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Original Article

Detection of *Hepatocystis* sp. Infection in Vervet Monkeys (*Chlorocebus Pygrythrus*) Imported from Tanzania Using Molecular and Microscopic Methods

Hablolvarid, M. H[1 *], Habibi, Gh[2]

1. Department of Animal Pathology and Epidemiology, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
2. Department of Parasite Vaccine Research and Production, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

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Corresponding Author: h.hablolvarid@rvsri.ac.ir

Abstract

The present study examined and reported *Hepatocystis sp.* infection in wild-caught vervet monkeys imported from Tanzania into the Razi vaccine and serum research institute (RVSRI). Polymerase chain reaction (PCR) targeting of 18S rRNA gene, followed by sequencing, Basic Local Alignment Search Tool (BLAST), and phylogenetic studies revealed that 82.8% of the imported monkeys were infected with *Hepatocystis*. Nevertheless, as illustrated by a routine parasitological examination of blood smears and histopathological examination of liver collected samples, the rates of *Hepatocystis* infection were obtained at 33.9% and 38.1%, respectively. Two isolated 18S rRNA gene sequences of *Hepatocystis* sp. from Tanzanian vervet monkeys were registered under the accession numbers OM281567 and OM281564 in GenBank. Although *Hepatocystis* infections do not cause clinical disease, they may interfere with the research data. The results of the current study pointed out that after proper nutrition and implementation of good physical environmental conditions for 3-4 months, the imported monkeys obviously gained weight and most of their hematological parameters, even in the presence of the parasite, returned to the normal levels and the experimental monkeys would be ready for use in studies.

Keywords: *Hepatocystis* sp., Molecular test, parasitological examination, Vervet monkey

1. Introduction

The assessment of the role of the wildlife animals in multi-host diseases is very valuable since it provides scientists with very useful information about virulence, host specificities, as well as genetic evolution and differentiation of pathogens (1). Free non-human primates (NHPs) in nature are now recognized as potential reservoirs of some human pathogens. This is especially important in the case of animals which live in the vicinity of human habitats. In fact, NHPs as a possible reservoir of some zoonotic pathogens have provided valuable clues to the origin and evolution of some human pathogens, such as *Plasmodium falciparum* (1-3). Furthermore, the presence of protozoan parasites in NHPs can lead to significant and unexpected effects on medical research by altering the immunological profile of the test animals (4). Therefore, there is always a need to implement surveillance programs on NHPs that live in the vicinity of humans and primates used in medical research.

A study was conducted in "Razi vaccine and serum research institute (RVSRI)" to monitor haemoprotozoan parasites of wild-caught vervet monkeys imported from Tanzania (project number: 2-

18-18-031-95035) for assesment of oral polio vaccine monkey neurovirulence test (MNVT). The results of the stated study approved the presence of some species of parasites belonging to both families of Plasmodiidae and Babesiidae using molecular and microscopic methods. The present study aimed to detect haemoprotozoan parasites infestation including; *Hepatocystis* sp. and piroplasms, in imported vervet monkeys by molecular and microscopic methods. In this article, we described the detection of *Hepatocystis* sp. infection in imported monkeys. Vervet monkey (*Chlorocebus pygerythrus*) is one of the most common research NHPs. These monkeys live in a variety of environmental conditions, including rainforests, forests, and plains known as the Savannah region in sub-Saharan Africa, over a wide range between Senegal in the west, as well as Ethiopia in the east and south.

Hepatocystis is a genus of parasites which are transmitted by the bite of the midges of the genus *Culicoides*. Both the phylogenetically close genera of *Hepatocystis* and *Plasmodium* belong to the phylum Apicomplexa, the order Coccidia, and the family Plasmodiidae. Hosts of *Hepatocystis* include old-world primates, bats, hippopotamus, and squirrels. Nonetheless, this genus is not found in the New World. There are currently 25 recognized species in this genus. The type species which is *Hepatocystis kochi*, formerly known as *Plasmodium Kochi*, was first observed as macroscopic cysts in the liver of vervet monkeys (5). Baboons and vervet monkeys have been reported as natural hosts of *Hepatocystis Kochi* in Kenya (6). The parasite does not cause clinical diseases but may interfere with the research data (7). In fact, Interference occurs when a substance or process falsely alters assay results. This may lead to an incorrect diagnosis, as well as inappropriate further tests or treatments with potentially unfavorable outcomes. *Hepatocystis Kochi* is the only type of *Hepatocystis* parasite whose life cycle is well known. The life cycle of *Hepatocystis* parasites is similar to that of the genus *Plasmodium*, except that the asexual stage (schizogony) takes place in the host liver instead of red blood cells. After biting of the carrier of the sexual stage of the parasite, *Culicoides* midges, the sporozoites enter the body and migrate to the liver where schizogony takes place, and typically cysts called merocysts forms within the liver parenchyma (7, 8). The mature merocysts are visible to the naked eye on the liver surface. Microscopically, a significant inflammatory response to the merocysts may or may not be present. Released merozoites from merocysts invade other hepatocytes or erythrocytes. The merozoites forms gametocytes within the erythrocytes. Mature gametocytes are larger than a normal erythrocyte and stain poorly, compared to other protozoa (8).

