

syndrome (GLICKMAN *et al.*, 1985). Small amounts of antigen, presumably circulating in these conditions, can stimulate the production of specific IgE rather than IgG. Our results would seem to support this hypothesis: the greater sensitivity of the IgE test makes it possible to recognize clinical cases of OLM which are missed with IgG ELISA. In a comprehensive laboratory diagnosis, both IgG and IgE antibody levels should therefore be determined.

C. GENCHI¹
M. TINELLI²
F. BRUNELLO³
P. FALAGIANI⁴

¹General Pathology Institute,
University of Milan, Italy

²Department of Infectious Diseases,
Pavia, Italy.

³S. Luigi Gonzaga Hospital,
Orbassano, Italy.

⁴Lofarma Research Laboratory,
Milan, Italy.

References

- Brunello, F., Genchi, C. & Falagiani, P. (1983). Detection of larva specific IgE in human toxocariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 77, 279-280.
- Genchi, C., Brunello, F., Falagiani, P., Sioli, C. & Almaviva, M. (1983). Larva specific IgE against *Toxocara* hatched larvae antigens in sera of toxocaral syndrome patients and healthy blood donors. In: *Immunological diagnosis and other diagnostic methods for parasitic infections: report of a conference sponsored by the first European congress of clinical microbiology*. Ivo de Carneri (Editor), pp. 27-29.
- Glickman, L. T., & Schantz, P. M. (1981). Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiological Reviews*, 3, 230-250.
- Glickman, L. T., Grieve, R. B., Lauria, S. S. & Jones, D. L. (1985). Serodiagnosis of ocular toxocariasis: a comparison of two antigens. *Journal of Clinical Pathology*, 38, 103-107.

Accepted for publication 14th February, 1986.

Could Crimea-Congo haemorrhagic fever be a biohazard in the Central African Republic?

In the past few years, occasional epidemics of Congo-Crimean haemorrhagic fever (CCHF) have occurred in several states of Australia and countries of West Africa (GEAR *et al.*, 1982; SALUZZO *et al.*, 1984, 1985).

Recently we had the opportunity to isolate a strain from a wild rodent (*Mastomys*), caught in the north-west part of the Central African Republic (CAR). This isolate was the fourth since that obtained by Sureau and his colleagues in 1973 in this country (SUREAU *et al.*, 1976) (Table I).

Today, no epidemic or enzootic manifestations have been notified in CAR. We decided to assess the risk of an enzootic occurring and spreading in this country. Our first serological survey failed to show as important the prevalence of CCHF antibodies in the human population (GEORGES *et al.*, 1980; GONZALEZ *et al.*, 1983).

Nevertheless, we were able to perform serological tests in various geographical zones, allowing a rough assessment of the disease. From 1979 to 1985, 2672

Table 1—Strains of Crimean-Congo haemorrhagic fever virus isolated in CAR

Strain	Collecting date	Origin (host)	Collecting place
BT 604	20/09/73	<i>Hyalomma nitidum</i>	Berberati
ArTB1232	15/10/75	<i>Boophilus decoloratus</i>	Bangui
ArTB958	10/11/75	<i>Amblyomma variegatum</i>	Bangui
HB76P493	09/10/76	Man	Bangui
AnB3881	10/06/83	<i>Mastomys</i> sp.	Boheng

human sera were checked for antibodies against viral haemorrhagic fevers (VHF) using a fluorescent antibody technique (JOHNSON *et al.*, 1981). We found positive sera for CCHF only among populations living on cattle raising and/or in cattle migration zones (Table II).

After 1980, we observed an extension of rinderpest in cattle; the epidemic extended from Sudan and Chad towards the CAR; the social and economic situation of some CAR regions was deeply changed because of important migrations of "Mbororo" (cattle raising) populations, caused by the political instability in Chad. A preliminary estimate showed more than a two-fold rise in the cattle population since 1980 in west CAR. It is precisely in that north-western zone of dry savanna that we have found human sera positive for CCHF virus and the only positive rodent of the *Mastomys* type among 300 tested (GONZALEZ *et al.*, 1983).

