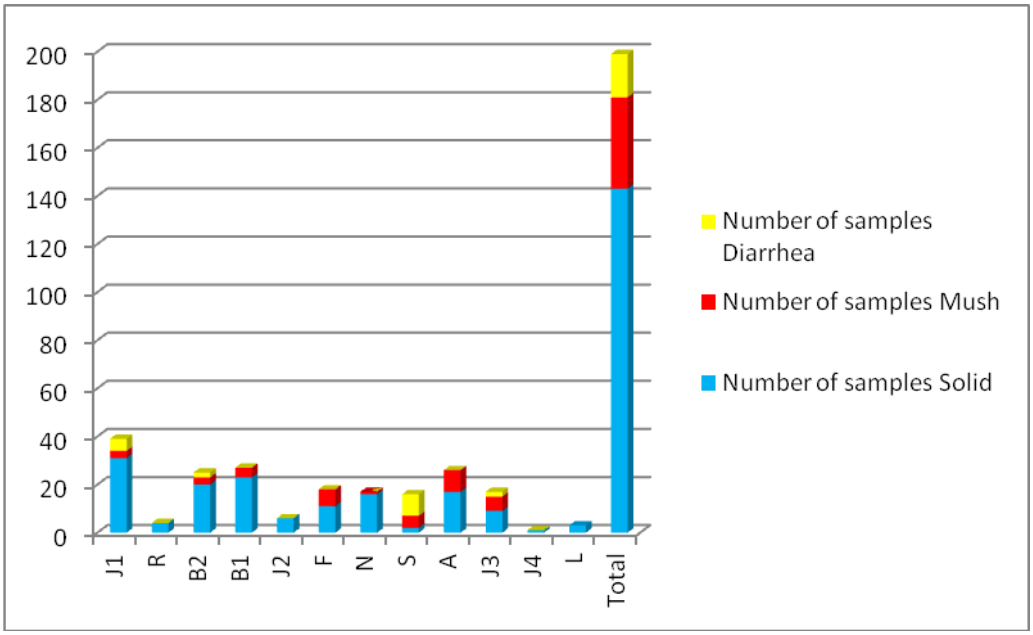


**Table 7 and graph 3. Number of fecal samples collected per group and total number of samples.**

| Group | Number of samples | | | |
|---|---|---|---|---|
| | Solid | Mush | Diarrhea | Total |
| J1 | 31 | 3 | 5 | 39 |
| R | 4 | 0 | 0 | 4 |
| B2 | 20 | 3 | 2 | 25 |
| B1 | 23 | 4 | 0 | 27 |
| J2 | 6 | 0 | 0 | 6 |
| F | 11 | 7 | 0 | 18 |
| N | 16 | 1 | 0 | 17 |
| S | 2 | 5 | 9 | 16 |
| A | 17 | 9 | 0 | 26 |
| J3 | 9 | 6 | 2 | 17 |
| J4 | 1 | 0 | 0 | 1 |
| L | 3 | 0 | 0 | 3 |
| Total | 143 | 38 | 18 | 199 |



First of all, is it debatable if there was a direct diarrhea problem during the study period. Because only 10% of the collected samples were diarrhea samples. Especially is it debatable if there was a real diarrhea problem for all the examined groups of primates, because the diarrhea samples were so different per group/week. The different outcomes between microscopy and multiplexPCR can have multipel causes. In the first place protozoa and especially different species of protozoa are not easy to recognize and discriminate by a microscope, especially not for a beginning researcher which was the case in this research (Polderman & Janse, 2005; Taylor, Coop, & Wall, 2007; Levecke, 2010). Further mistakes could have been made in the process of preparing the samples for microscopic examination, with the direct centrifuge sedimentation flotation method with sucrose. Because the direct centrifuge sedimentation flotation method is a very precise process. Less mistakes can be made in the process of preparing the samples for examination by multiplexPCR, because there are less steps to prepare the samples compared to the preparations for microscopic examination. The only mistake in the process of preparing the samples for examination by multiplexPCR could have been made when measuring the quantity of feces and the quantity of Cobas buffer that was used. Further flaws in the process of preparing the samples for examination by multiplexPCR could have been made in the duration of the time these samples were in the freezer before they could be examined by multiplexPCR.
Some things that can speak against detection of protozoa in samples by multiplexPCR is that samples that are examined in duplo by multiplexPCR in the first week had different outcomes, see attachment 2. Also the multiplexPCR is developed for examination of human feces samples, so it is unclear if it works the same for primate feces samples. In the manual of the multiplexPCR is not described whether the multiplexPCR can be used for animals and or primate stool samples. So it is difficult to say which of these two examination methods can be seen as the golden standard in this research. Concluding that both methods should be combined and could be complementary.
As said before there could be other influences that could have caused the diarrhea, like social stress and medication/sedation. Further research has to be done to examine these influences. Primates can also carry the three species of protozoa without having clinical signs, so maybe that was the case in this population. Or some primates show clinical signs when they carry the protozoa and have diarrhea and other primates do not have diarrhea when they carry the protozoa.
During this research the food and housing and deep litter, were comparable for all the primates during the study period. So in this research it is not possible to say anything about the influence of food and housing, deep litter, on the cause of diarrhea. Further research has to be done to say anything about the influence of food and housing in relation to diarrhea. Although the food could contain ingredients, like beet, that cause a red color of the feces it is not proved that the food also contained ingredients that could have caused diarrhea. See further attachment 1,2 and 4 for the medication/sedation during the study period.
One thing that stands out on the tables and graphs above is the high number of diarrhea, and mush, samples in group S) (*Macaca sylvanus*). Both, by microscopy and by multiplexPCR, there was no significant relation between the protozoa found in group S and the diarrhea in that group. It stays unclear where this higher incidence of diarrhea came from, see attachment 1 and 2. Also the other influences like changes in group structure and medication and/or sedation were comparable for group S as for the other groups. Group S for example did not even get medication and/or sedation that could cause diarrhea, see attachment 4. So also these outcomes cannot explain the high number of diarrhea, and mush, samples in group S. Group J1 (*Macaca fascicularis*) has a higher number of diarrhea samples, it is also unclear where this higher number of diarrhea samples came from.