The main objective of this study was to detect *Hepatocystis* sp. infections in wild-caught vervet monkeys imported from Tanzania into the Razi vaccine and serum research institute, Karaj, Iran. This study reports a series of molecular and microscopic investigations focusing on evaluating the occurrence of *Hepatocystis* sp.

2. Materials and Methods

2.1. Animals

A total of 65 wild-caught vervet monkeys were imported from Tanzania into RVSRI for MNVT in 2015. All the purchased monkeys were aged 2-5 years and weighed 1.5-3 Kg. These monkeys were housed in the quarantine of primates in RVSRI for three months from transportation, provided with balanced environmental conditions (e.g., light and temperature), and fed a diet consisting of various nutrients that supplied all nutritional needs of the tested animals. During the quarantine period, a tuberculin skin test (TST) was performed three times on all monkeys, two weeks apart, yielding negative results. Moreover, the ELISA (enzyme-linked immunosorbent assay) method for the detection of simian immunodeficiency virus (SIV) infection was applied twice on collected samples of monkeys' serums, demonstrating negative results in all cases.

2.2. Weighting and Measurement of Hematological Parameters

To this end, 40 days after arrival, all the monkeys were anesthetized by intramuscular (IM) injection of 10 mg/kg of body weight ketamine solution. The anesthetized animals were initially weighed; subsequently, their blood sample was drawn through the femoral vein using disposable syringes. A total of 3 ml of blood was taken from each animal and drained in one sampling tube containing Ethylenediaminetetraacetic acid (EDTA) anticoagulant. A complete blood count (CBC) was performed on each blood sample, and the blood was then kept at -20°C until molecular testing. Hemoglobin level, hematocrit, red blood cell (RBC) count, erythrocyte index, platelet count, and white blood cell count were measured and reported by "Sysmex XT-1800i, japan" hematology analyzer, and differential white blood cell count was performed manually. One more time on day 80 after arrival, blood samples were drawn from 11 remaining alive monkeys, and blood parameters were evaluated and compared with the results obtained on day 40. Meanwhile, two thick and thin blood smears were prepared using the conventional method on glass slides from each blood sample. After fixation by methanol, the blood smears were stained by the Giemsa method and examined by a light microscope (LM) for the identification of hemoprotozoan parasites.

2.3. Histopathological Examination

After sacrificing the monkeys at the end of MNVT, the liver samples from animals were collected for histopathological examination and kept in a 10% formalin solution. Upon the completion of fixation, the resected samples were processed and embedded in paraffin; thereafter, 5-µm-thick tissue sections were cut and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

2.4. Molecular Tests

2.4.1. DNA Isolation

For molecular examination of blood samples, DNA was extracted by proteinase K and phenol-chloroform method; subsequently, the DNA samples were stored in a freezer at -20°C for polymerase chain reaction (PCR) (9). The optimization of PCR was performed using different PCR programs by a gradient thermal cycler, PCR mixes, as well as different concentrations of enzymes, specific DNA primers, and different replication cycles.

