

# Possible human bartonellosis in the Sudan : clinical and microbiological observations

Autor(en): **Wernsdorfer, Gunther**

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# Possible Human Bartonellosis in the Sudan. Clinical and Microbiological Observations

GUNTHER WERNSDORFER, Cairo

## 1. Introduction

Human disease caused by *Bartonella bacilliformis*, known under the names of Oroya Fever, Carrion's Disease, Guáitara Fever and Verruga Peruana, is believed to occur only in South America, there again restricted to latitudes between 2° N and 16° S (GEIGY & HERBIG 1955, GRADWOHL, SOTO & FELSENFELD 1951, MANSON-BAHR, 1966, MÜLLER 1946, TOPLEY & WILSON 1957, JOYEUX & SICÉ 1950, MACKIE, HUNTER & WORTH 1954, APHA 'Report on Communicable Diseases in Man' 1960).

Own observations and subsequent investigations at least suggest that human bartonellosis may also exist on a continent other than the American. This report relates to observations in the Sudan (Africa).

## 2. General Information

The classification of *Bartonella bacilliformis* is still disputed and the object of a considerable number of publications (ALZAMORA 1945, FREI 1950, GROOT 1951, HORSFALL 1962, MAYER et al. 1926, NAUCK et al. 1950, NIVEN et al. 1952, NOGUCHI 1926 a-d, NOGUCHI & BATTISTINI 1926, PETERS & WIGAND 1951, 1955, SCHLOSSBERGER et al. 1952, SPLITTER 1952, STRONG 1942, STRONG et al. 1915, TOPLEY & WILSON 1957, WEINMAN 1944, 1957, WIGAND 1956 a, 1956 b, 1958, 1966). The majority of observers, though, tends to consider *Bartonella* a distinct genus of intermediate position between true bacteria and rickettsiae.

*Bartonella bacilliformis* displays a high degree of pleomorphism. It is gram-negative in fresh cultures, highly motile, and has particular nutritional requirements which do not permit growth on ordinary bacteriological media. The studies of GEIMAN (1944), GROOT (1951), MANSON-BAHR (1961, 1966), WIGAND & PETERS (1952) and WIGAND (1966) provide much detail on morphological and physiological characteristics of the microorganism.

It is assumed that natural transmission of *Bartonella bacilliformis* relies exclusively on the bites of females of *Phlebotomus* spp. In Peru *P. verrucarum* was incriminated as a vector (NOGUCHI et al. 1929, BATTISTINI 1929, 1931). Here also *P. noguchii* and

*P. peruensis* may play a role in transmission (PINKERTON & WEINMAN 1937 a, 1937 b). *Phlebotomus* spp. are also likely to be the vectors in Colombia (GROOT 1951), where RISTORCELLI & DAO 1941 (cited from GROOT 1951) encountered a rich *Phlebotomus* fauna out of which *P. columbianus* appears to be the most probable main vector. Detail on the mechanics of transmission can be found in the publications of HERTIG (1939), HORSFALL (1962) and NOGUCHI (1926 d).

The main reservoir of the disease may well be man, who shows according to WEINMAN & PINKERTON (1937) a full range of tolerance including carrier state over prolonged periods. Asymptomatic infection of man with *Bartonella bacilliformis* is by no means rare (WIGAND 1966). From the incidence of Carrion's Disease HORSFALL (1962) infers a basically sylvatic origin – probably with field mice as carriers – and a sylvatic-human phase.

### 3. Own Observations

Hitherto all experience speaks against the occurrence of human bartonellosis outside South America. The observation, in December 1962, of a disease similar to if not identical with human bartonellosis in a young Sudanese woman, and the results of the ensuing microbiological studies have stimulated preparation of this report.

#### 3.1 Case Notes

Patient: N/S. I., female.

Age: 23 years.

Residence: village 1 mile from Singa (Northern Fung, Blue Nile Province); never left the area of Singa, except on the transfer to Khartoum Hospital.

Previous illnesses: none of relevance.

Pregnancies: two, followed by normal deliveries.

This illness: November 1962 start of an acute febrile disease. Admission to Singa Hospital, where hepato- and splenomegaly were found. Kala Azar was clinically diagnosed, but parasitologically not confirmed. Under specific treatment for Kala Azar no improvement of condition. The latter aggravated, and in early December 1962 the patient was transferred to Khartoum Civil Hospital. Also here the clinical picture was suggestive of Kala Azar and specific chemotherapy continued.

The patient appeared emaciated and apathic, corresponding to the serious clinical picture.

The body temperature (oral) varied between 38.5° and 40°C, with occasional profuse sweating. During the intervals the skin appeared dry.

The patient complained from severe headache, frequently she was in a somnolent state. On admission to Khartoum Civil Hospital she suffered from mild diarrhoea which later disappeared. Anorexia was marked, vomiting occurred occasionally. Dyspnoea was prominent.

General examination revealed the following main findings (10 December, 1962):

Skin: low turgor, greyish, no depigmentations; petechiae on arms and trunk in the abdominal region. They also appeared after mechanical provocation (Rumpel-Leede). Otherwise no exanthema.

Sclerae: normal colour.

Tongue: tip pale, centre thickly coated, margins reddened.

Heart: tachycardia, at the time of examination no signs of pericarditis.

Lungs: no pathological findings.

Kidneys: on palpation no pathological signs, no pain.

Liver: approx. 7 cm below the right costal margin, rather soft, slightly painful on palpation.

Spleen: slightly enlarged (Spleen 1–2 accd. to WHO/Hackett classification).

Abdomen: besides hepato- and splenomegaly on palpation no pathological signs, no ascites.

Lymph nodes: general moderate enlargement, rather firm.

Long bones: all painful, particularly those of the lower extremities.

Urin: Albumen +++, RBC in sediment +++, Urobilinogen ++++ (!).