## Conclusion

There were different species of protozoa found in the feces samples of the primates of Stichting AAP, especially *Giardia*, *Cryptosporidium* and *Entamoeba*. But no link was found between having diarrhea and infection with gastrointestinal protozoa in the primates of Stichting AAP. By microscopic examination many more *Cryptosporidium* and *Giardia* cysts were found. Furthermore there were three species of examined protozoa found and also all combinations of all three species of protozoa were present. The protozoa found most by multiplexPCR was *E. hitolytica/dispar*. The two samples in which *Giardia* was found were too few to include in this study. The percentage of *E. histolytica/dispar* in the diarrhea samples was 10% less than the percentages of *E. histolytica/dispar* in mush and normal feces samples, so without any statistical analysis it could be concluded that there was no link between having diarrhea and having gastrointestinal protozoa by multiplexPCR. See table 1 and attachment 2. (Quantitative) statistical analysis of the samples examined by microscope could not prove a link between gastrointestinal protozoa and diarrhea in the primates either. See table 2 and attachment 1. Other influences that can be seen as a cause of the diarrhea are mentioned, but further research has to be done to examine these influences. So it stays unclear what the cause of the diarrhea of these primates at Stichting AAP was. Unfortunately the diarrhea was not extensive during the test period.
Also the differences between examination by multiplexPCR and by microscopy could not be explained by this research. More information on the sensitivity and sensibility of the multiplexPCR is necessary to explain these differences.

## Attachments

**1**

| Group and time | Freshness | Consistency | Found protozoa and quantity | | |
|---|---|---|---|---|---|
| | | | *Giardia* | *Cryptosporidium* | *Entamoeba* |
| J41 17-10 (9:40) | | Solid | - | - | - |
| L1 17-10 (9:35) | | Solid | - | - | - |
| J31 17-10 (10:30) | | Solid | - | - | - |
| J32 17-10 (10:30) | | Solid | - | - | - |
| J33 17-10 (10:30) | | Solid | - | - | - |
| B11 18-10 (9:15) | average | Solid | - | - | - |
| B12 18-10 (9:15) | average | Solid | - | - | +/- |
| B131 18-10 (11:30) | average | Solid | - | - | - |
| B132 18-10 (11:30) | average | Solid | - | - | - |
| N1 18-10 (11:45) | average | Solid | - | - | - |
| N2 18-10 (11:45) | average | Solid | - | - | - |
| N3 18-10 (11:45) | average | Solid | - | - | - |
| B21 17-10 (11:30) | | Solid | - | - | +/- |
| B22 17-10 (11:30) | | Solid | - | - | +/- |
| B23 17-10 (11:30) | | Diarrhea | - | - | - |
| B24 17-10 (11:30) | | Diarrhea | - | - | - |
| B25 17-10 (11:30) | | Solid | - | - | - |
| A1 19-10 (11:50) | average | Solid | - | - | - |
| A2 19-10 (11:50) | average | Solid | - | - | - |
| A3 19-10 (11:50) | average | Solid | - | - | - |
| A4 19-10 (11:50) | average | Solid | - | - | - |
| J11 17-10 (9:30) | | Solid | - | - | - |
| J12 17-10 (9:35) | | Solid | - | - | - |
| J13 17-10 (9:40) | | Solid | - | - | - |
| J14 17-10 (9:45) | | Solid | - | - | +/- |
| J15 17-10 (9:45) | | Solid | - | - | - |
| | | | | | |
| #2L1 24-10 (9:35) | | | - | - | - |
| #2B21 24-10 (10:00) | | Solid | - | - | - |
| #2B22 24-10 (10:00) | | Solid | - | - | - |
| #2B23 24-10 (9:50) | | Solid | - | - | - |
| #2B24 24-10 (9:40) | | Solid | - | - | - |
| #2N1 24-10 (10:55) | | | - | - | - |
| #2N3 24-10 (10:55) | | | - | - | - |
| #2N4 24-10 (10:55) | | | - | - | - |
| #2J21 24-10 (11:20) | | | - | - | - |
| #2J22 24-10 (11:20) | | | - | - | - |
| #2J11 24-10 (13:05) | | Solid | - | - | - |
| #2J13 24-10 (12:50) | | Solid | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| #2J122 24-10 (13:00) | | Diarrhea | - | - | +/- |
| #2B11 25-10 (10:30) | average | Solid | +/- | +/- | - |
| #2B12 25-10 (10:30) | average | Solid | +/- | - | - |
| #2B131 25-10 (14:15) | average | Solid | - | - | - |
| #2B132 25-10 (14:15) | average | Solid | - | - | - |
| #2A1 25-10 (10:30) | average | Solid | - | - | - |
| #2A2 25-10 (10:30) | average | Solid | - | - | - |
| #2A3 25-10 (10:30) | average | Solid | - | - | - |
| #2A4 25-10 (10:30) | average | Solid | +/- | - | - |
| #2S1 26-10 (15:15) | average | Diarrhea | - | +/- | - |
| #2S2 26-10 (15:15) | average | Diarrhea | - | - | - |
| #2S3 26-10 (15:10) | average | Diarrhea | +/- | +/- | - |
| #2J31 26-10 (12:50) | average | Diarrhea | +/- | - | +/- |
| #2J32 26-10 (12:50) | average | Solid | +/- | - | - |
| #2F1 26-10 (10:30) | average | Solid | +/- | - | - |
| #2F2 26-10 (10:30) | average | Solid | - | +/- | - |
| #2F3 26-10 (10:30) | average | Solid | - | - | - |
| | | | | | |
| #3B21 1-11 | average | Solid | - | - | - |
| #3B22 1-11 | average | Solid | - | +/- | - |
| #3B23 1-11 | average | Solid | - | +/- | - |
| #3B24 1-11 | average | Solid | - | - | - |
| #3N1 1-11 | average | Solid | - | - | - |
| #3N2 1-11 | average | Solid | - | - | - |
| #3N3 1-11 | average | Solid | +/- | - | - |
| #3J11 1-11 | average | Solid | - | - | - |
| #3J12 1-11 | average | Solid | - | - | - |
| #3J13 1-11 | average | Solid | +/- | - | +/- |
| #3J14 1-11 | average | Solid | - | +/- | - |
| #3J15 1-11 | average | Solid | - | - | - |
| #3S1 1-11 | average | Mush | - | - | - |
| #3S2 1-11 | average | Mush | - | - | - |
| #3S3 1-11 | average | Mush | - | - | - |
| #3L1 1-11 | average | Solid | - | - | +/- |
| #3J21 1-11 | average | Solid | +/- | - | - |
| #3J22 1-11 | average | Solid | - | - | +/- |
| #3J31 2-11 | average | Solid | - | - | - |
| #3J32 2-11 | average | Solid | - | - | - |
| #3B11 2-11 | average | Solid | - | +/- | - |
| #3B12 2-11 | average | Solid | - | +/- | - |
| #3B13 2-11 | average | Solid | - | +/- | - |
| #3B14 2-11 | average | Mush | - | - | +/- |
| #3A1 2-11 (12:15) | average | Mush | - | - | - |
| #3A2 2-11 (12:15) | average | Mush | - | - | - |
| #3A3 2-11 (12:15) | average | Solid | - | - | +/- |