2.4.2. Polymerase Chain Reaction

The detection of *Hepatocystis* DNA was performed using specific PCR assays to amplify the 18S rRNA gene sequence. The assay used the Plgen 18SF: 5'- TAT TAA AAT TGT TGC AGT TAA ARC G -3' and a reverse primer Plgen18SR: 5'- ATC TGT CAA TCC TAC TCT TGT C -3' the 734 bp fragment of 18S rRNA gene sequence (10), a semi-nested forward primer, nP18F 5'- AGA TTT TCT GGA GAC AAA CAA CTG C, was also applied to increase the sensitivity of the PCR (360 bp). Each PCR contained DNA, 10 pmole forward and reverse primers, as well as2X PCR Super Master Mix (Yekta Tajhiz Azma, Iran). Amplification used cycles of 95°C for 90 sec, 35 cycles of 94°C for 10 sec, 52°C for 20 sec, and 72°C for 30 sec; final extension at 72 °C for 5 min; and holding at 4 °C by gradient thermal cycler (Corbett CGI-96, Palm-Cycler Thermal Cycler, Australia).

2.4.3. Polymerase Chain Reaction product detection and sequencing

The PCR products were electrophoresed on 1.5% agarose gel and visualized through in-gel staining using RedGel (Biotium, Inc. Hayward, CA) and visualized by UV Transillumination (Uvidoc, Gel Documentation System, Cambridge, UK). The PCR products were cleaned up and extracted from agarose gel and submitted for bidirectional DNA sequencing using the chain termination method (Takapouzist, Bioneer, South Korea).

2.4.4. Nucleotide Sequencing and Phylogenetic Analysis

The PCR product for the *Hepatocystis* 18S rRNA gene was sequenced and identified using the Basic Local Alignment Search Tool (BLAST) at the

National Center for Biotechnology Information (NCBI). The DNA sequences of the 18S rRNA gene were obtained from studied *Hepatocystis* samples and 19 sequences of 18S rRNA gene sequences, including 6 *Hepatocystis* spp., 12 *Plasmodium* spp., and one *Theileria annulata* as outgroup were accessed from GenBank. The sequences were aligned by Clustal W multiple alignments program. The phylogenetic tree was constructed by the DNADist Neighbor-Joining method (MEGA 7.0 Software).

3. Results

3.1. Results Of Weighting And Complete Blood Count

The results of assessing the weight and blood parameters of 52 monkeys on days 40 and 80 after arrival are displayed in tables 1 and 2.

Table 1. Mean and range of the weight and blood parameters of the 52 imported vervet monkeys on day 40 after arrival into RVSRI

Genus (No)	Weight (Gram)	WBC ($\times10^3$)	RBC ($\times10^6$)	Platlet ($\times10^3$)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Male and female (52)	1814±503 1000-2900	12.5±11.7 3.76-75.2	4.7±0.42 3.77-5.73	430±182 9-884	10.5±0.97 8.3-13.1	36.3±2.9 29.6-44.8	77.4±2.85 70.2-84.7	22.4±1.12 19.6-25.4	29±0.95 27.5-31.4
Male (30)	1867±501 1100-2900	12.57±9.55 3.76-47.72	4.75±0.4 3.77-5.65	451±176 28.2-884	10.5±0.84 8.5-11.7	36.2±2.57 29.6-39.8	76.5±2.6 70.2-83.6	22.1±0.99 19.6-24.1	28.9±0.85 27.5-30.9
Female (22)	1744±506 1000-2600	12.4±14.3 5.87-75.21	4.63±0.45 3.87-5.73	401±189 9-732	10.6±1.14 8.3-13.1	36.3±3.36 29.9-44.8	78.6±2.8 75.5-84.7	22.9±1.14 21.1-25.4	29.1±1.1 27.5-31.4

Table 2. Increases in mean and range of the weight and blood parameters of the 11 imported vervet monkeys due to better nutrition and reduction of stress level on days 80 after arrival into RVSRI, in comparison with day 40

