Table 1: Findings of seroprevalence studies investigating presence of ebolavirus immunoglobulin G antibodies in 'asymptomatic' populations, 1961 - 2016

A = populations with household contact or known case contact

B = populations without known contact but in outbreak areas or villages with cases

C = general population – no known outbreak exposure or contact

Location	**Year sera collected**	**Population type** (as described in paper)	**Group**	**No. of samples**	**# IgG +ve**	**Species % +ve**	**Assay type**	**Cut-off** (as described in paper	**Antigen & validation information**	**Notes**
Assab, Awash, Blue Nile, Illubor, & Ogaden regions, Ethiopia[1]	1961/62	Asymptomatic individuals from area not affected by ongoing Yellow Fever epidemic	C	178	42	EBOV 24%	IFA(w)	≥1:16	Antigens: Polyvalent ELM (Ebola-Lassa-Marburg viruses) & monovalent EBOV (Mayinga): all sera reacting with ELM also reacted with monovalent EBOV antigen but not with monovalent Marburg and Lassa.	Samples from symptomatic Yellow Fever-negative individuals also tested: 12% positive.
N.W. Zaire (now Democratic Republic of Congo DRC)[2]	1972-78	General population from area west of Yambuku (site of 1st recorded outbreak in 1976)	C	251	43 26	EBOV 17.1% 10.4%	IFA(w)	≥1:16 ≥1:64	Antigen: EBOV (May. 80826)	
Harbel, Bong town, Yekepa, Liberia[3]	1973	Staff & family members of rubber and mining companies	C	592	83	Ebolavirus 14.0%	ELISA/ WB	Not stated	Antigen not stated	Sera taken in 1973 and investigated for Lassa and ebolavirus in ~1986. Ebola statistic reported without further information.
Northern Rhodesia (now Zimbabwe)[4]	1975	"Control group"	C	243	2 0	EBOV 0.8% 0.0%	IFA(w)	≥1:8 ≥1:64	Antigen not specified in report: Kuhn[52] notes antigen as EBOV	Areas sampled had no known outbreak
Yambuku, Zaire (DRC)[5]	1976	Asymptomatic contacts of cases	A	404	10	EBOV 2.5%	IFA(w)	≥1:64	Antigen: EBOV Validation: Repeat testing of the 4 antibody positive with no known contact gave the same results. 32/33 (97%) positive samples (including samples from cases) confirmed positive by CDC (US)[4] (p141)	
		Residents from villages with cases but no known contact	B	448	4	EBOV 0.9				
		Residents from 4 neighbouring villages with no cases	C	442	5	EBOV 1.1%				
Maridi, Sudan (now South Sudan)[6]	1976	Close family contacts	A	93	13	SUDV 14.0%	IFA(w)	≥1:8	Antigen: SUDV Validation: 42 of 48 clinically diagnosed survivors from Nzara (87%) were considered positive using the same IFA protocol. Several samples from Nzara were retested and confirmed positive by CDC (US) using the same protocol.	9 antibody positive family contacts had symptoms and have been excluded from these figures. Not clear if all subjects in this group were interviewed about symptoms.
		Asymptomatic Maridi schoolboys with no known contact	B	29	3	SUDV 10.3%				
		Asymptomatic hospital staff with probable/possible contact	A	64	7	SUDV 10.9%				4 nurses; 1 cleaner, 1 toilet cleaner, 1 water carrier were positive