| | | | | | |
|---|---|---|---|---|---|
| #3F1 2-11 | average | Solid | - | - | - |
| #3F2 2-11 | average | Solid | - | - | - |
| #3F3 2-11 | average | Mush | - | - | - |
| | | | | | |
| #4A31 9-11 (10:30) | fresh | Mush | - | - | - |
| #4J142 9-11 (10:30) | fresh | Mush | +/- | - | - |
| #4A32 9-11 (10:30) | fresh | Mush | - | +/- | - |
| #4B111 9-11 (10:30) | fresh | Mush | +/- | - | +/- |
| #4J151 9-11 (10:30) | fresh | Mush | +/- | - | +/- |
| #4N1 8-11 | average | Solid | ++ | - | - |
| #4N2 8-11 | average | Solid | - | - | - |
| #4N3 8-11 | average | Solid | - | + | - |
| #4J21 8-11 | average | Solid | - | - | +/- |
| #4J22 8-11 | average | Solid | - | - | - |
| #4L1 8-11 | average | Solid | - | - | - |
| #4S1 8-11 | average | Mush | - | - | + |
| #4S2 8-11 | average | Mush | +/- | - | +/- |
| #4S3 8-11 | average | Solid | - | - | +/- |
| #4B21 9-11 (12:00) | fresh | Mush | + | +/- | - |
| #4B22 9-11 (12:00) | fresh | Mush | ++ | - | - |
| #4B23 9-11 (12:00) | fresh | Solid | + | +/- | +/- |
| #4B24 9-11 (12:00) | fresh | Mush | ++ | - | - |
| #4A1 9-11 (10:30) | fresh | Mush | + | +/- | - |
| #4A2 9-11 (10:30) | fresh | Mush | +/- | +/- | - |
| #4A33 9-11 (10:30) | fresh | Mush | + | - | - |
| #4B112 8-11 | average | Solid | + | - | - |
| #4B12 8-11 | average | Solid | + | - | - |
| #4B13 8-11 | average | Solid | + | - | +/- |
| #4B14 8-11 | average | Solid | ++ | - | - |
| #4J11 8-11 | average | Solid | ++ | - | +/- |
| #4J12 8-11 | average | Solid | ++ | - | - |
| #4J13 8-11 | average | Solid | + | - | +/- |
| #4J141 8-11 | average | Solid | +/- | - | - |
| #4J152 8-11 | average | Mush | + | - | +/- |
| #4F1 8-11 | average | Mush | - | - | - |
| #4F2 8-11 | average | Mush | +/- | - | - |
| #4F3 8-11 | average | Solid | +/- | - | - |
| | | | | | |
| #5J21 15-11 (11:40) | average | Solid | + | +/- | +/- |
| #5J22 15-11 (12:00) | fresh | Solid | + | +/- | +/- |
| #5B11 15-11 (12:00) | fresh | Mush | +/- | +/- | - |
| #5B12 15-11 (11:40) | fresh | Mush | +/- | +/- | +/- |
| #5B131 15-11 (11:30) | fresh | Solid | + | +/- | - |
| #5B132 15-11 (11:30) | fresh | Solid | + | - | + |
| #5J31 15-11 (11:45) | fresh | Mush | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| #5J32 15-11 (11:45) | fresh | Mush | - | - | + |
| #5J33 15-11 (11:45) | fresh | Mush | - | - | - |
| #5A1 15-11 (9:00) | average | Solid | +/- | +/- | - |
| #5A2 15-11 (9:00) | fresh | Solid | + | + | - |
| #5A3 15-11 (9:00) | average | Solid | - | ++ | - |
| #5A4 15-11 (9:00) | average | Mush | +/- | - | - |
| #5B21 15-11 (10:00) | average | Solid | - | +/- | + |
| #5B22 15-11 (10:00) | average | Solid | + | - | + |
| #5B23 15-11 (10:00) | average | Solid | - | - | + |
| #5B24 15-11 (10:00) | average | Solid | +/- | - | +/- |
| #5S1 15-11 (10:00) | fresh | Diarrhea | + | - | - |
| #5S2 15-11 (10:00) | fresh | Diarrhea | - | +/- | - |
| #5S3 15-11 (10:00) | fresh | Diarrhea | - | - | - |
| #5F1 15-11 (9:45) | fresh | Mush | - | +/- | - |
| #5F2 15-11 (9:45) | fresh | Mush | - | - | - |
| #5F3 15-11 (9:45) | fresh | Mush | - | - | - |
| #5N1 16-11 (9:50) | fresh | Solid | - | +/- | - |
| #5N2 16-11 (9:50) | fresh | Solid | +/- | +/- | - |
| #5N3 15-11 (9:35) | fresh | Solid | - | - | - |
| #5J11 15-11 (12:00) | fresh | Solid | +/- | +/- | - |
| #5J12 15-11 (12:00) | fresh | Solid | - | +/- | +/- |
| #5J13 15-11 (12:00) | fresh | Solid | +/- | +/- | +/- |
| #5J14 15-11 (12:00) | fresh | Diarrhea | +/- | +/- | +/- |
| #5J15 15-11 (12:00) | fresh | Solid | - | - | - |
| | | | | | |
| #6R1 21-11 (14:00) | fresh | Solid | +/- | - | +/- |
| #6R2 21-11 (14:00) | fresh | Solid | +/- | - | + |
| #6R3 21-11 (14:00) | fresh | Solid | - | - | + |
| #6R4 21-11 (14:00) | fresh | Solid | +/- | +/- | - |
| #6N1 21-11 (9:30) | fresh | Solid | + | +/- | - |
| #6N2 21-11 (9:30) | fresh | Mush | + | +/- | - |
| #6N3 21-11 (10:30) | fresh | Solid | - | - | - |
| #6A11 22-11 (11:40) | fresh | Solid | + | - | + |
| #6A21 22-11 (11:40) | fresh | Solid | ++ | - | ++ |
| #6J11 22-11 (10:00) | fresh | Solid | +/- | - | ++ |
| #6J12 22-11 (10:00) | fresh | Solid | +/- | +/- | ++ |
| #6J13 22-11 (10:00) | fresh | Diarrhea | - | - | + |
| #6J14 22-11 (10:00) | fresh | Diarrhea | + | - | ++ |
| #6J15 22-11 (10:00) | fresh | Solid | - | - | - |
| #6F1 22-11 (11:40) | fresh | Solid | +/- | - | - |
| #6F2 22-11 (11:40) | fresh | Mush | +/- | - | - |
| #6F3 22-11 (11:40) | fresh | Mush | +/- | - | - |
| #6B11 22-11 (10:30) | fresh | Solid | +/- | - | + |
| #6B12 22-11 (10:30) | fresh | Solid | +/- | - | + |
| #6J21 22-11 (12:15) | fresh | Solid | +/- | - | - |

