Correspondence

**Comments on *Leishmania major* in Gorilla Feces**

To the Editor—As a group of experts with a long-term experience working in the field of leishmaniasis research, we wish to comment on the highly original finding by Hamad et al [1] about the presence of *Leishmania major* promastigotes and amastigotes in gorilla feces in southern Cameroon.

The finding of *L. major*, the agent of zoonotic cutaneous leishmaniasis, in southern Cameroon, a forested area, is indeed surprising. *Leishmania* alternate between a mammalian host and an insect vector (the phlebotomine sandfly) to complete their life cycle. Each *Leishmania* species is harbored by obligate and more or less adapted/specific mammalian hosts. The life cycle of *L. major* is particularly well known: it always takes place in arid or semiarid areas and has never been reported in humid tropical forests. Its reservoir hosts are the gerbillid rodents *Psammomys obesus* and *Meriones shawi* in pre-Saharan Africa [2], and its proven vectors are *Phlebotomus papatasi* and *Phlebotomus duboscqi* [3, 4]. In West and Central Africa, the area of *L. major* endemcity has been defined as a shrub savanna limited to the south by the isohyet denoting annual precipitation of 1250 mm [4].

As Hamad et al mentioned, no case of cutaneous leishmaniasis has been reported in the forested southern region of Cameroon where gorilla feces was collected, contrasting with the northern region of Mokolo, a semi-arid area, where *L. major* has been identified, as well as its suspected vector, *P. duboscqi* [5, 6]. Therefore, the article appears to contradict previous robust epidemiological and ecological data, including those from Cameroon.

The presence of both promastigotes (the insect forms of the parasite) and amastigotes (the mammalian-adapted forms) in stool specimens is also a surprising finding. The authors suggest that apes may ingest sandflies harboring promastigotes as a part of their daily diet. To our knowledge, this has never been reported. Sandflies, which typically weigh 0.1–0.2 mg, are much smaller and more difficult to catch than termites and other insects that have been identified in gorilla diets. They are nocturnal insects whose larvae stay deep in the soil and rodent burrows. Furthermore, the authors cannot explain how intact promastigotes might survive a fierce digestion process: they are highly fragile organisms that cannot withstand changes in osmotic pressure, whether from desiccation or the addition of water to a preparation.

Finally, promastigotes are not found in the mammalian hosts. In support of their finding, the authors refer to 2 publications reporting “promastigote-like forms” in cutaneous leishmaniasis. In our opinion, the article by Correa et al [7] does not provide any evidence of promastigotes in the electron microscopy pictures provided, and the article by Daboul et al [8] (published in *LabMedicine* and wrongly referenced as published in *Science*) clearly misidentifies gross artifacts, essentially collagen fibers, as promastigotes. Another hypothesis raised by Hamad et al is that promastigote-infected sandflies might agglutinate on feces. Sandfly females do not feed on feces. Apart from the blood meal necessary for females, adults feed on flowers, nectar and honeydew [9].

On the other hand, amastigotes are purely intracellular stages; in humans and other primates tested so far, *L. major* amastigotes stay in the skin at the site of the sandfly bite [10]. If *L. major* has been found disseminating in an immunosuppressed patient [11], this cannot be viewed as a common pathophysiological process. It is, therefore, highly surprising to detect this dermotropic parasite in high numbers in intestinal products, particularly because gorillas are claimed by Hamad et al to be asymptomatic carriers of the pathogen.

Polymerase chain reaction is well known to lead to high rates of carryover contamination, particularly when it is used for diagnosis or detection, both of which involve repeated use of the same DNA primers. Accordingly, the authors probably used fluorescence in situ hybridization (FISH) targeting 18S ribosomal DNA to confirm their initial molecular detection. One of our groups is specialized in using FISH to analyze *Leishmania* organisms [12], and taking into account all of the procedures associated with processing and investigating cryopreserved stool samples, we find the evidence of absolutely superb and intact promastigotes presented by Hamad et al far from convincing. Finally, the mere presentation of 2 fluorescent round-shaped forms placed side by side as evidence of amastigotes does not appear to be sufficient evidence to support claim that these forms were present in the stool specimens. A serological survey would be much more convincing in showing that these animals were infected by *Leishmania* organisms.

In summary, it appears that there are too many extraordinary findings in this article for it to reflect a true exception to the epidemiological evidence. The main conclusion of Hamad et al, that “great apes might play a role as reservoir hosts for...
L. major parasites,” (p. 272) appears highly speculative and, in our opinion, surely cannot be “viewed as a point of concern for public health in the region” (p. 272).

Notes

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