Genus (No)	Time	Weight (Gram)	WBC ($\times10^3$)	RBC ($\times10^6$)	Platelet ($\times10^3$)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Male and female (11)	day 40	1950±232.6 1700-2500	12.3±8.85 5.76-39.4	4.8±0.3 4.2-5.3	466±143 159-662	10.9±0.73 9.6-12.1	37.1±2 33.4-40.9	78±2.32 75.7-83.6	22.8±0.6 21.7-23.5	30.2±2.83 28-38.8
	day 80	2258±375.8 1880-3050	7.34±1.65 4.92-9.98	5.2±0.4 4.6-5.6	459±117 281-582	12.4±0.67 11.4-13.2	40.5±2.52 36.1-43.2	78.7±2.37 74.6-82.4	24.1±0.83 22.4-25.3	30.6±1.17 28.4-32.1
Male (8)	day 40	1994±258 1700-2500	13±13.2 5.76-39.4	4.8±0.56 4.45-5.18	462±101.6 321-591	10.9±0.57 9.9-11.7	37.13±1.43 34.9-39.5	77.5±2.43 75.7-83.6	22.74±0.68 21.7-23.5	30.6±3.21 28-38.8
	day 80	2351±406 1880-3050	7.67±1.85 4.92-9.98	5.2±0.35 4.59-5.58	450±104 319-578	12.46±0.64 11.6-13.2	40.9±2.2 37.8-43.2	78.7±2.66 74.6-82.4	23.97±0.97 22.4-25.3	30.49±1.34 28.4-32.1
Female (3)	day 40	1883±47 1800-1900	9.7±2 6.92-11.2	4.67±0.45 4.15-5.26	463±218 159-662	10.8±1 9.6-12.1	37.1±3.1 33.4-40.9	79.4±1.2 77.8-80.5	23.1±0.1 23-23.2	29.1±0.37 28.7-29.6
	day 80	2010±53 1950-2050	6.45±0.3 6.24-6.76	5±0.38 4.71-5.44	481±174 281-582	12.2±0.85 11.4-13.1	39.5±3.5 36.1-43.1	78.6±1.8 76.6-80	24.3±.27 24.1-24.6	30.9±0.6 30.4-31.6

3.2 Results of Parasitological Examination

The microscopic evaluation of Giemsa-stained blood smears of the imported vervet monkeys that was performed by two examiners, A and B, using an LM for the identification of *Hepatocystis* and piroplasms, revealed the presence of gametocyte stages of *Hepatocystis* and intraerythrocytic asexual stages of piroplasms parasites (Table 3). Accordingly, in 31 (58.5%) positive cases reported by examiner A, both the ring and gametocyte forms in 14 (26.4%) cases, while in 13 (24.5%) and 4 (7.5%) cases, only ring and gametocyte forms were observed, respectively. Therefore, based on the gametocytes observation criteria, the total rate of *Hepatocystis* infection was obtained at 33.9% (26.4%+7.5%) (Figures 1-3). Regarding the results that were reported as suspicious, in all cases, only ring shapes were observed.

Table 3. Results of microscopic evaluation of Giemsa-stained blood smears of the vervet monkeys for the detection of *Hepatocystis* and piroplasms by two examiners

Examiners	Negative cases/total (%)	Suspected cases/total cases (%)	Positive cases/total cases (%)
A	19.53 (35.9%)	3.53 (5.7%)	31.53 (58.5%)

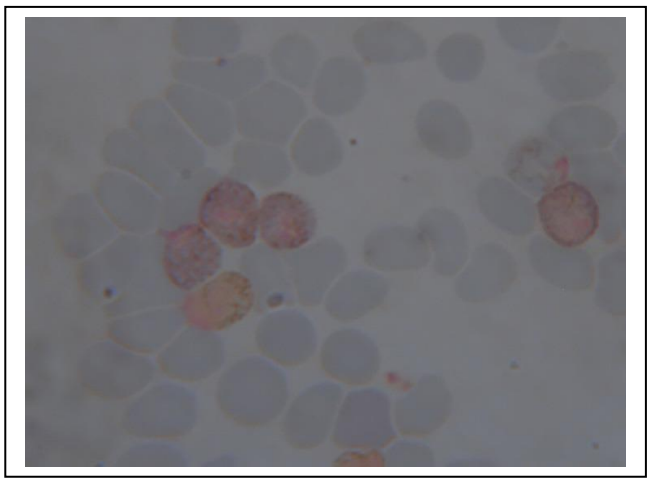

Figure 1. Presence of several gametocytes of the *Hepatocystis* observed in the bloodstream of a vervet monkey (Giemsa-staining×1200)