| | | | | | |
|---|---|---|---|---|---|
| #6J22 22-11 (12:15) | fresh | Solid | +/- | - | + |
| #6S1 22-11 (10:30) | fresh | Diarrhea | +/- | +/- | - |
| #6S2 22-11 (10:30) | fresh | Diarrhea | +/- | - | + |
| #6S3 22-11 (10:30) | fresh | Diarrhea | - | - | +/- |
| #6J31 22-11 (12:30) | fresh | Mush | +/- | - | - |
| #6J32 22-11 (12:30) | fresh | Mush | - | - | - |
| #6J33 22-11 (12:30) | fresh | Diarrhea | - | - | +/- |
| #6A12 28-11 (12:00) | fresh | Solid | - | - | - |
| #6A22 28-11 (11:40) | fresh | Mush | + | - | + |
| #6A3 28-11 (12:00) | fresh | Solid | - | - | - |
| #6A4 28-11 (12:00) | fresh | Solid | +/- | +/- | - |
| | | | | | |
| #7N1 5-12 | average | Solid | ++ | - | + |
| #7N2 5-12 | average | Solid | + | - | + |
| #7J11 5-12 | average | Solid | - | + | - |
| #7J12 5-12 | average | Solid | - | + | - |
| #7J13 5-12 | average | Solid | +/- | - | +/- |
| #7J14 6-12 | average | Solid | - | - | +/- |
| #7J15 6-12 | average | Solid | - | - | + |
| #7N3 6-12 | average | Solid | + | +/- | - |
| #7B131 6-12 | average | Solid | - | - | + |
| #7B132 6-12 | average | Solid | + | - | ++ |
| #7J31 6-12 | average | Solid | + | +/- | + |
| #7J32 6-12 | average | Solid | - | - | - |
| #7J33 6-12 | average | Solid | +/- | +/- | - |
| #7B11 6-12 | average | Solid | - | +/- | - |
| #7B12 6-12 | average | Solid | +/- | - | + |
| #7B21 6-12 | average | Solid | - | - | - |
| #7B22 6-12 | average | Solid | +/- | +/- | - |
| #7B23 6-12 | average | Solid | ++ | - | ++ |
| #7B24 6-12 | average | Solid | + | - | + |
| #7F1 6-12 | average | Solid | +/- | - | - |
| #7F2 6-12 | average | Solid | +/- | - | - |
| #7F3 6-12 | average | Solid | - | +/- | - |
| #7F4 6-12 | average | Solid | - | - | - |