Blood: Leukocytes 10. 12. 1962 11,000/mm<sup>3</sup>, 12. 12. 1962 22,000/mm<sup>3</sup>, 15. 12. 1962 15,000/mm<sup>3</sup>. Erythrocytes 10. 12. 1962 2,900,000/mm<sup>3</sup>, 12. 12. 1962 2,500,000/mm<sup>3</sup>, 15. 12. 1962 2,400,000/mm<sup>3</sup>, Haemoglobin 10. 12. 1962 32% Sahli (5.1 g%), 15. 12. 1962 25% Sahli (4.0 g%), ESR 10. 12. 1962 40 mm 1st hour (Westergreen).

In 10 December, 1962 five bone-marrow specimens (sternum) were taken and sent for examination to the Department of Bacteriology and Parasitology, Faculty of Medicine, University of Khartoum, where they were registered under the number 1989. The clinical diagnosis was stated as "severest form of Kala Azar", refractory to all hitherto applied specific medication.

The BM-smears were of medium quality. After fixation with absolute methanol staining according to Giemsa, search for Leishmania Bodies (LB), conducted for more than one hour, yielded negative results. Instead a conspicuous "precipitate" of rather homogeneous appearance was noticed. Its nature was still unknown.

The report to the hospital stated "negative for *Leishmania donovani*", along with a request for repeated supply of specimens. The Giemsa stock solution and materials used for the first staining were checked by staining of normal blood and BM-smears. No precipitate was observed.

Re-examination of the patient's BM-smears showed that only BM-cells and particularly the incidentally present RBC contained the conspicuous "precipitate". The other parts of the slide were largely free of the "precipitate".

This did not as yet give any indication regarding cause and nature of the stained particles. For further investigation and for exclusion of an accidental nature of the findings, blood slides were taken daily during the next following days, and blood cultures initiated at the same time. Also BM-smears were prepared again.

### 3.2 Laboratory Findings

The blood films and the BM-smears were stained with Giemsa solution, varying the staining period between 45 and 120 minutes. The BM-smears were again negative for LB. The results were the same as in the specimens of 10/12/1962 (fig. 1), now also seen in the blood films (fig. 2 and fig. 3): besides very marked polychromasy, aniso- and poikilocytosis, 80–90% of the RBC were



found to be occupied by one or more of what by now for certain appeared to be a microorganism. The latter was seen in form of small rounded bodies and rods which almost exclusively had intracellular position. The microorganism seemed to be differentiated into a slightly bluish-pink cytoplasm and intracytoplasmatic granules of an intensively red tint (chromatin granules?). The majority of microorganisms appeared ovoid or elongated, in groups of rarely more than four, and often arranged in V- or Y-shapes or in short, slightly curved chains of up to four rods in sequence. The "chromatin-granules" are, as a constant feature, separated from the next members of the chain by a bluish-pink seam of cytoplasm which seems to be slightly elongated towards the pole.

In gram-stained blood films the microorganisms appeared gram-labile, somewhat more intensively stained granules are visible. The gram-staining of blood films has in our opinion no further diagnostic value and was applied only for orientation.

The first blood culture with simple broth was negative. The next following blood cultures with liver extract broth and on blood agar (resulting from admixture) were positive. Growth of a still unidentified microorganism became visible after 3–5 days.

On blood agar and liver extract agar of normal consistence growth was initially scanty, especially at an incubation temperature of 37°C. Understandably it was normal at first to incubate at 37°C since type and characteristics of the microorganisms were not known at the time.

Subsequently optimum growth was achieved on semi-solid media containing blood, liver extract or serum. The most suitable incubation temperature was 25–28°C. The cultures then usually showed after 2 days small colonies of 0.2–0.4 diameter. The colonies were shiny, smooth, transparent and slightly opalescent, initially without a tendency towards confluence. On direct illumination the colonies appeared light grey to yellowish-grey. Older colonies assumed a slightly reddish reflection, especially when grown on blood agar or liver extract agar.

On blood agar haemolysis became distinct after 2–5 days, sharply defined around every colony. The haemolysis was obviously complete since the plates discoloured completely and became transparent.

In older cultures the colonies became more prominent and showed confluence. These features were the same through all passages (up to no. 32).

The microorganism was an obligatory aerobe. But cultures which were, after the 24th passage, exposed to considerable though not complete oxygen deficit continued to grow. The oxygen deficit was

accidentally induced through the action of the laboratory's cleaning personnel.

Phase contrast microscopy, unusually fast movement of the cultured microorganism in all directions, and rotation around its own axis, indicated the presence of flagellae or an intracorporal mechanism of motility. The motility test, carried out by suspending culture material in broth or tap water, showed intensive motility between 25° and 40°C, the maximum at 30°C. Frequently two or more of the microorganisms were seen to move, attached to each other, in serpentine or rotating fashion. The single microorganism usually moved straight or in long drawn curves, over distances of up to 5 mm. The motility of the cultured microorganism was a constant feature in all passages up to the last (no. 32). If grown with other microorganisms it soon lost the motility. Cultures in which the microorganism showed reducing motility used to die within a short space of time.

In heparinized fresh blood the phase contrast method revealed slow movement of the intra-erythrocytic microorganisms. Also the free microorganisms, ovoid and elongated forms, showed movement which though was much slower than that of the cultured microorganisms. At first this was thought to be partially the result of the high plasma viscosity, but the velocity was not significantly altered by diluting the heparinized fresh blood 1:1, 1:2 or 1:10 with physiological saline resp. Polyvinylpyrrolidon solution.

From fresh cultures the microorganism stained gram-negative (fig. 4) and showed near one pole a dense aggregation of dark red to violet granules. In older cultures there was an alteration towards gram-lability, but also here the granular structures were clearly discernible within the cytoplasm. The gram-lability was more apparent in microorganisms grown on media containing liver extract.