Nzara, Sudan (now South Sudan)[6]	1976	Asymptomatic cotton factory workers (site of index case but reportedly no direct contact)	B	109	7	SUDV 6.4%	IFA(w)	≥1:8		Among factory workers titre range was 1:16 - 1:32
		Close family contacts of clinically diagnosed cases	A	78	1	SUDV 1.3%				Only 6/31 (19.4%) of clinically diagnosed subjects were antibody positive, and none had levels > 1:32
San Blas Islands, Panama[4]	1977	San Blas Indians	C	200	1	EBOV 0.5%	IFA(w)	≥1:64	Antigen: not specified in report: Kuhn[52] records antigen as EBOV	Areas sampled had no known outbreak. Method of selection not known
Tandala, Zaire (DRC)[7]	1977-78	Missionaries and 'a few' hospital staff with case contact (1977)	A	50	0	EBOV 0%	IFA(w)	≥1:16	Antigen: EBOV	One doctor, who tested positive in 1977 and 1978 and had history of severe illness after attending the autopsy of a haemorrhagic fever victim in 1972, is excluded. Some individuals gave samples in both the 1977 and 1978 groups
		Hospital staff with case contact (1978)	A	71	0	EBOV 0%				
		Residents of villages with confirmed /suspect cases	B	346	21	EBOV 6.1%				
		Residents of other villages in same area	B	750	58	EBOV 7.7%				
Liberia [8]	1978-79	Random rural general population in multiple counties	C	433	26	Ebolavirus 6.0%	IFA(w)	≥1:16	Antigens: unspecified ebolavirus, Marburg & Lassa viruses. No sera positive for ebolavirus was positive for MARV or LASV.	No known outbreak. Titre range: 1:16 to 1:1024
Nzara/Yambio, (South) Sudan[9]	1979	Asymptomatic adult family members of cases with known physical contact	A	38	12	Ebolavirus 32%	IFA(w)	≥1:16	Antigen: unspecified	
		Asymptomatic adult family members of cases who denied physical contact	B	23	3	Ebolavirus 13%				
		Adults from families without known cases in same area	B	45	8	Ebolavirus 18%				Unknown if these people were exposed in 1976 outbreak, which could explain the high prevalence
Bangassou, Central African Republic (CAR)[10]	1979	General population in forest and semi-forest zones	C	499	10 3	Ebolavirus 2.0% 0.6%	IFA(w)	≥1:16 ≥1:64	Antigen: Polyvalent of unspecified ebolavirus, MARV & LASV, followed by monovalent test for positive samples. Positive sera sent to CDC (US) for repeat testing; results not reported.	Areas sampled had no known outbreak
Moloundou, Lolodorf Bipindi, Lomie, Yaounde & Pete, Cameroon[11]	1980	General population in five regions (forest, pre Sahelian savannah and the capital) and different ethnic groups	C	1517	147	Ebolavirus 9.7%	IFA(w)	≥1:16	Antigens: unspecified ebolavirus provided by CDC (US)	No known outbreak. Positives in all areas, range 3%-23%. Highest in Pygmies and rain forest farmers. 6% in the capital, Yaoundé. Report to OCEAC in the same year gave positivity of 6.2% (51/821) in Moloundou, compared to 13.2% for the same location in this study, and 29% (20/70) in Mbatika, but positivity threshold used is not reported.[63]