**2**

| Group | CT value found protozoa | | |
|---|---|---|---|
| | *Giardia* spp | *Cryptosporidium* spp | *Entamoeba* |
| #2J12 | >45 | >45 | 29,13 |
| #2J13 | >45 | >45 | 31,70 |
| #2J14 | >45 | >45 | 24,77 |
| #2J121 | >45 | >45 | 23,58 |
| #2B21 | >45 | >45 | 26,00 |
| #2L1 | >45 | >45 | 27,38 |
| #2B22 | >45 | >45 | 30,76 |
| #2B11 | >45 | >45 | >45 |
| #2J21 | >45 | >45 | 24,26 |
| #2B23 | >45 | >45 | 24,73 |
| #2J22 | >45 | >45 | 25,21 |
| #2B24 | >45 | >45 | >45 |
| #2N3 | >45 | >45 | 28,31 |
| #2N4 | >45 | >45 | 27,07 |
| #2B12 | >45 | >45 | 30,83 |
| #2B131 | >45 | >45 | 26,89 |
| blanco | | | |
| #2B132 | >45 | >45 | 26,49 |
| A4 | >45 | >45 | 31,52 |
| A4 | >45 | >45 | 30,59 |
| blanco | | | |
| A3 | >45 | >45 | 25,58 |
| A3 | >45 | >45 | 26,50 |
| A2 | >45 | >45 | >45 |
| A2 | >45 | >45 | >45 |
| A1 | >45 | >45 | >45 |
| #4J12 | >45 | >45 | 29,69 |
| #4J152 | >45 | >45 | 27,43 |
| #4F2 | >45 | >45 | >45 |
| #4F3 | >45 | >45 | >45 |
| #4B13 | >45 | >45 | 29,19 |
| #4B111 | >45 | >45 | 27,11 |
| #4B14 | >45 | >45 | 29,14 |
| #4J141 | >45 | >45 | 29,86 |
| #4J11 | >45 | >45 | 32,63 |
| #4F1 | >45 | >45 | >45 |
| #4B12 | >45 | >45 | 31,21 |
| #4J13 | >45 | >45 | 30,04 |
| #2S3 | >45 | >45 | >45 |
| #2S1 | >45 | >45 | 34,72 |
| #2S2 | >45 | >45 | >45 |

| | | | |
|---|---|---|---|
| #4N3 | >45 | >45 | 24,55 |
| #4N1 | >45 | >45 | >45 |
| #4N2 | >45 | >45 | >45 |
| #4A32 | >45 | >45 | >45 |
| #4A31 | >45 | >45 | >45 |
| #4J151 | >45 | >45 | 27,71 |
| #4B112 | >45 | >45 | 30,95 |
| #4J142 | >45 | >45 | 34,84 |
| #4A1 | >45 | >45 | >45 |
| #4A2 | >45 | >45 | 25,02 |
| #4A33 | >45 | >45 | 31,32 |
| #4L1 | >45 | >45 | 28,64 |
| #4J21 | >45 | >45 | 26,47 |
| #4J22 | >45 | >45 | 29,85 |
| #4S1 | >45 | >45 | 25,11 |
| #4S2 | >45 | >45 | 25,49 |
| #4S3 | >45 | >45 | 32,19 |
| #4B21 | >45 | >45 | >45 |
| #4B22 | >45 | >45 | 33,50 |
| #4B23 | >45 | >45 | 27,78 |
| #4B24 | >45 | >45 | >45 |
| #2F3 | >45 | >45 | 27,21 |
| #2F2 | >45 | >45 | >45 |
| #2F1 | >45 | >45 | >45 |
| #2J31 | >45 | >45 | >45 |
| #2J32 | >45 | >45 | >45 |
| #3N1 | >45 | >45 | 30,58 |
| #3F2 | >45 | >45 | 36,54 |
| #3F3 | 36,83 | >45 | 33,79 |
| #3S3 | >45 | >45 | >45 |
| #3S1 | >45 | >45 | >45 |
| #3S2 | >45 | >45 | 35,00 |
| #3N2 | >45 | >45 | 27,21 |
| #3N3 | >45 | >45 | 27,77 |
| #3A1 | >45 | >45 | 29,06 |
| #3A2 | >45 | >45 | 34,28 |
| #3A3 | >45 | >45 | 31,95 |
| #3F1 | >45 | >45 | 26,76 |
| #3L1 | >45 | >45 | 27,70 |
| #3B21 | >45 | >45 | 26,19 |
| #3B22 | >45 | >45 | 29,00 |
| #3B23 | >45 | >45 | >45 |
| #3B24 | >45 | >45 | >45 |
| #3J15 | >45 | >45 | 25,03 |
| #3B11 | >45 | >45 | 29,21 |