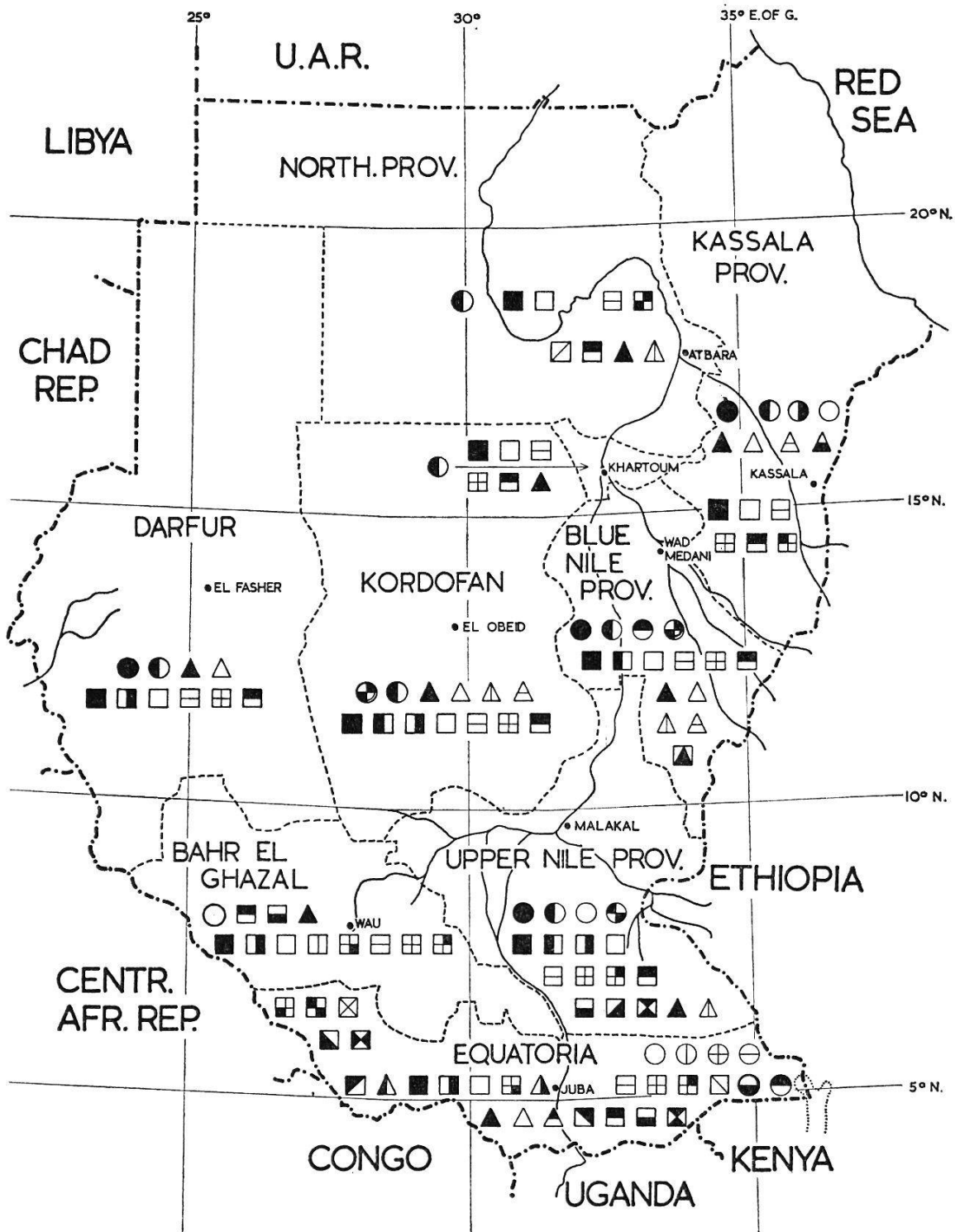
With Giemsa staining the microorganism from the culture had an appearance very similar to that from the fresh blood specimens (fig. 5).

Morphological and physiological features of the microorganisms found in blood specimens and culture are summarized in the following table.

### *3.3 Further Observations*

During the two months following the observation of the first case (see para 3.1), parasitological specimens of five more patients of Khartoum Civil Hospital contained the same intra-erythrocytic microorganism as described under 3.2. In all these patients, none

	<i>Blood forms</i> (figs. 1-3)	<i>Culture forms</i> (figs. 4-6)
<i>Dimensions</i>	Length 0.6-3 microns, diameter 0.3-0.5 micron, round forms: $0.8-0.9 \times 1.0$ micron.	Length 1.0-3 microns, diameter 0.3-0.5 (-0.6) micron.
<i>Pleomorphism</i>	Very marked.	Very marked.
<i>Appearance after Giemsa staining</i>	Plump bodies and rods, cytoplasm bluish-pink, granula mostly deep red; intracellular situation (RBC, in BM also endothelial cells); groups of 2-3, rarely 4 microorganisms, often in V and Y shapes or short chains; here granula near free ends; single and several separated microorganisms in RBCs not rare.	Small rods, granula clearly differentiated, but only $\frac{1}{2}-\frac{2}{3}$ the size of those in the blood forms; cytoplasm bluish to bluish-pink.
<i>Appearance after Gram staining</i>		Gram-negative; granula less distinct. In old cultures gram-lability (intermediate tint). In culture forms not rarely more than one granula in one microorganism. V and Y forms are found, as well as short chains (fig. 6), if culture material is not too much deranged while handling.
<i>Motility</i>	Fresh blood: microorganisms show under phase contrast slow movement within or on the erythrocytes.	Very marked motility, leading over considerable distances; it is often observed that the same microorganisms collide repeatedly. Intensive motility at 25-40°C, maximum at 30°C. Motility is a constant feature (observed to the last, i.e. 32nd passage).
<i>General Features</i>	Rarely more than 4 microorganisms in the same RBC. Erythrocytes never "over-filled", whereas lymphocytes and neutrophilic granulated leukocytes often appear to be completely stuffed with the microorganism. Erythrocytes are highly fragile, show polychromasy, aniso- and poikilocytosis.	Fresh colonies 0.2-0.4 mm diameter, prominent, transparent, shiny surface, pale grey to yellowish-grey, on blood-agar plates occasionally pinkish-grey. Older cultures (four weeks and more) show confluent, shiny colonies with "terrace"-growth: main colonies higher, periphery only $\frac{1}{3}-\frac{1}{2}$ in height; colonies transparent. Blood-agar media are haemolysed around the colonies; haemolysis becomes complete once growth has extended peripherally. Growth is aerobic, but on semi-solid media considerable oxygen deficit is tolerated. Optimum incubation temperature 25-28°C. Growth occurs only on media containing blood, liver extract or serum; semi-solid media yield best results.



of whom had ever left the Sudan, the suspected diagnosis was Kala Azar, but the clinical symptomatology was very similar to that of the first case, and search for LB yielded negative results. All 5 patients, 3 female and 2 male, were aged between 17 and 25 years. Two of the female patients, aged 17 and 20 years respectively, developed on trunk, neck and face skin efflorescences similar to verrugae. Further observation of these cases was unfortunately not feasible.