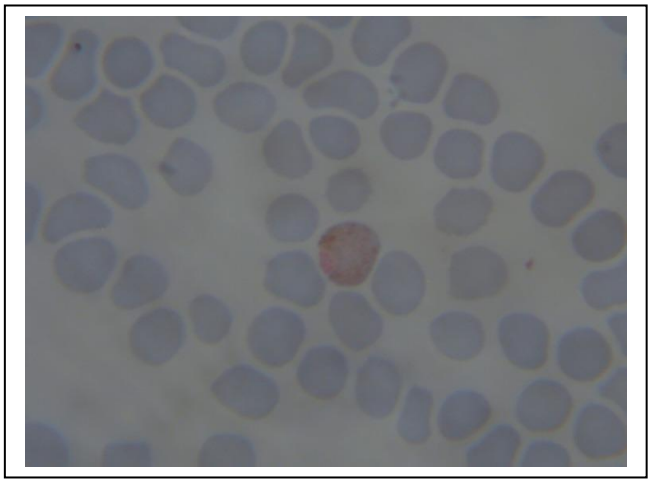

Figure 2. A microgemocyte of *Hepatocystis* with a large pale pink nucleus (Giemsa- staining×1200)

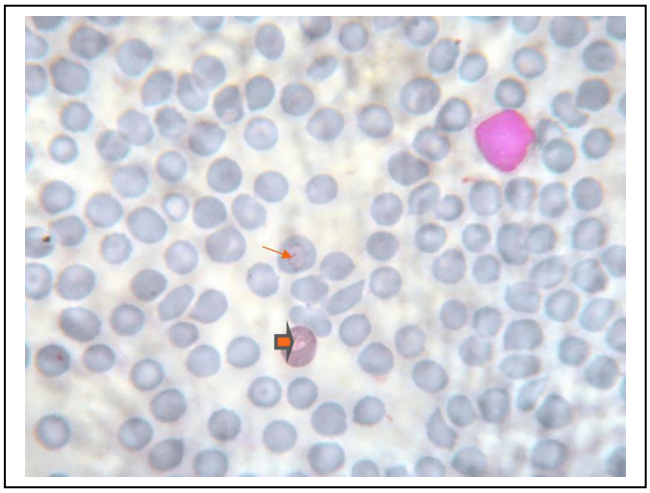

Figure 3. A macrogametocyte of *Hepatocystis* with a nucleus of dense chromatin located in a pale pink area in the peripheral blood of a vervet monkey (arrowhead) is observed. A ring form also is also depicted in this figure (arrow). (Giemsa- staining×1000)

3.3 Results of Histopathological Examination

Histopathological evaluation of the liver tissue specimen of the 42 vervet monkeys for the presence of *Hepatocystis* merocysts and granulomatous reactions revealed that in 16 cases (38.1%), there were foci of *Hepatocystis* merocysts, with or without granulomas reactions, which was suggestive of *Hepatocystis* as the etiologic agent causing the lesions (Figures 4 and 5).

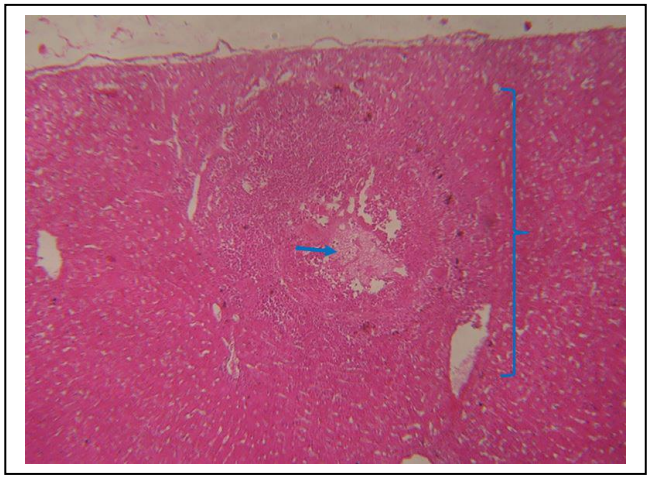

Figure 4. Hepatocystosis, liver of a vervet monkey-granulomatous reaction (bracket) around a degenerated merocyst (arrow) (H&E×100)

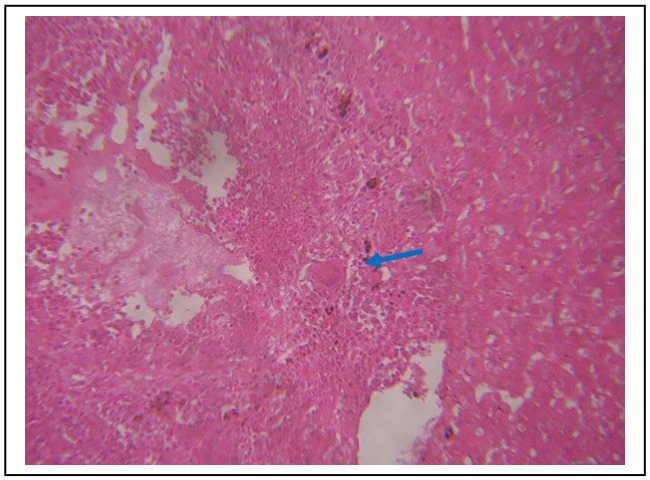

Figure 5. Hepatocystosis, liver of a vervet monkey, the presence of a Langhans giant cell (arrow) inside granulomatous reaction around a degenerated merocyst (H&E×200)