| | | | |
|---|---|---|---|
| #3B12 | >45 | >45 | >45 |
| #3B13 | 35,79 | >45 | 30,56 |
| #3J21 | >45 | >45 | 30,63 |
| #3J22 | >45 | >45 | 24,12 |
| #3J31 | >45 | >45 | >45 |
| #3J32 | >45 | >45 | >45 |
| #3J13 | >45 | >45 | 29,45 |
| #3J14 | >45 | >45 | >45 |
| #3J11 | >45 | >45 | 30,69 |
| #3J12 | >45 | >45 | >45 |
| #3B14 | >45 | >45 | >45 |
| #6N2 | >45 | >45 | 34,65 |
| #6R4 | >45 | >45 | 32,67 |
| #6R1 | >45 | >45 | 31,73 |
| #6R2 | >45 | >45 | 32,67 |
| #6N1 | >45 | >45 | 34,82 |
| #6R3 | >45 | >45 | 33,15 |
| #5N2 | >45 | >45 | 29,14 |
| #5N1 | >45 | >45 | 28,90 |
| #5J21 | >45 | >45 | 25,13 |
| #5J22 | >45 | >45 | 24,52 |
| #5B11 | >45 | >45 | 31,83 |
| #5B12 | >45 | >45 | 33,44 |
| #5F1 | >45 | >45 | 30,96 |
| #5B131 | >45 | >45 | 34,08 |
| #5B132 | >45 | >45 | 35,85 |
| #5N3 | >45 | >45 | 25,51 |
| #5J31 | >45 | >45 | >45 |
| #5J32 | >45 | >45 | 35,50 |
| #5J33 | >45 | >45 | 31,48 |
| #5A1 | >45 | >45 | >45 |
| #5A2 | >45 | >45 | >45 |
| #5A3 | >45 | >45 | >45 |
| #5A4 | >45 | >45 | 31,79 |
| #5B21 | >45 | >45 | 28,47 |
| #5B22 | >45 | >45 | 33,14 |
| #5B23 | >45 | >45 | 34,26 |
| #5B24 | >45 | >45 | 26,93 |
| #5S1 | >45 | >45 | 26,06 |
| #5S2 | >45 | >45 | 30,03 |
| #5S3 | >45 | >45 | 26,02 |
| #5F2 | >45 | >45 | 35,35 |
| #5F3 | >45 | >45 | 31,54 |
| #5J11 | >45 | >45 | 35,32 |
| #5J12 | >45 | >45 | 28,61 |

| | | | |
|---|---|---|---|
| #5J13 | >45 | >45 | 27,72 |
| #5J14 | >45 | >45 | 28,26 |
| #5J15 | >45 | >45 | 27,14 |
| #7B132 | >45 | >45 | 26,45 |
| #7B11 | >45 | >45 | 30,87 |
| #7F3 | >45 | >45 | 30,55 |
| #7N3 | >45 | >45 | >45 |
| #7F4 | >45 | >45 | 31,82 |
| #7B23 | >45 | >45 | 29,09 |
| #7F1 | >45 | >45 | 29,72 |
| #7B24 | >45 | >45 | 32,01 |
| #7B131 | >45 | >45 | 25,22 |
| #7J31 | >45 | >45 | 33,85 |
| #7J32 | >45 | >45 | 29,27 |
| #7B12 | >45 | >45 | 32,95 |
| #7B21 | >45 | >45 | 34,61 |
| #7F2 | >45 | >45 | 32,77 |
| #7B22 | >45 | >45 | 34,31 |
| #7J33 | >45 | >45 | 29,73 |
| #6S1 | >45 | >45 | 27,63 |
| #6J32 | >45 | >45 | >45 |
| #6S3 | >45 | >45 | 27,25 |
| #6J22 | >45 | >45 | 24,15 |
| #6J13 | >45 | >45 | 26,32 |
| #6J31 | >45 | >45 | >45 |
| #6B11 | >45 | >45 | 25,76 |
| #6A21 | >45 | >45 | 27,74 |
| #6J11 | >45 | >45 | 26,90 |
| #6J15 | >45 | >45 | 26,58 |
| #6A11 | >45 | >45 | 29,31 |
| #6J12 | >45 | >45 | 27,68 |
| #6N3 | >45 | >45 | 35,93 |
| #6F1 | >45 | >45 | 27,60 |
| #6S2 | >45 | >45 | 26,61 |
| #6B12 | >45 | >45 | 33,10 |
| #6J33 | >45 | >45 | >45 |
| #6J21 | >45 | >45 | 35,96 |
| #6J14 | >45 | >45 | 24,10 |
| #6F2 | >45 | >45 | 33,77 |
| #6F3 | >45 | >45 | 34,86 |
| #6A22 | >45 | >45 | >45 |
| #6A12 | >45 | >45 | >45 |
| #6A4 | >45 | >45 | >45 |
| #6A3 | >45 | >45 | >45 |
| #7J11 | >45 | >45 | 30,61 |