# PHLEBOTOMES OF THE SUDAN

(DISTRIBUTION BY PROVINCES)

## PHLEBOTOMUS (PHLEBOTOMUS)

- P. ORIENTALIS
- ◐ P. PAPATASI
- ◑ P. PAPATASI VAR. BERGEROTI
- ◒ P. ROUBAUDI VAR. FOURTONI
- ◓ P. ALEXANDRI
- ⊕ P. SERGENTI VAR. SAEVUS
- ⊖ P. MARTINI
- ⊗ P. LONGIPES
- ◐ P. LESLEYAE
- ⊕ P. HEISCHI
- P. RODHAINI

## PHLEBOTOMUS (SINTONIUS)

- ▲ P. CLYDEI
- △ P. AFFINIS INCL. VAR. VORAX
- △ P. ADLERI
- △ P. CHRISTOPHERSI VAR. CALCARATUS
- ▲ P. TIBERIADIS
- ▲ P. THOMSONI
- ▲ P. CHRISTOPHERSI
- ▲ P. SUBERECTUS

## PHLEBOTOMUS (SPELAEOMYIA)

- ▲ P. DARLINGI

## PHLEBOTOMUS (SERGENTOMYIA)

- P. BEDFORDI INCL. VAR. DISTINCTUS
- ◼ P. BEDFORDI VAR. BEREIRI
- ◼ P. BEDFORDI VAR. CONGOLENSIS
- ◻ P. BUXTONI
- ◼ P. COWLANDI
- ◼ P. SCHOUTEDENI VAR. PUNGENS
- ◻ P. ANTENNATUS INCL. VAR. CINCTUS & OCCIDENTALIS
- ◼ P. SIMILLIMUS
- ⊗ P. YUSAFI
- ◻ P. SCHWETZI INCL. VAR. NIGRICANS
- ◻ P. FREETOWNENSIS INCL. VAR. SUDANICUS
- ◼ P. FREETOWNENSIS VAR. NIGER
- ◼ P. FREETOWNENSIS VAR. EREMITIS
- ◼ P. FREETOWNENSIS VAR. MAGNUS
- ◻ P. INGRAMI
- ⊗ P. KIRKI
- ◼ P. SERRATUS
- ◼ P. COLLARTI
- ⊗ P. DECIPIENS
- ◻ P. LEWISI
- ◼ P. HUNTI
- ◼ P. HORGANI
- ◻ P. SQUAMIPLEURIS
- ◼ P. SQUAMIPLEURIS VAR. INERMIS
- ⊗ P. RUTTLEDGEI

From all the cases the microorganism was also cultured. The findings fully conformed with those described under para 3.2.

Sugar fermentation tests were carried out with the microorganisms from all 6 patients, with the following results.

The sugar fermentation tests, carried out in media which do not normally permit the microorganisms' striving, indicate that either the microorganism is not able to ferment lactose, mannitol and sucrose, or that the metabolic functions were at very low level, what is also indicated by the glucose fermentation which gave only in 4 of the cultures positive results: a very slight acidification; gas production did not take place. The motility tests though indicated that the microorganisms were alive in all tubes until the end of the fermentation test.

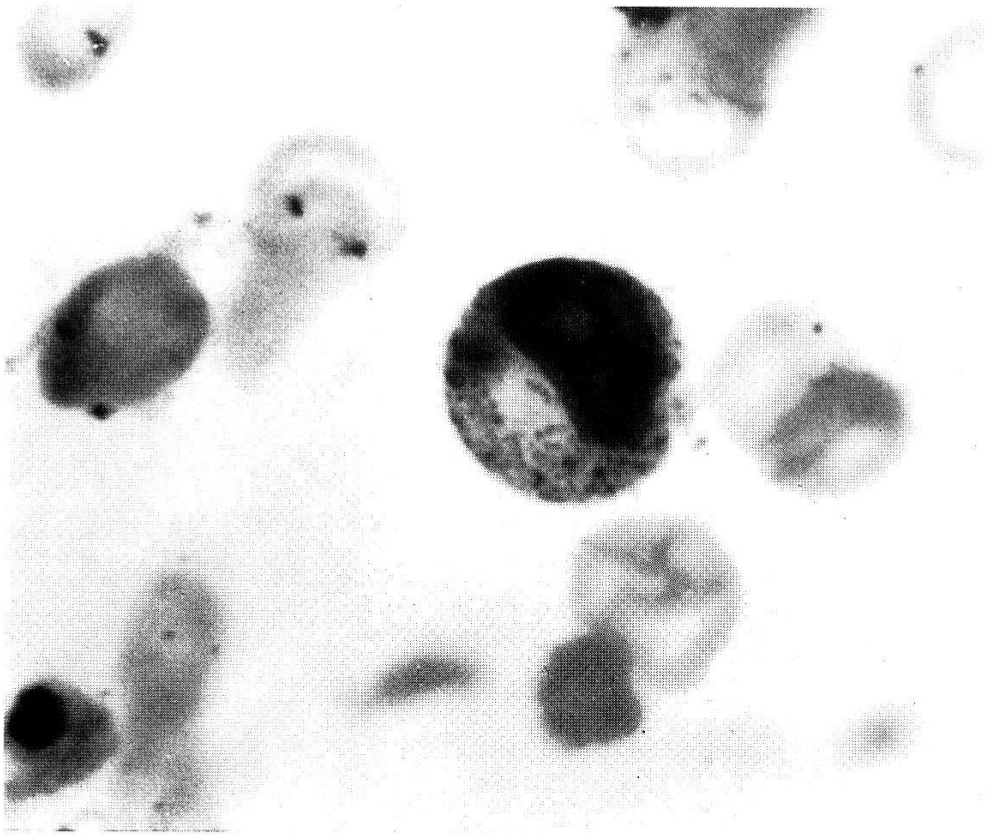
Cultures of the microorganisms in question were sent to the University Departments of Hygiene and Bacteriology at Cologne