3.4 Results of Molecular Examination

The results of PCR on blood samples obtained from 29 vervet monkeys for the detection of *Hepatocystis* sp. infection pointed out that in 14 (48.3%) cases, there was a mixed infection of *Hepatocystis* with piroplasms; nonetheless, a single infection with *Hepatocystis* was detected in 10 (34.5%) cases. Furthermore, no hemoprotozoan parasite infection was found in 5 (17.2%) cases. These data indicated that 82.8% (48.3+34.5) of the imported vervet monkeys were infected with *Hepatocystis* sp. (Figure 6).

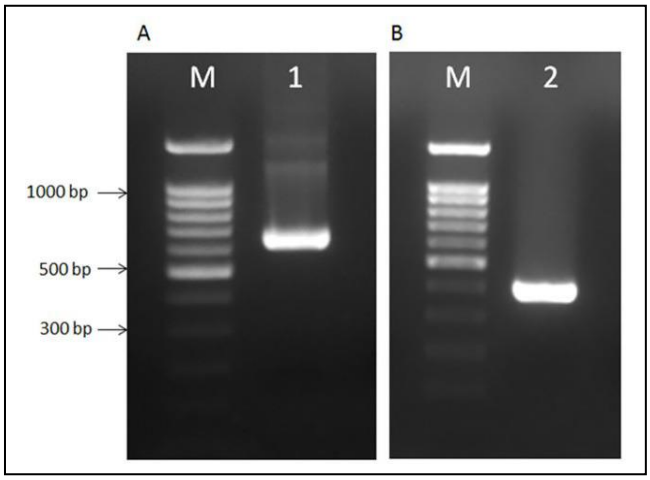

Figure 6. Gel agarose electrophoresis of the vervet monkey, DNA samples amplified for *Hepatocystis* sp. infection. Polymerase chain reaction for *Hepatocystis* 18S rRNA gene using external primers (lane 1 in Part A) and semi-nested primers (lane 2 in Part B), the positive amplified PCR products in the first and second rounds of the PCR were 734 and 360 bp, respectively. Lane M is the 100 bp DNA size marker for both gels

3.4.1 Nucleotide Sequence Accession Numbers

The 18S rRNA gene sequences of *Hepatocystis* sp. isolated from Tanzanian vervet monkeys were submitted to GenBank and registered under the accession numbers OM281567 and OM281564.

3.4.2 Result of Nucleotide Sequencing and Phylogenetic Analysis

The 144 bp nucleotide sequence of *Hepatocystis sp* 18S rRNA gene was compared with 18 related and one outgroup 18S rRNA gene sequences in the GenBank database. Nucleotide BLAST(blast-n) analysis showed a high percentage of similarity (100%) to other published sequences for *Hepatocystis* spp. 18S rRNA, and 99% identity to Plasmodium spp. 18S rRNA gene sequences. Phylogenetic analysis was performed based on the partial-length sequence of the 18S rRNA gene. The isolate from the current study was clustered in a subclade of isolates from Malaysia, Borneo (JX090222 and JX090231) and Thailand (MK078098) (Figure 7).

This tree shows that *Hepatocystis* spp. isolated from Iran (is marked) is located close to six other *Hepatocystis* spp. in one clade and apart from different clades of *Plasmodium* spp. One corresponding sequence from *T. annulata* Iran vaccine strain

(HM628581) served as the outgroup. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree is drawn to scale and indicates the number of substitutions per site. The analysis involved 20 nucleotide sequences and all positions containing gaps, as well as missing data, were eliminated. There was a total of 144 positions in the final dataset. Evolutionary analyses were conducted in MEGA software (version 7). A brief comparison of the results of a different diagnostic test for the detection of *Hepatocystis* sp. are displayed in table 4.

