Ann. Soc. belge Méd. trop.
1979, **59**, 87-92

MEASUREMENT OF ANTIBODIES TO EBOLA VIRUS IN HUMAN SERA FROM N. W.-ZAIRE

by

G. VAN DER GROEN & S. R. PATTYN

Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium

Summary — Two hundred and fifty one human sera, obtained in the period between 1972 and 1978, in the North Western part of Zaire, were screened for Ebola antibody by the indirect immunofluorescence antibdoy test (IFA). Thirteen percent of the sera were positive. Ebola is endemic in the North Western part of Zaire since 1972.

KEYWORDS : Ebola Virus; Antibodies, Viral; Indirect Fluorescent Antibody Technic; Zaire.

Introduction

Ebola haemorrhagic fever was first recognized in South Sudan and North Central Zaire in 1976 (Breman *et al.*, 1978; Francis *et al.*, 1978).

Ebola virus antibody surveys in various population groups showed that the geographic spread of Ebola virus is greater than the epidemic area of 1976 (van der Groen *et al.*, 1978).

This paper presents the results of an Ebola antibody survey in the North Western part of Zaire on sera obtained between 1972 and 1978.

Material and methods

Antibodies to Ebola virus were measured by the indirect fluorescent technique (IFA) (van der Groen *et al.*, 1978).

Viral antigen

Gamma and U. V. irradiated multispot slides with Ebola virus infected Vero cells (type 80826 Mayinga) were kindly provided by the Center for Disease Control, Atlanta, Georgia, U. S. A.

Conjugate

Sheep antiserum against human immunoglobulin G conjugated with fluorescein isothiocyanate manufactured by Wellcome (Lot no. K 4715) was used at a dilution of 1 : 100. Evans blue in a final concentration of 0.2 percent (W/V) was used as counterstain.

Sera

The sera were obtained in the North Western part of Zaire by members of a Belgian scientific team working in Gemena. They were kindly provided by Dr. F. De Lange and P. Bourdoux, Laboratory for Radioisotopes, University of Brussels, Belgium.

A reference immune serum with human anti-Ebola (no. 096029 CDC) was kindly provided by CDC, Atlanta.

Readings were performed with a Leitz microscope waterimmersion lense (× 50) and ocular × 6.3.

Results

The sera were initially screened at a dilution of 1 : 16, because at lower dilutions non specific fluorescence was frequent. It was characterized by a very pronounced yellow illumination of all the cells on the slide. This is in contrast with the positive control serum in which only 40 percent of the cells contained the characteristic green-yellow fluorescent inclusions while the cells not containing Ebola virus antigen were coloured dark red without any trace of fluorescence. Moreover non specific reactions sometimes showed up in all the cells as green fluorescent wormlike structures totally different from the characteristic fluorescent Ebola virus inclusions. About 30 percent of the sera showed these aspecific reactions.

At a dilution of 1 : 16 it was possible to differentiate between the aspecific and characteristic fluorescent inclusions. Similar non specific reactions with sera from Zaire were observed by D. Heymann (personal communication). Aspecific fluorescence was completely absent when European sera were screened.

The aspecific fluorescence can be somewhat troublesome for the interpretation of the IFA test and a different method for detection of specific Ebola virus antibodies is needed to confirm these results.

Of a total of 251 sera investigated, 43 (13 percent) were positive for Ebola virus antibodies (table 1).

Of the 43 positive sera, 17 (31 percent) had a titer of 1 : 16, 22 (41 percent) 1 : 64, 3 (5.5 percent) 1 : 256 and 1 (2 percent) 1 : 1,024 (table 1).

The village Boyabo Libenge showed the highest percentages of positive sera (table 1).

The highest titer was observed in a serum obtained in 1974 in Bodiawa and normally reflects a relatively short time interval after infection.

The distribution of antibodies in different age groups for both sexes is shown in table 2. No significant differences could be detected. It may be seen that antibodies are acquired between 9 and 19 years whereafter the prevalence remains stable around 20 percent.

Discussion

The percentage (13 percent) of Ebola antibody positive sera in Western Equateur Province of Zaire agreement with 8 percent found in the epidemic

area of Yambuku, Zaire in 1976 (van der Groen *et al.*, 1978), and with 18 percent positives among a population of Pygmees in the central part of Cameroun (personal communication, D. Heymann).