| | | | |
|---|---|---|---|
| #7N1 | >45 | >45 | 30,68 |
| #7J13 | >45 | >45 | 26,79 |
| #7J12 | >45 | >45 | 28,86 |
| #7J14 | >45 | >45 | 30,88 |
| #7N2 | >45 | >45 | 29,91 |
| #7J15 | >45 | >45 | 27,74 |
| #2A4 | >45 | >45 | 34,20 |
| #2A3 | >45 | >45 | 29,21 |
| #2A2 | >45 | >45 | 33,63 |
| #2A1 | >45 | >45 | >45 |
| A1 | >45 | >45 | >45 |
| N1 | >45 | >45 | 32,27 |
| N1 | >45 | >45 | 33,92 |
| N2 | >45 | >45 | 38,48 |
| N2 | >45 | >45 | 33,49 |
| N3 | >45 | >45 | 36,51 |
| N3 | >45 | >45 | 34,78 |
| B11 | >45 | >45 | 26,90 |
| B11 | >45 | >45 | 28,73 |
| B12 | >45 | >45 | 23,32 |
| B12 | >45 | >45 | 23,30 |
| B131 | >45 | >45 | 33,60 |
| B131 | >45 | >45 | 35,61 |
| B132 | 36,11 | >45 | 36,01 |
| B132 | 36,62 | >45 | >45 |
| B21 | >45 | >45 | 21,48 |
| B21 | >45 | >45 | 22,65 |
| B22 | >45 | >45 | 33,93 |
| B22 | >45 | >45 | 32,86 |
| B23 | >45 | >45 | 22,47 |
| B23 | >45 | >45 | 23,54 |
| B24 | >45 | >45 | 24,89 |
| B24 | >45 | >45 | 23,75 |
| L1 | >45 | >45 | 25,69 |
| L1 | >45 | >45 | 25,27 |
| J41 | >45 | >45 | 21,87 |
| J41 | >45 | >45 | 20,91 |
| J11 | >45 | >45 | 26,35 |
| J11 | >45 | >45 | 24,26 |
| J12 | >45 | >45 | 27,56 |
| J12 | >45 | >45 | 27,23 |
| J13 | >45 | >45 | 25,27 |
| J13 | >45 | >45 | 25,84 |
| J14 | >45 | >45 | 25,97 |
| J14 | >45 | >45 | 25,59 |

| | | | |
|---|---|---|---|
| J15 | >45 | >45 | 27,33 |
| J15 | >45 | >45 | 27,02 |
| J16 | >45 | >45 | 22,01 |
| J16 | >45 | >45 | 21,88 |
| J31 | >45 | >45 | >45 |
| J31 | >45 | >45 | >45 |
| J33 | >45 | >45 | 26,84 |
| J33 | >45 | >45 | 28,01 |
| J32 | >45 | >45 | >45 |
| J32 | >45 | >45 | >45 |