Serial no. (patient)	No. of prev. passage	Lactose	Glucose	Mannitol	Sucrose	Motility
(a) Series of 5 August, 1963 (broth, reading after 24, 48, 120 hours)						
1	25	neg.	neg.	neg.	neg.	+++
2	25	neg.	neg.	neg.	neg.	+++
3	18	neg.	Acid + No gas	neg.	neg.	+++
4	19	neg.	Acid + No gas	neg.	neg.	+++
5	17	neg.	neg.	neg.	neg.	+++
6	16	neg.	Acid + No gas	neg.	neg.	+++
(b) Series of 25 August, 1963 (broth, reading after 24, 48, 96 hours)						
1	27	neg.	neg.	neg.	neg.	+++
2	28	neg.	Acid after 96 h + No gas	neg.	neg.	+++
3	20	neg.	neg.	neg.	neg.	+++
4	21	neg.	Acid + No gas	neg.	neg.	+++
5	19	neg.	neg.	neg.	neg.	+++
6	19	neg.	neg.	neg.	neg.	+++
(c) Series of 26 August, 1963 (pepton water, reading after 24, 48, 72, 120 h)						
1	27	neg.	neg.	neg.	neg.	+++
2	28	neg.	neg.	neg.	neg.	+++
3	20	neg.	neg.	neg.	neg.	+++
4	21	neg.	Acid + No gas	neg.	neg.	+++
5	19	neg.	neg.	neg.	neg.	+++
6	19	neg.	neg.	neg.	neg.	+++

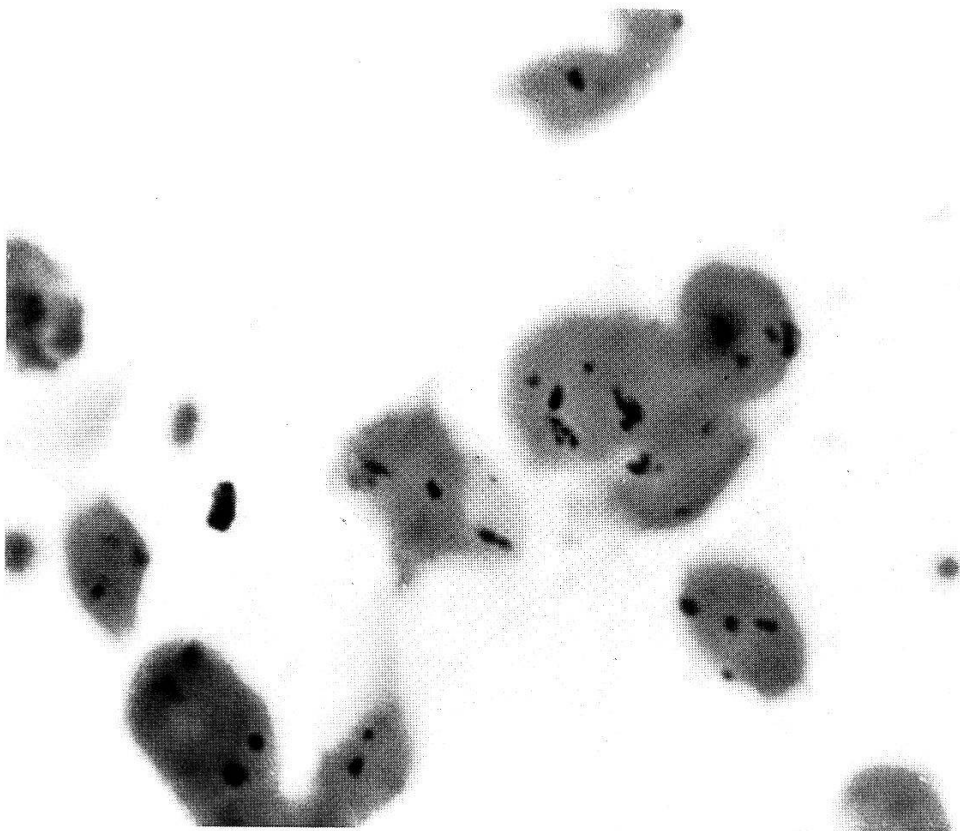
and Erlangen. Both Departments could unfortunately not classify the microorganism.

The cultures (6) were maintained until 21 October 1963, when an accident caused the destruction of all material. At that time the cultures from the first two patients were carried to the 32nd passage. In this context it should also be pointed out that the maintenance of the cultures was a task of considerable difficulty. Serious obstacles were encountered in the preparation of standardized, uncontaminated media, since installations, equipment and technical auxiliary personnel were below the required level.





*Fig. 1.* Bone marrow, sternum, Giemsa (pat. N/S.I., ref. no. 1989), oil imm. 100  $\times$ , green filter.



*Fig. 2.* Blood (pat. N/S.I., ref. no. 1989), Giemsa, oil imm. 100  $\times$ , green filter.

#### 4. Discussion and Conclusions

The patient, whose case notes are given in para 3.1, was initially believed to suffer from Kala Azar. Repeated laboratory examinations did not confirm this diagnosis. Also the *ex juvantibus* treatment failed. The clinical picture differed considerably from that of Kala Azar, whether in the classical form or in one of the locally observed modified courses.

In blood and bone marrow intra-erythrocytic microorganisms were observed which were subsequently isolated by means of blood culture. The finding of pleomorphic intra-erythrocytic microorganisms and the similarity of the disease's symptomatology with that of South-American Oroya Fever suggested that the disease may be caused by Bartonellae. The clinical picture much more conformed with that of Oroya Fever than it did with Kala Azar.