TABLE 1

Prevalence of persons with antibody to Ebola virus, Zaire

Villages	Year of sampling	Number of persons surveyed	Number of persons with antibody titer 1 : 16	1 : 64	1 : 256	1 : 1,024	Total	
Bogene	1978	10	1	2			3	30 %
Motuba	1978	10		1	1		2	20 %
Bodango	1978	10		1	1		2	20 %
Bokpulunu	1978	10		2			2	20 %
GBWA	1978	10					0	
Bowanga	1977	5					0	
Wodjo	1977	5		1			1	20 %
Boyabutui	1977	5					0	
Seavoro	1977	5	1				1	20 %
Bokalakiti	1977	5					0	
Bolombo	1977	5	1				1	20 %
Linzamba	1977	5					0	
Bango	1977	4		2			2	50 %
Gwaka (plantation)	1977	5					0	
Bogene	1977	5					0	
Bokalakiti	1977	1					0	
Bogene	1976	5	1				1	20 %
Bobanga	1976	5					0	
Bolingo	1976	5					0	
Komozi	1976	5	1				1	20 %
Djiba (plantation)	1976	5		1			1	20 %
Libobi	1976	5					0	
Bokungu	1976	5	1				1	20 %
Kundu	1976	5			1		1	20 %
Dombo	1976	5		2			2	40 %
Engbonda	1976	5					0	
Bombaliswe	1975	9	2	2			4	44 %
Tondo	1975	9	2				2	22 %
Bopingina	1975	7					0	
Boyabo Libenge	1975	9		5			5	56 %
Bodiawa I	1974	5		1		1	2	40 %
Bodika	1974	5	1				1	20 %
Dambalia	1974	5						
Diobo	1974	7	1				1	14 %
Busu Gobe I	1974	2						
Boyalulia	1973	1					0	
Niaki	1973	7	1	2			3	43 %
Wagbodo	1972	8	1				1	13 %
Boyagbokoto	1972	8					0	
Boyabadua	1972	8	1				1	12.5 %
Boyalulia	1972	4	1				1	25 %
Boyagomobwa	1972	7	1				1	14 %
Total		251	17	22	3	1	43	13 %

TABLE 2

Prevalence of antibody to Ebola virus by age group and sex, Zaire

Age group	Male		Female	
	Number surveyed	Number with antibody	Number surveyed	Number with antibody
0 - 4	0	0	0	0
5 - 9	6	0	5	0
10 - 14	23	2 (9 %)	17	2 (12 %)
15 - 19	30	3 (10 %)	31	7 (23 %)
20 - 29	45	11 (24 %)	35	7 (21 %)
30 - 39	15	2 (13 %)	16	1 (6 %)
40 and +	10	2 (20 %)	4	1 (25 %)
Total (*)	129	20 (15.5 %)	108	18 (17 %)

(*) Because the age of every person surveyed was not known there is a difference for the total number surveyed, between tables 2 and 1.

The geographic area where Ebola virus antibodies occur is very large : Tondo (two positive sera out of nine tested) in the South is situated 700 km from Seavoro (one positive serum out of five) in the North (figure 1). As all sera tested came from an area west of Yambuku, it would be highly interesting to obtain similar information from an area east of Yambuku and from the right bank of the Ubangi river.

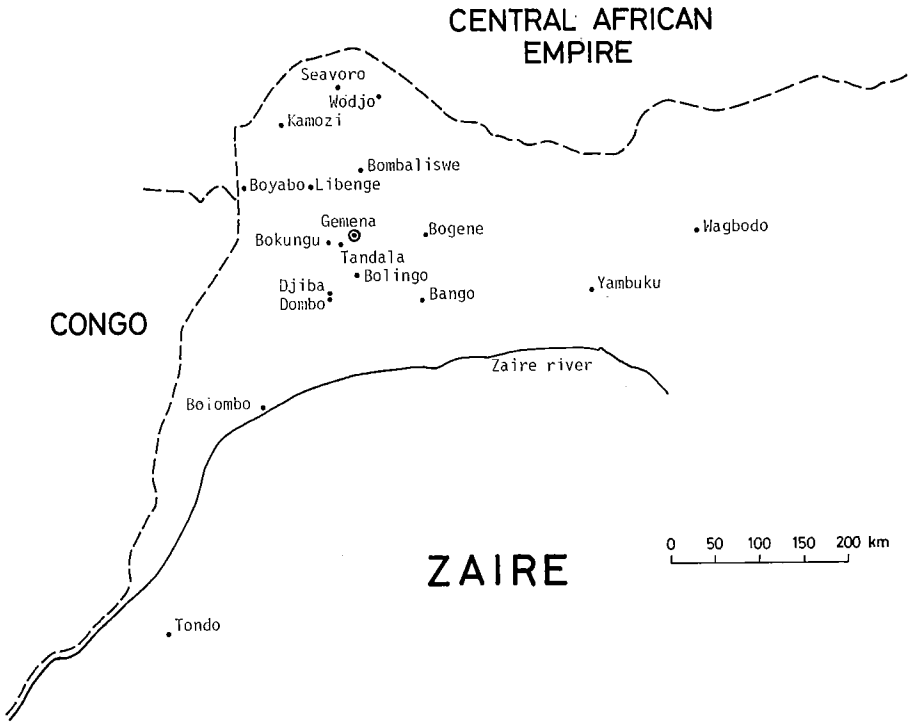

Figure 1.

Geographic spread of Ebola antibody in the North Western part of Zaire.

Our knowledge about the geographical spread of Ebola antibodies up till now is summarized in table 3. If the antibodies detected are specific and not the result of an infection by a cross reacting agent, Ebola virus infection is much more widespread, both in terms of incidence and of geographic distribution than was previously thought.