Lugulu, western Kenya[12]	1980	Family and close neighbours of an IFA confirmed case (asymptomatic?)	A	84	4	Ebolavirus 4.8%	IFA (?)	≥1:16	Antigens: monospecific, triple (unspecified ebolavirus, MARV, LASV) and poly-antigen (CCHFV, RVFV, ebolavirus, LASV, MARV). Validation: sera examined at National Institute of Virology, Johannesburg and CDC(US): labs used different thresholds, so positive confirmed only where both found ≥1:16	Area in Western Kenya, close to Nzoia. Samples collected during investigation of 2 MARV suspect cases who were later shown to be ebolavirus positive.
Kenya[13]	1980	Different studies in 5 regions of Kenya: - Lodwar, Laisamis, Masia, Malindi/Kilifi - Nzoia	C	1058 841	18 9	EBOV/SUDV 1.7% 1.1%	IFA(w)	≥ 1:16	Antigens: inactivated unspecified ebolavirus, MARV, CCHFV, RVFV, & LASV; positives tested against EBOV(May) & SUDV (Boniface & Maleo). Authors report 'most' of the Nzoia samples were only tested against EBOV(May)	No known outbreak but Nzoia cohort reported to include suspected cases and their contacts. Highest prevalence: Lodwar 7.8% (north-west Kenya). Note referenced paper includes some sera reported on in other papers.[72]
Haute Ogooue, Gabon[14]	1980	General population in outbreak area but no known contact	B	253	16 8 5 1 21 8	EBOV 6.3% 3.2% SUDV 2.0% 0.4% EBOV/SUDV 8.3% 3.1%	IFA(w)	≥ 1:16 ≥1:64 ≥ 1:16 ≥ 1:64 ≥ 1:16 ≥1:64	Antigens: inactivated polyvalent unspecified ebolavirus/LASV MARV: positives tested against EBOV(802850) & SUDV(802681).	Samples from the Occupational Health Services, plus 28 women & their newborns. One sample was positive ≥1:64 on both EBOV & SUDV
Northern Rhodesia (now Zimbabwe)[15]	1980	Asymptomatic schoolboys (8-10y): no known outbreak	C	486	9 4	EBOV 1.9% 0.8%	IFA(g)	≥ 1:8 ≥ 1:128	Antigen: polyvalent CCHFV, RVFV, LASV, MARV, unspecified ebolavirus slides provided by CDC (US): positives tested against individual antigens (EBOV & SUDV) Validation: 4 of 5 positive samples sent to CDC (US) were ≥1:128 in repeat IFA testing.	None were positive for SUDV.
Pool, Congo-Brazzaville (now Republic of Congo)[16]	1981	Children from 20 villages aged 3-15 years and unvaccinated for smallpox	C	790	119	Ebolavirus 15.0%	IFA(?)	Not stated	Antigen: polyvalent CCHFV, RVFV, LASV, MARV, unspecified ebolavirus; also tested against monovalent antigens	Pool region is on the border with DRC. Areas sampled had no known outbreak but populations were selected for close contact with animals
Grand Bassa, Liberia[17]	1981-82	Individuals asymptomatic for EVD consisting of 106 epilepsy patients; 87 healthy relatives of these patients; 32 unrelated geographically matched controls.	C	225	26 4 29	EBOV 11.6% SUDV 1.8% Overall 12.9%	IFA(w)	unclear	Antigen: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon) (known as CRE_2LM); positives retested against individual antigens. Validation: Difficulties with non-specific binding led researchers to replicate and use blinded observers to read results. Only samples with unequivocally positive results by 2 observers were considered positive.	No known outbreak. Similar proportion positive in each participant group. 38% epilepsy patients had a febrile illness 1-4 weeks before onset of epilepsy, but no significant difference in seroprevalence with & without febrile history. Paper says 30 positives in total but one counted twice (positive for EBOV and SUDV). Titres ranged from 32-128 for EBOV; 6/29 had antibodies to more than 1 virus.