The microbiological findings very much suggest that the microorganism belongs to the Bartonellae; this conclusion is based on the following characteristics:

*Dimensions:*  $0.3-0.5 \times 0.6-3$  microns (rods, blood form),  $0.3-0.6 \times 1.0-3$  microns (rods, culture forms).

*Pleomorphism:* very marked in blood and culture forms.

*Morphological features:* Giemsa staining shows plump bodies and rods in which bluish-pink "cytoplasm" and deep red granula are visible. Short chains and typical V and Y forms are frequent in blood and culture forms. Intra-erythrocytic position in blood forms.

*Gram staining:* negative, giving place to gram-lability in older cultures and those containing liver extract.

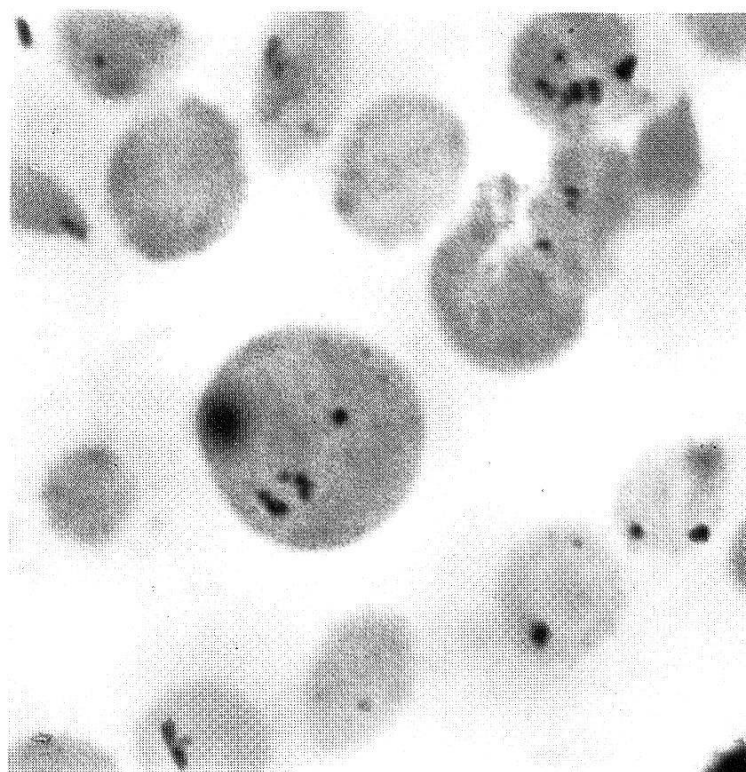
*Motility:* blood forms show in phase contrast microscopy slow intra-erythrocytic movement. Culture forms are highly motile, the movement covering comparatively long distances.

*Optimum growth temperature:*  $25^{\circ}-28^{\circ}\text{C}$ .

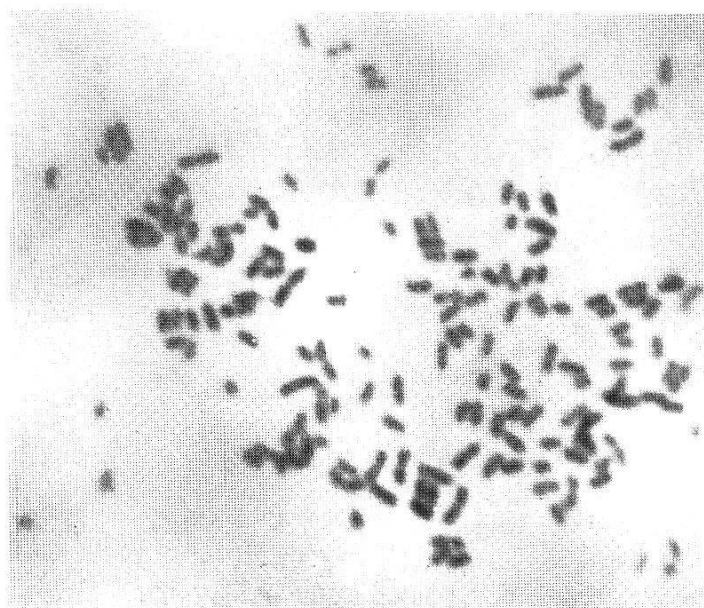
*Growth:* not occurring on media lacking blood, liver extract or serum. Fastest and richest growth on semi-solid blood and liver-extract media.

The microorganism was isolated from five further patients, all of whom were supposed to suffer from drug-resistant Kala Azar. Two of these cases developed later skin efflorescences resembling verrugae.

The author acknowledges with thanks the kind interest and suggestions of Prof. Shozo Inoki, Research Institute for Microbial Diseases, Osaka University, Japan, known as an authority in the



*Fig. 3.* Blood (pat. N/S.I., ref. no. 1989), Giemsa, oil imm. 100  $\times$ , green filter.



*Fig. 4.* Microorganisms from culture (prim., ref. no. 1989), Gram, oil imm. 100  $\times$ , green filter.

field of bartonellosis. Prof. Inoki examined the demonstrated material during a visit to Khartoum in August 1963 and came to the conclusion that the microorganism was *Bartonella bacilliformis*.

Due to the untimely ending of the cultures, re-confirmation of the findings will have to be the objective of further studies and surveys.

If the observed cases were indeed bartonellosis, and the microbiological findings are extremely suggestive, it still is the question whether they were caused by *Bartonella bacilliformis* or a nearly related, other *Bartonella*. The latter seems to be possible since haemolysis in culture media is obviously a rare occurrence with *Bartonella bacilliformis*. Thus it may well be that the cases were not the result of introduced *Bartonella bacilliformis* from South-America, but of an infection with a local *Bartonella*.

As regards mode of transmission it is very likely that *Phlebotomus* may have played a role. The patients all came from Kala Azar areas (Northern Fung, Blue Nile Province).