In conclusion, the factors making possible an endemic manifestation of CCHF disease are present in CAR. Potential vectors (*Hyalomma* and *Amblyomma* ticks) are widely distributed in the country; the total cattle herd is increasing each year and this also increases the risk of enzootic manifestation. For the

Table 2—Serological survey of human populations in the CAR (fluorescent antibodies against CCHF)

Date of sampling	Geographical origin	Total Sera tested	Positive Sera
Dec. 1978	Mongoumba	497	0/0*0
Jan. 1979	Bangassou	499	0/0/0
May 1980	Bozo	100	0/0/0
Jun. 1980	Birao	280	0/0/0
Apr. 1980	Bambari	90	2/2/2
Jun. 1980	Bouar	184	0/0/0
Jun. 1981	Nola	80	0/0/0
Jun. 1981	Gomoka	83	0/0/0
Jun. 1981	Bambari	44	0/0/0
Jun. 1981	Botambi	52	0/0/0
Feb. 1983	Bangassou	22	0/0/0
Mar. 1983	Boheng/ Boguila	35	1/2/9
Jun. 1983	Paoua	96	1/1/0
Jun. 1983	Bangui	101	0/0/0
Jan. 1985	Balembé	31	0/0/0
Jan. 1985	Bouar	112	4/3/6
Jan. 1985	Dika	75	4/5/3
Jan. 1985	Dongo Bodama	59	3/5/1
Jan. 1985	NDongue	10	0/0/0
Jan. 1985	Zemio	140	0/0/0
Jan. 1985	Bambouti	82	0/0/0

*Number of positive sera (antibody titre ≥ 16) percentage positive.

second time a wild rodent, *Mastomys*, has been found positive for CCHF virus antibody (SALUZZO *et al.*, 1984). Consequently, *Mastomys* sp. could be an occasional host for the virus, presenting a real risk to humans because of its domestic behaviour. There is a need for an intensive serological survey to be developed in man and cattle, with a survey of tick populations for virus isolation.

A. J. GEORGES¹
J. P. GONZALEZ²

¹Institut Pasteur
B.P. 923, Bangui
Central African Republic

²Institut Français de Recherche
Scientifique pour le
Développement en Coopération
B.P. 893
Bangui, Central African Republic

References

- Gear, J. H. S., Thomas, P. D., Hop, M., Andronikou, S., Cohn, R. J., Ledger, J. & Berkowitz, F. E. (1982). Congo-Crimean hemorrhagic fever in South African. Report of a fatal case in the Transvaal. *South African Medical Journal*, 62, 576-850.
- Georges, A. J., Saluzzo, J. F., Gonzalez, J. P. & Dussarat, G. (1980). Arboviroses en Centrafrique: Incidences et aspects diagnostiques chez l'homme. *Médecine Tropicale*, 40, 561-568.
- Gonzalez, J. P., McCormick, J. B., Saluzzo, J. F. & Georges, A. J. (1983). Les fièvres hémorragiques africaines d'origine virale. Contribution à leur étude en République Centrafricaine. *Cahiers O.R.S.T.O.M., Série Entomologie Médicale et Parasitologie*, 21, 119-130.
- Johnson, K. M., Elliot, L. H. & Heymann, D. (1981). Preparation of polyvalent viral immunofluorescent intracellular antigens and use in human serosurveys. *Journal of Clinical Microbiology*, 14, 527-529.
- Saluzzo, J. F., Digoutte, J. P., Cornet, M., Bauden, D., Roux, J. & Robert, V. (1984). Isolation of Crimean-Congo hemorrhagic fever and Rift Valley fever viruses in Upper Volta. *Lancet*, 8387, 1179.
- Saluzzo, J. F., Aubry, P., Aubert, H., & Digoutte, J. P. (1985). La Maladie à virus CHF-Congo en Afrique. A propos d'un cas à manifestations hémorragiques en Mauritanie. *Bulletin de la Société de Pathologie Exotique*, 78, 164-169.
- Sureau, P., Cornet, J. P., Germain, M., Camicas, J. L. & Robin, Y. (1976). Enquête sur les arbovirus transmis par les tiques en République Centrafricaine (1973-1974). Isolement des virus Dugbe, CHF-Congo, Jos et Bhandja. *Bulletin de la Société de Pathologie Exotique*, 69, 28-33.