**3**

| Number | Protozoa found by microscope | | | Protozoa found by PCR | | | Number of samples the same |
|---|---|---|---|---|---|---|---|
| | *Giardia* | *Cryptosporidium* | *Entamoeba* | *Giardia* | *Cryptosporidium* | *Entamoeba histolytica/dispar* | |
| J41 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| L1 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| J31 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| J32 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| J33 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| B11 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| B12 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| B131 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| B132 | 0 | 0 | 0 | 1 | 0 | 1 | 4 |
| N1 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| N2 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| N3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| B21 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| B22 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| B23 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| B24 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| A1 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| A2 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| A3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| A4 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| J11 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| J12 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| J13 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| J14 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| J15 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| | | | | | | | 118 |
| #2L1 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2B21 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2B22 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2B23 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2B24 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| #2N3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2N4 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2J21 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2J22 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2J13 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2J14 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2J12 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2B11 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| #2B12 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #2B131 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2B132 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2A1 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2A2 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2A3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #2A4 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #2S1 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| #2S2 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| #2S3 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| #2J31 | 1 | 0 | 1 | 0 | 0 | 0 | 2 |
| #2J32 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| #2F1 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| #2F2 | 0 | 1 | 0 | 0 | 0 | 0 | 4 | |
| #2F3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| | | | | | | | | 96 |
| #3B21 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3B22 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | |
| #3B23 | 0 | 1 | 0 | 0 | 0 | 0 | 4 | |
| #3B24 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #3N1 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3N2 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3N3 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | |
| #3J11 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3J12 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #3J13 | 1 | 0 | 1 | 0 | 0 | 1 | 4 | |
| #3J14 | 0 | 1 | 0 | 0 | 0 | 0 | 4 | |
| #3J15 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3S1 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #3S2 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3S3 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #3L1 | 0 | 0 | 1 | 0 | 0 | 1 | 6 | |
| #3J21 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | |
| #3J22 | 0 | 0 | 1 | 0 | 0 | 1 | 6 | |
| #3J31 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #3J32 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #3B11 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | |
| #3B12 | 0 | 1 | 0 | 0 | 0 | 0 | 4 | |
| #3B13 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | |
| #3B14 | 0 | 0 | 1 | 0 | 0 | 0 | 4 | |
| #3A1 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3A2 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3A3 | 0 | 0 | 1 | 0 | 0 | 1 | 6 | |
| #3F1 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #3F2 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #3F3 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | |
| | | | | | | | | 126 |
| #4A31 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #4J142 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | |
| #4A32 | 0 | 1 | 0 | 0 | 0 | 0 | 4 | |
| #4B111 | 1 | 0 | 1 | 0 | 0 | 1 | 4 | |
| #4J151 | 1 | 0 | 1 | 0 | 0 | 1 | 4 | |
| #4N1 | 1 | 0 | 0 | 0 | 0 | 0 | 4 | |
| #4N2 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| #4N3 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | |
| #4J21 | 0 | 0 | 1 | 0 | 0 | 1 | 6 | |
| #4J22 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #4L1 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| #4S1 | 0 | 0 | 1 | 0 | 0 | 1 | 6 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| #4S2 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #4S3 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| #4B21 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| #4B22 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #4B23 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| #4B24 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| #4A1 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| #4A2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #4A33 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #4B112 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #4B12 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #4B13 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #4B14 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #4J11 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #4J12 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #4J13 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #4J141 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #4J152 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #4F1 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| #4F2 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| #4F3 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| #5J21 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| #5J22 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| #5B11 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #5B12 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| #5B131 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #5B132 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #5J31 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| #5J32 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| #5J33 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #5A1 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| #5A2 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| #5A3 | 0 | 1 | 0 | 0 | 0 | 0 | 4 |
| #5A4 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #5B21 | 0 | 1 | 1 | 0 | 0 | 1 | 4 |
| #5B22 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #5B23 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| #5B24 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #5S1 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #5S2 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| #5S3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #5F1 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| #5F2 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #5F3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #5N1 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| #5N2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #5N3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #5J11 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #5J12 | 0 | 1 | 1 | 0 | 0 | 1 | 4 |
| #5J13 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| #5J14 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| #5J15 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| | | | | | | | 90 |
| #6R1 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6R2 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6R3 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| #6R4 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #6N1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #6N2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #6N3 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #6A11 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6A21 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6J11 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6J12 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| #6J13 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| #6J14 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6J15 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #6F1 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #6F2 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #6F3 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #6B11 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6B12 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6J21 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #6J22 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6S1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #6S2 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #6S3 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| #6J31 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| #6J32 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| #6J33 | 0 | 0 | 1 | 0 | 0 | 0 | 4 |
| #6A12 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| #6A22 | 1 | 0 | 1 | 0 | 0 | 0 | 2 |
| #6A3 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| #6A4 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| | | | | | | | 106 |
| #7N1 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #7N2 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #7J11 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| #7J12 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| #7J13 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #7J14 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| #7J15 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| #7N3 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| #7B131 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| #7B132 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #7J31 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| #7J32 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #7J33 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #7B11 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| #7B12 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #7B21 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| #7B22 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| #7B23 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #7B24 | 1 | 0 | 1 | 0 | 0 | 1 | 4 |
| #7F1 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #7F2 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| #7F3 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| #7F4 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |

74

726

Total outcomes protozoa : 199 volution 2 volution 3 = 1194.
Found outcomes protozoa the same between examination by multiplexPCR and microscopic examination: 726.
Percentage of protozoa the same: 726/1194 volution 100 = 60,8%.

**4**

| **Species** | **Group** | Name | Ivermectin (date) | Other treatment |
|---|---|---|---|---|
| **Crab eating macaques** | **Jakob (5)** | Jakob | 11/9 | |
| | | Bamar | 11/9 | |
| | | Nenek | 11/9 | 9/11 sedation |
| | | Lunak | 11/9 | 9/11 sedation |
| | | Ratu | 11/9 | 9/11 sedation |
| | **Roland (2)** | Roland | 10/9 | 11/11 metacam, 11 days (d) |
| | | Paham | 29/4 | |
| | **Besar (2)** | Besar | 10/9 | 18/10 Clavubactin/metacam 16 d |
| | | Kurus | 10/9 | |
| | **Berani (4)** | Berani | 11/9 | 9/11 sedation |
| | | Kawan | 11/9 | 9/11 sedation |
| | | Asing | 20/7 | 15/10 clavubactin/metacam 7d |
| | | Istri | 15/6 | 31/10 clavubactin/metacam 22d |
| | **Joelie (2)** | Joelie | 10/9 | 20/11 metacam 16 d/clavubactin 8d |
| | | Pantai | 10/9 | |
| **Barbary macaques** | **Fugitive (3)** | Fugitive | 10/9 | |
| | | Chiviruca | 10/9 | 4/10 clavubactin/metacam 14 d |
| | | Fellah | 10/9 | |
| | **Nepal (3)** | Nepal | 10/9 | |
| | | Shizo | 10/9 | |
| | | Xuda | 10/9 | 17/10 clavubactin/metacam 18 d, 8/11 clavub. 8d |
| | **Sjeik (3)** | Sjeik | 10/9 | |
| | | Kaïd | 10/9 | |
| | | Meimond | 12/9 | |
| | **Anou (3)** | Anou | 10/9 | |
| | | Mouki | 10/9 | 18/11 clavub/metacam 11 d |
| | | Yilda | 10/9 | 9/11 sedation |
| | | Nanushka | 10/9 | 11/10 metacam 6 d |
| | **Jonk (2)** | Jonk | 10/9 | |
| | | Ndidi | 10/9 | 27/10 metacam 5 d |
| | **Lùlu (1)** | Lùlu | 10/9 | |

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