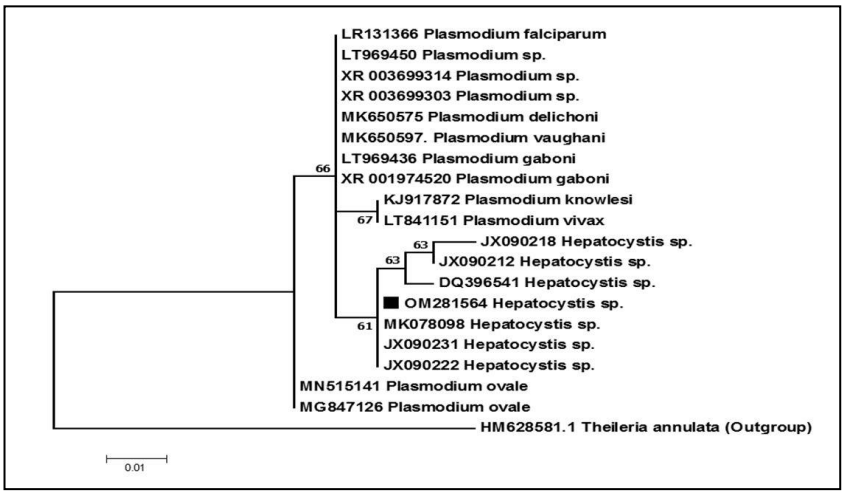

Figure 7. Phylogenetic relationships among *Hepatocystis* spp., *Plasmodium* spp., and new isolated *Hepatocystis* sp. from a vervet monkey imported into RVSRI, based on 18S rRNA gene sequences

Table 4. Results of molecular tests, in comparison with parasitological and liver histopathological examinations of the imported vervet monkeys for the detection of *Hepatocystis* sp.

	Number of cases/total number of examined (%)	**Molecular test results**	**Results of Parasitological/histopathological examinations**
Parasitological test	15/29 (51.7%)	Positive	Positive
Histopathological examination	13/25 (52%)		
Parasitological test	5/29 (17.3%)	Negative	Negative
Histopathological examination	4/25 (16%)		
Parasitological test	9/29 (31%)	Positive	Negative
Histopathological examination	8/25 (32%)		

4. Discussion

The importance of information obtained from parasitic infections in NHPs in medical research has been long overlooked (1). Nevertheless, we have recently witnessed an opinion shift regarding the role of wild animals in the transmission of parasites due to research conducted on wildlife in protected areas, as well as the implementation of surveillance programs for zoonotic and emerging zoonotic pathogens (3, 11, 12). Based on the results of the present study, the imported vervet monkeys from Tanzania were naturally infected with *Hepatocystis sp.* and piroplasms. Active infections with *Hepatocystis* are mainly chronic with a small number of parasites, and in many cases, they are in the form of mixed infections with piroplasms. The diagnosis of chronic *Hepatocystis* infection and confirmation of mixed infections with piroplasms in vervet monkeys provide useful information on the use of these animals in medical research. Approximately 200 species of mammals, reptiles, and birds are potential hosts to protozoa of the genus *Plasmodium*. Primates represent more than half of the host species. Currently, 33 parasite species are recognized as simian malaria, occurring in multiple species of prosimians, New World and Old World monkeys, as well as African and Asian apes (10).

As illustrated by the routine parasitological examination of blood smears and histopathological examination of liver collected samples, the rates of *Hepatocystis* infection were obtained at 33.9% and 38.1%, respectively. Different prevalence proportions of *Hepatocystis kochi* infection in African green monkeys have been determined and reported by microscopic examination of blood smears, for instance, Jeneby (1), (13) reported the infection rates of 18% and 29%, respectively. Nevertheless, in other reports, the prevalence rates of *Hepatocystis kochi* in non-human primates of the western and eastern borders of central Africa have been reported as 42%-56% and 40%-75%, respectively (8, 14). In fact, in this kind of studies, differences in the number of trapped animals, variation in the sample collected areas, and density of the insect vectors could affect the probability of infection with blood parasites and, as a result, different prevalence reports (1).