Sud-Ubangi sub-region, DRC (includes Tandala)[18]	1981-85	Age/sex matched controls from the same villages as reported cases	C	137	2	Ebolavirus 1.5%	IFA(w)	≥1:64	Antigen: polyvalent CRE_2LM; positives tested against unspecified ebolavirus-specific antigens.	In addition 188 contacts of possible and probable cases were tested; 28 were positive at ≥1:64 but all had had symptoms fitting the definition of a possible or clinical case. It is not clear how many of the other contacts had symptoms.
Northeastern, southeastern & western Gabon[19]	1981-1997	Six rural communities (Makokou, Doussala, Doussieousou, Matadi-Ngoussa, Moukoro, Latoursville): sera gathered during onchocerciasis research	C	1147	14	EBOV 1.2%	ELISA (k)	Mean +3 SD of OD of negative controls	Antigen: EBOV Validation: In 2003, 6 of original 14 positives were re-bled (others unavailable): 2 were still positive. 14 controls (relatives and 'cohorts') were unreactive	6 seropositives were from north-eastern Gabon where outbreaks had occurred; 8 were from western communities more than 500km from known epidemics. Authors also investigate and correlate animal with human outbreaks. Conclude that less virulent strains of EBOV affected western areas.
Madina-Ula, Guinea[20]	1982-83	Healthy adults sampled during an outbreak of an unknown disease	C	138	11 4 2	EBOV 7.8% 2.9% 2.2%	ELISA(r) ELISA(r) IFA(b) ELISA(r) IFA (b)	≥ 1:8 ≥ 1:512 ≥ 1:16 ≥ 1:512 ≥ 1:64	Antigen: EBOV	Areas sampled had no known outbreak
Benin[21]	1983	General population, non-outbreak country	C	603	2	EBOV or SUDV? 0.3%	IFA (?)	≥ 1:64	Antigen: EBOV, SUDV	Unpublished data cited by Gonzalez *et al* (2005): no further information, not specified which ebolavirus antigen samples were reactive to.
Ethiopia, Awash valley[1]	1983	Unexposed children	C	250	0	EBOV 0.0%	IFA(w)	≥ 1:16	Antigens: Polyvalent ELM (Ebola-Lassa-Marburg viruses) & monovalent EBOV (May)	Areas sampled had no known outbreak
Karamoja, Uganda[22]	1984	'Healthy' adults 20-40y recruited during visits to a health centre, excluding any with current or recent fever	C	132	4 4	EBOV 3.0% SUDV 3.0%	IFA(w)	unclear	Antigen: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon) (CRE_2LM); positives retested against individual antigens.	No known outbreak. Not clear if some samples had antibodies to both EBOV & SUDV. Not specified how many > 1:64. All samples < 1: 128.
Mobai, Sierra Leone & unspecified location Sudan[23]	<1984	No information on population type: general population assumed from description - Mobai, Sierra Leone - Sudan	C	556 284	10 10 1 0	EBOV (a) 1.8% (b) 1.8% (a) 0.35 (b) 0	(a) ELISA +ve/IFA+ ve (b) ELISA +ve/ IFA -ve or unclear	ELISA: +ve within 2 SD of +ve ref. sera; -ve within 1 SD of negative ref sera IFA ≥ 1:100	Antigen: EBOV Validation: All? samples were tested using both assays	Year of sample collection is not recorded. Paper reports a high level of cross reactivity with MARV, lasting a number a number of years after infection.

Haute Ogooue, Gabon[24]	1985	Inhabitants of Ambinda village	C	213	20 6 2 1 22 7	EBOV 9.4 % 1.4% SUDV 0.9 % 0.5% Overall 10.3% 3.3%	IFA (j)	≥ 1:16 ≥ 1:64 ≥ 1:16 ≥ 1:64 ≥ 1:16 ≥ 1:64	Antigen: polyvalent CCHFV (IB-AR-10200 Nigeria), RVFV (ZH-501 Egypt), LASV (Josiah), MARV (Musoki), EBOV (May), SUDV(Bon) (CRE_2LM); positives retested against individual antigens.	No known outbreak
Nola,Ikaumba, Bozo, Bangassou, Mbre & Birao, Central African Republic [25]	1984-85	General population from 5 ecological regions including one close to Zaire/DRC outbreak area	C	836	152 63	EBOV 18.2 % SUDV 7.5 %	IFA(j)	≥ 1:16	Antigens: EBOV (May), SUDV (Bon), MARV (Mus)	No known outbreak but Zemio which borders DRC accounted for 43% of EBOV positives
Nola,Ikaumba, Bozo, Bangassou, Mbre & Birao, Central African Republic [26]	1984-85	Asymptomatic general population from 5 ecological distinct zones selected on accessibility: additional villages wer chosen where multiple ethnic groups coexisted.	C	4295* 4078*	681 209 853 259 914 335	EBOV 15.9% 5.1% SUDV 19.8% 6.4% Overall 21.3% 8.2%	IFA (j)	≥ 1:16 ≥ 1:128 ≥ 1:16 ≥ 1:128 ≥ 1:16 ≥ 1:128	Antigens: polyvalent EBOV (May), SUDV (Bon), MARV (Mus), LASV (Jos), CCHFV (10200), RVFV(ZH501); positives (≥ 1:16) retested against monovalent antigen. Validation: 185 samples were reanalysed by ELISA in 1996: results confirmed original analysis.[21]	Study linked to one above in CAR[25] but using different set of sera sampled in the same period. * Two different denominators are cited: 4296 people are reported for all titre levels; 4078 people are reported in the table describing only samples showing titres ≥ 1:128. Highest prevalence in woods and forest regions. 86% of titres ≥ 1:64 but reached as high as 1:2048
Nkongsamba, Cameroon[27]	1985	Randomly selected urban general population (15-44 years)	C	375	7 5	Ebolavirus 1.9% 1.3%	IFA (?)	≥ 1:16 ≥ 1:64	Antigens: polyvalent unspecified ebolavirus, CCHRV, RVFV, MARV)	These samples were included in the following multi-country study which used a different threshold.[28] One sample was positive for both ebolavirus and RVFV
Central Africa[28] (now Middle Africa)	1985-87	Randomly selected sera collected in: Cameroon (Mora, Maroua, Nkongsamba) Central African Republic (Bangui) Chad (N'djamena) Republic of Congo (Pointe Noire, Brazzaville) Equatorial Guinea (Bioco Island, Nsork) Gabon (Libreville, Port-Gentil, Ogooue-Ivindo, Haut Ogooue, Ngounie)	C	1152 327 334 728 688 1841	89 107 334 51 111 259	EBOV/SUDV 7.7% 32.7% 3.6% 7.0% 16.1% 14.0%	IFA (w)	≥ 1:16	Antigens: polyvalent EBOV (May), SUDV (Bon), MARV (Mus), LASV (Jos), CCHFV (10200), RVFV(ZH501); positives (≥ 1:16) retested against monovalent antigen.	Areas sampled had no known outbreak