TABLE 3

Ebola IFA antibodies in human sera from selected geographic regions

Area	Number of samples tested	Total number of positive anti-Ebola IFA sera	Reference
Zaire :			
— Yambuku epidemic area	984	78 (8 %)	van der Groen *et al.* (1978)
— Control area	442	21 (5 %)	Idem
— Libela	36	3 (8 %)	Idem
— N. W. Zaire			
with Gemena as center	251	43 (13 %)	Present study
of the area	1,168	108 (9 %)	D. Heymann, person. commun.
Sudan :			
— Maridi	214	71 (33 %)	van der Groen *et al.* (1978)
— Nzara	218	14 (6.4 %)	Idem
Uganda :			
— N. Bosoga	1	1	Idem
Rhodesia :			
— Northern zone	243	6 (3 %)	Idem
Cameroun	—	(18 %)	Heymann, D. person. commun
Central America :			
— Panama	200	4 (2 %)	van der Groen *et al.* (1978)

The serum of a Belgian physician who was sent five times to Gemena, in the endemic zone, between 1975 and 1978 had a titer of 1 : 64 for Ebola virus antibodies. He was ill from 5 to 11 August 1978, with fever, sore throat, headache and muscle pain all over the body. He was never bitten by animals. On 25-26 of July he examined a large number of people at the mission at Bominenge.

That Ebola virus is sporadically active in this area was shown by the isolation of Ebola virus from a post-mortem blood specimen of a 9 year old girl who died with a haemorrhagic fever syndrome at the Tandala Mission Hospital in June 1977 (Johnson, personal communication).

Since Ebola virus circulated already at least since 1972 in N. W.-Zaire, and since an epidemic similar to the one of 1976 in Yambuku was never reported in this region it must be assumed that Ebola virus infection is much more common and much less serious than during the 1976 epidemics in Maridi and Yambuku. It is most improbable that extensive epidemics characterized by high mortality would have gone unnoticed in this region. This leeds to the hypothesis that the high mortality rate of the Zaire-Sudan Ebola virus outbreak in 1976, was exceptional and due to the artificial spread of the virus (Breman *et al.*, 1978).

Ebola virus infection in the local communities is probably characterized by a much lower infectivity, a lower attack rate and a higher frequency of non apparent infection.

The N. W. part of Zaire seems to be convenient for research concerning the virus reservoir of Ebola virus.

Acknowledgements — *The authors thank Dr. F. De Lange and Dr. P. Bourdoux, Laboratory of Radioisotopes, University of Brussels (U.L.B.), Belgium, who kindly provided the sera. The authors are indebted to Dr. K. M. Johnson, for inactivated Ebola antigen slides.*

Bepaling van antistoffen tegen Ebola virus in mensensera afkomstig uit N.W.-Zaïre.

Samenvatting — Tweehonderd éénenvijftig mensensera, tussen 1972 en 1978 in Noord-West-Zaïre verzameld, werden op aanwezigheid van Ebola antistoffen onderzocht met behulp van de indirecte immunofluorescentietechniek.

Dertien procent van de sera waren positief.

Ebola is endemisch in het Noord-westelijk gedeelte van Zaïre minstens sedert 1972.

Détermination des anticorps contre le virus Ebola dans des sérums humains provenant de la partie nord-ouest du Zaïre.

Résumé — Deux cent cinquante et un sérums humains, collectés entre 1972 et 1978 dans le nord-ouest du Zaïre ont été examinés pour la présence d'anticorps envers le virus Ebola par la méthode d'immunofluorescence indirecte.

Treize pourcent des sérums étaient positifs.

Ces résultats montrent que le virus Ebola est endémique dans le partie nord-ouest du Zaïre.

Received for publication on February 8, 1979.

REFERENCES

Breman, J. G., Piot, P., Johnson, K. M., White, M. K., Mbuyi, M., Sureau, P., Heymann, D. L., Van Nieuwenhove, S., McCormick, J. B., Ruppol, J. P., Kintoki, V., Isaäcson, M., van der Groen, G., Webb, P. A. & Nguete, K. (1978) : The epidemiology of Ebola fever in Zaire, 1976. *in* : Ebola virus haemorrhagic fever. ed. S. R. Pattyn, p. 103-121. Elsevier/North-Holland Biomedical Press. Amsterdam. New York.

Francis, D. P., Smith, H. D., Highton, R. B., Simpson, D. I. H., Pacifico Lolik, Isiaih Mayom Deng, Lagu Gillo, A., Idris, A. A., El Tahir, B. (1978) : Ebola fever in the Sudan, 1976 : Epidemiological aspects of the diseases. *in* : Ebola virus haemorrhagic fever. ed. S. R. Pattyn, pp. 129-135. Elsevier/North-Holland Biomedical Press. Amsterdam. New York.

Van der Groen, G., Johnson, K. M., Webb, F. A., Wulff, H. & Lange, J. (1978) : Results of Ebola antibody surveys in various population groups. *in* : Ebola virus haemorrhagic fever. ed. S. R. Pattyn, pp. 203-205. Elsevier/North-Holland Biomedical Press. Amsterdam. New York.