In molecular detection and characterization of the blood protozoa of primates, the use of primers targeting Cty-b and 18S rRNA genes has become very widespread (12, 15-17). These intra-species conserved genes have been recommended in epidemiological studies related to the interspecies diversity of blood parasites (18). The PCR has been widely used for epidemiological studies on malaria infections (19). In general, PCR is more sensitive and specific than the examination of thick or thin blood smears, particularly in cases of low parasitemia. More cases of *Plasmodium* and *Hepatocystis* infections, as well as concomitant hemoprotozoan parasites infections, in Asian macaque monkeys, have been detected, as compared to the routine microscopic assessment of blood smears (12, 20). The results obtained from molecular examination of whole blood samples of the imported vervet monkeys revealed that 82.8% of them were infected singly with *Hepatocystis* sp. or in the form of mixed infections with piroplasms. This was more than the maximum rate of infection (75%) reported by Strait, Else (8) in the east side of Africa.

Along the same lines, Jeneby (1) reported the overall prevalence of single and mixed infection of *Hepatocystis* by PCR testing as 61% in three species of monkeys. The analysis of the Cyt-b gene demonstrated that there was a difference in sequences between *Hepatocystis* and *Plasmodium* isolates (12). The phylogenetic results of the present study are in agreement with those obtained by Jeneby (1) who used Cyt-b gene sequences and demonstrated that baboons, African green monkeys, and Sykes monkeys in the wildlife of Kenya were infected with *Hepatocystis*; nonetheless, no *Plasmodium* infections were detected. According to Garnham (5), free baboons in wildlife host parasites close to *Plasmodium*; nonetheless, they did not host *Plasmodium*.

The comparison between the results of the microscopic examination of blood smears and

molecular tests of the 29 imported vervet monkeys revealed that in 69% of cases, the results were entirely consistent with each other (51.7% both positive+17.3% both negative); however, in 31% of cases, there was inconsistency in the results. Furthermore, the comparison between the results of histopathological examination of the liver of 25 imported vervet monkeys and molecular tests indicated that in 68% of cases, the results were entirely in accordance with each other (52% both positive+16% both negative); nonetheless, in 32% of cases, a discrepancy was detected in the results (Table 4). In general, the results of the current study pointed out that molecular tests are the most sensitive method for the identification of *hepatocystis* infection. Moreover, the rate of *Hepatocystis* infection was very high (82.8%) among vervet monkeys imported from Tanzania. Moreover, the results of histopathological examination of the liver and also microscopic examinations of blood smears of imported vervet monkeys in 68%-69% of cases were in agreement with the results of molecular tests in the detection of *Hepatocystis sp*. infections.

Blood parameters reflect the health condition of animals and are particularly useful in the selection of animals for research and testing. The present study pointed out that the weight of the imported vervet monkeys and most hematological related parameters were significantly improved during the quarantine period. In fact, the use of a proper diet, improvement of physical environmental conditions, adaptation to a new environment, reduction of stress, and gradual improvement of the psychological status of the monkeys were the main causes of increasing the weight and attainment of the normal level of blood parameters (21). Based on another finding of this research, the improvement and stabilization of blood parameters lessened the effect of *Hepatocystis* and piroplasms infections on the health status of monkeys.

In a similar vein, a decrease in white blood cells could be attributed to the reduction of stress and cortisol levels in the bloodstream of monkeys since it has been found that fear and mental disorders after catching monkeys from nature could cause leukocytosis. Comparable mechanisms may be involved in decreased platelet counts by reducing the release of platelets from the spleen and lungs. Previous studies have indicated that vervet monkeys take a long time to be adapted to the new environments; nonetheless, most blood parameters could attain a stable status after four months (21). As evidenced by the results of the present study, it can be concluded that quarantine of wild-caught vervet monkeys for 3-4 months could provide adequate time for stabilizing good health status, even in the presence of *Hepatocystis* and/or piroplasms infections and the experimental monkeys would be ready for use in studies.

Authors' Contribution

Study concept and design: M. H. H.
Acquisition of data: M. H. H. and Gh. H.
Analysis and interpretation of data: M. H. H.
Drafting of the manuscript: M. H. H.
Critical revision of the manuscript for important intellectual content: Gh. H.
Statistical analysis: M. H. H.
Administrative, technical, and material support: M. H. H. and Gh. H.
Study supervision: M. H. H.

Ethics

All the animals used in this study were housed and maintained in facilities of Razi institute, according to the animal welfare committee of the research deputy of RVSRI and National Ethical Framework for Animal Research in Iran, the Ministry of Health and Medical Education (TUMS, reference code: 91-01-159-18022).

Conflict of Interest

The authors declare that they have no conflict of interest.

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