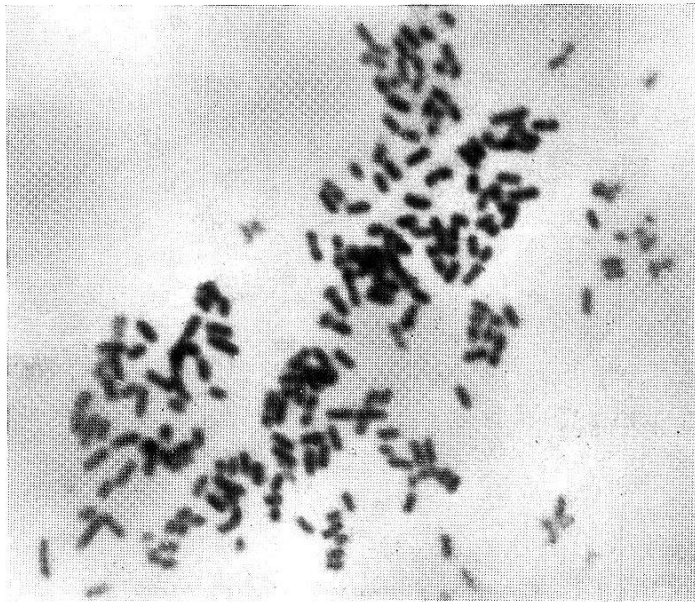
*Phlebotomus verrucarum* and the other New-World spp. of vectorial *Phlebotomus* were not encountered in the Sudan. This does not exclude, however, that one or the other of the local *Phlebotomus* spp. may be capable of transmitting *Bartonella bacilliformis* or another, hitherto unknown, human-pathogenic local *Bartonella*. Relevant experiments were not yet undertaken.

The *Phlebotomus* fauna of the Sudan is copious. The Kala Azar problem in the Sudan has stimulated very intensive taxonomic studies which were almost exclusively carried out by LEWIS and KIRK and resulted in the identification of 36 different species and 13 varieties of *Phlebotomus* (KIRK & LEWIS 1940, 1946, 1947, 1948, 1949, 1950, 1952; LEWIS & KIRK 1949, 1951, 1954, 1957).

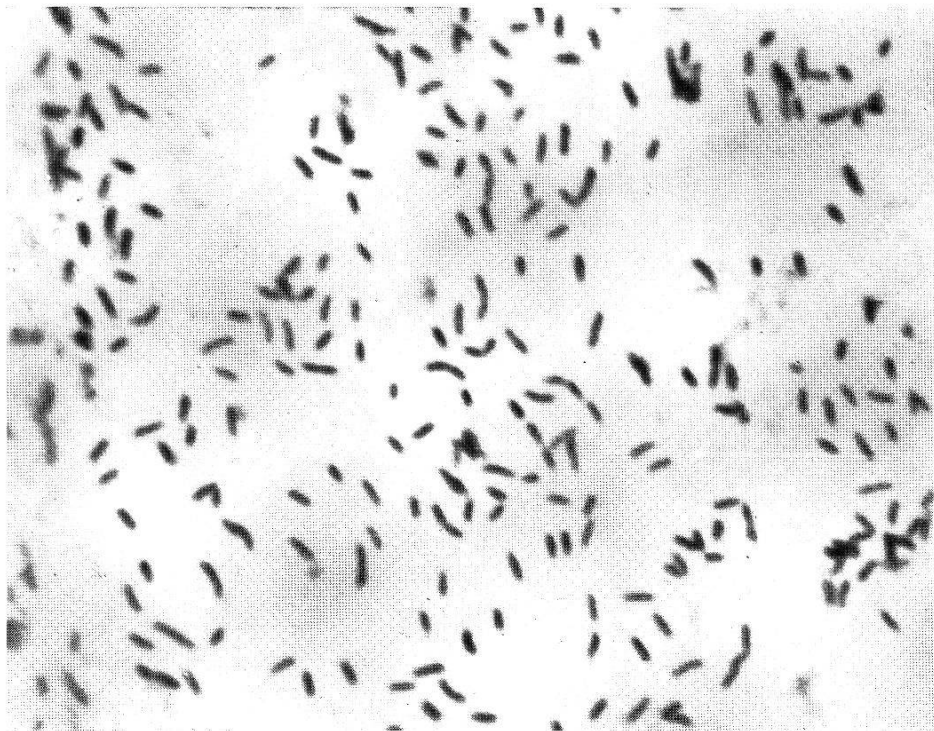
Map 1, which was compiled from the data of above quoted papers of LEWIS and KIRK and those of HOOGSTRAAL et al. (1962) and DIETLEIN (1964), shows the known distribution of *Phlebotomus* spp. in the Sudan, on Province basis.

In Blue Nile Province, the origin of the six patients, so far the following *Phlebotomus* spp. were found:

- P. (Phlebotomus) orientalis*
- P. (Phlebotomus) papatasi*
- P. (Phlebotomus) roubaudi* var. *fourtoni*
- P. (Phlebotomus) lesleyae*
- P. (Sergentomyia) bedfordi*
- P. (Sergentomyia) bedfordi* var. *bereiri*
- P. (Sergentomyia) antennatus*
- P. (Sergentomyia) schwetzi*



*Fig. 5.* Microorganisms from culture (12th pass., ref. no. 1989), Giemsa, oil imm. 100  $\times$ , green filter.



*Fig. 6.* Microorganisms from culture (12th pass., ref. no. 1989), Giemsa, oil imm. 100  $\times$ , green filter.



- P. (Sergentomyia) freetownensis* (= *africanus*)
- P. (Sergentomyia) squamipleuris*
- P. (Sintonius) adleri*
- P. (Sintonius) affinis*
- P. (Sintonius) christophersi* var. *calcaratus*
- P. (Sintonius) clydei*
- P. (Spelaeomyia) darlingi*.