Modifying the method by the use of a stepwise ionic strength gradient we were able to isolate different strains of *T. cruzi* in sufficient yields for biochemical studies.

Briefly, γ -irradiated (580 rads) Lewis rats (90 to 120 g) were inoculated with 1×10^5 blood trypomastigotes of YuYu, CL and Colombian strains. When a high parasitaemia was reached ($1.5-2.5 \times 10^7$ trypomastigotes ml^{-1}) the rodents (20 to 30) were bled under anaesthesia using heparin (2.5 mg ml^{-1}) as anticoagulant. To remove most of the erythrocytes, the suspension was centrifuged at 100 g for 10 min at room temperature and the tubes transferred to a 37°C water bath for 20 min. The plasma (60 ml) containing the trypanosomes was separated and the sediment was resuspended in 30 ml of PSG, pH 7.45 ($I = 0.145$) and the whole procedure repeated twice. The supernatants were pooled giving a volume of 120 ml and poured on top of a DEAE-cellulose (Whatman, DE-52) column pre-equilibrated with PSG, pH 7.45 ($I = 0.145$) and contained in a Buchner funnel 8 cm diameter \times 6 cm height. The organisms were eluted using 200 ml quantities of PSG solutions of increasing ionic strength, namely, $I = 0.145$; 0.181; 0.217; 0.253; 0.289. The eluate was collected in 40 ml fractions in centrifuge tubes containing inactivated foetal calf serum to give a final 5% concentration.

The YuYu strain was recovered in the fraction eluting between the addition of PSG $I = 0.181$ and 0.217 and the CL strain in the fraction 0.217-0.253. The broad forms of the Colombian strain were eluted after the first 150 ml of PSG at $I = 0.253$ was added, whereas slender forms were eluted only at $I = 0.289$. Recovery rates were 85% for YuYu, 80% for CL and 70% for the Colombian. Total yields ranged from $1.0-2.0 \times 10^9$ blood forms for each separation and the organisms were 100% pure.

Our results confirm previous reports that *T. cruzi* shows differences in its surface charge according to the strain (SOUZA, 1981; BOGLIOLO, 1983) and that these differences can occur among sub-populations of the same strain (SOUZA, 1981).

This study received financial support from the UNDP/World Bank/WHO, CNPq, and FINEP.

E. A. EVANGELISTA¹
E. CHIARI²
A. R. BOGLIOLO¹
A. A. SILVA PEREIRA¹

¹Departamento de Bioquímica-Imunologia,
²Departamento de Parasitologia,
Instituto de Ciências Biológicas,
Universidade Federal de Minas Gerais,
31.270 Belo Horizonte, Brazil

Elution characteristics of bloodstream forms of three strains of *Trypanosoma cruzi* during isolation on DEAE-cellulose

The isolation of trypanosomes from rodent blood using a DEAE-cellulose column was introduced by LANHAM (1968). GUTTERIDGE *et al.* (1978) used this technique to isolate *Trypanosoma cruzi*, Sonia strain, from blood from rats and chinchillas by eluting the organisms with phosphate-saline-glucose (PSG) solution at pH 7.5, $I = 0.206$.

References

- Bogliolo, A. R. (1983). *Comparative behavioural and life cycle studies on different zymodemes of Trypanosoma cruzi*. Ph.D. Thesis, University of London.
- Gutteridge, W. E., Cover, B. & Gaborak, M. (1978). Isolation of blood and intracellular forms of *Trypanosoma cruzi* from rats and other rodents and preliminary studies of their metabolism. *Parasitology*, 76, 159-176.
- Lanham, S. M. (1968). Separation of trypanosomes from the blood of infected rats and mice by anion-exchangers. *Nature*, 218, 1273-1274.

Accepted for publication 28th February, 1986.