In this connection it is interesting to note that *P. orientalis* and *P. heischi* were incriminated as vectors of Kala Azar in the Sudan (HOOGSTRAAL & DIETLEIN 1964). The search for natural reservoir of *Leishmania donovani* was successfully conducted by HOOGSTRAAL et al. (1963a, 1963b, 1964), who incriminated Nile Grass Rat (*Arvicanthus niloticus luctuosus*), Senegal Genet (*Genetta genetta senegalensis*), Sudanese Serval Cat (*Felis serval phillipsi*), Spiny Mouse (*Acomys* spp.) and *Rattus rattus*. In Malakal town it was the two last mentioned rodents; here obviously a rodent-vector-rodent cycle was in operation which, due to the absence of *P. orientalis*, did not normally extend to man, but would do so after disturbance of the vectorial equilibrium.

The association of Phlebotomus and rodents is very intensive in the Sudan, especially in the central clay plain with its seasonally enormous subterranean environment. This may also have a major bearing on the question of bartonellosis, since in South-America rodents appear to be a natural reservoir of *Bartonella bacilliformis* (HORSFALL 1962). It would seem possible that rodents in the Sudan are naturally infected with a human-pathogenic Bartonella, but that the rodent-vector-rodent cycle rarely extends to man, especially if the transmitting Phlebotomus species is highly zoophilic. This would also explain sporadic occurrence and scarcity of human infections.

## 5. Recommendations

The above-mentioned observations are submitted for discussion. For further clarification and substantiation the following course of action is suggested:

- (a) Detection and examination of suspected and doubtful cases, referred and notified by hospitals, health centres, dispensaries, dressing stations of the areas concerned.



- (b) Confirmation of suspected cases
  - i. by microscopic methods, examining blood, bone marrow, spleen punctate, lymph node aspiration material.
  - ii. by culture, incl. differentiation and identification.
- (c) Epidemiological investigation
  - i. Environment of cases:
    - family and close contacts, neighbours and further contacts, village and village contacts incl. migrants.
  - ii. Vectorial and reservoir studies:
    - incrimination (cultural methods), vector/reservoir contact and equilibrium, vector distribution, physiology and seasonality.

Since the area concerned has rather unstable meteorological conditions, which in consequence cause a high degree of instability as regards vector-borne diseases (e.g. Kala Azar, malaria), it may be necessary to conduct the screening over prolonged periods. The same would apply to post-incrimination point and period prevalence surveys.

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A first, brief report on the subject of this paper was given in 20th August, 1963, under reference circular CR/265-2, within the research report for the academic year 1962/63 to the Dean, Faculty of Medicine, University of Khartoum.

### Zusammenfassung

Im Dezember 1962 kam im Khartoum Civil Hospital eine Patientin mit Verdacht auf akute, atypische Kala Azar zur Beobachtung. Die Diagnose ließ sich parasitologisch nicht bestätigen. Spezifische Chemotherapie gegen Kala Azar blieb erfolglos.

In Knochenmark und Blut fanden sich intraerythrozytäre Mikroorganismen. In der Folge gelang Kultur auf Blut-, Leberextrakt- und Serum-Medien. Blut- und Kulturformen des Mikroorganismus zeigten hochgradige Pleomorphie, mit einem Vorherrschen von kokkoiden Strukturen ( $0,8-0,9 \times 1 \mu$ ) oder Stäbchen ( $0,3-0,6 \times 1-3 \mu$ ). Die Stäbchen bildeten häufig kurze Ketten, V- und Y-Formen. Im Frischblut zeigte der Mikroorganismus langsame intraerythrozytäre Bewegung, die Kulturformen wiesen außerordentlich rasche Beweglichkeit auf. Das Wachstum war obligat aerob, mit einem Optimum bei  $25-28^{\circ}\text{C}$ .

Die Eigenschaften des Mikroorganismus wiesen deutlich auf *Bartonella bacilliformis* hin. Gleichermassen entsprach die klinische Symptomatologie mehr dem Oroya-Fieber, einer bisher nur in Südamerika bekannten Krankheit, als derjenigen von Kala Azar.

Innerhalb von zwei Monaten nach Beobachtung des ersten Falles wurde der gleiche Mikroorganismus von weiteren 5 Patienten isoliert, bei welchen der Verdacht auf atypische, Chemotherapie-resistente Kala Azar bestand.

Alle 6 Patienten stammten aus der südlichen Blaunil-Provinz, einer für endemische Kala Azar und reiche Phlebotomenfauna bekannten Gegend.

### Résumé

En décembre 1962, une patiente vint consulter à l'hôpital civil de Khartoum pour un cas aigu, mais atypique, de Kala Azar. Le diagnostic parasitologique fut négatif. Une chimiothérapie spécifique resta sans succès.

Dans la moelle osseuse et dans le sang, on trouva des microorganismes intra-érythrocytaires que l'on réussit, par la suite, à cultiver sur différents milieux : sang, sérum, extraits hépatiques. Les formes sanguines et de culture du micro-organisme montrèrent un pléomorphisme très marqué avec des formes en bâtonnets ( $0,3-0,6 \times 1-3 \mu\text{m}$ ) et une prédominance de formes en coques ( $0,8-0,9 \times 1 \mu\text{m}$ ). Les bâtonnets formèrent souvent de courtes chaînes et des arrangements en V ou en Y. Dans le sang frais, les microorganismes montrèrent de lents mouvements intraérythrocytaires, alors que ceux-ci étaient très rapides en culture. Leur croissance n'était possible qu'en milieu à température optimum de  $25-28^{\circ}\text{C}$ .

Les propriétés du microorganisme en question font penser à *Bartonella bacilliformis*. Par ailleurs, les symptômes cliniques correspondaient plus à la fièvre Oroya (maladie jusqu'ici connue qu'en Amérique du Sud) qu'au Kala Azar.

Au cours des deux mois qui suivirent ce premier cas, le même microorganisme fut isolé de 5 autres patients chez qui on soupçonnait également un Kala Azar atypique, résistant à la chimiothérapie.

Les 6 patients provenaient de la province sud du Nil bleu, une région réputée endémique pour le Kala Azar et connue pour sa faune riche en Phlébotomes.