Chobe, Northern Botswana[29]	1984-86	1984: 52 asymptomatic villagers) 1985: 25 villagers with non-specific or ictero-haemorrhagic symptoms 1986: 77 asymptomatic villagers	C	154	0	EBOV/SUDV 0%	IFA (J)	≥ 1:16	Antigens: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon): positives re-tested against monovalent antigens.	Areas sampled had no known outbreak. Unable to separate results for the symptomatic group. Only reaction found was against RVFV. Testing performed in Paris.
Lobaye, Central African Republic[30]	1987	Asymptomatic general population, Lobaye district: Pygmy hunter-gathers	C	127	31	EBOV/SUDV 24.4%	IFA (J)	≥ 1:128	Antigens: polyvalent EBOV (May), SUDV (Bon), MARV (Mus), LASV (Jos), CCHFV (10200), RVFV(ZH501); positives (≥ 1:16) retested against monovalent antigen, considered reactive if ≥ 1:128. Validation: 296 samples from this study and 185 samples from the CAR 1984-85 study above were re-analysed in 1996 using ELISA (≥1:400 & sum of 4 ODs ≥ 1.000). 6.2% were Ebola IgG positive (30/481) compared to 6.4% in these samples previously by IFA.[21,26]	Area with no known outbreak. Of the positives, 45 reacted to both EBOV & SUDV: it is not possible to identify how this splits between the groups.
		Asymptomatic general population Lobaye district: Mozombo/Mbati subsistence farmers	C	300	42	EBOV/SUDV 14.4%				
Nigeria[31]	1988	Asymptomatic general population in different locations	C	1677	30 22	SUDV 1.8% EBOV/SUDV 1.3%	IFA(w)	≥ 1:10	Antigens: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon): positives with titre ≥ 1:10 retested against monovalent antigens. Known positive/negative controls used	Areas sampled had no known outbreak. All positive samples came from savannah areas (Benue/Gongola) Of the positives, none reacted to EBOV alone.
Antanarivo, Mandoto, andasibe, Tsiroanomandidy & Ampijoroa, Madagascar[32]	1989	Asymptomatic adults from 5 different areas (urban & rural, cattle-lands, forested)	C	381	17	EBOV 4.5%	IFA (j)	≥ 1:16	Antigens: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon): positives retested against monovalent antigens	Areas sampled had no known outbreak. Range of titres: 1:16 to 1:512; highest prevalence in the capital Antanarivo 13.3%.
United States[33]	1990	CDC (US) employees with current or previous occupational exposure to monkeys. None ill.	B	550	42	EBOV/SUDV/ RESTV/MARV 7.6%	IFA (?)	≥ 1:16	Antigens: EBOV, SUDV, Reston ebolavirus (RESTV), MARV. Validation: confirmed by western Blot	This paper summarises 2 others [73,74] Results are for positivity to at least one of the four antigens, which include Marburg.
		Adult primary care outpatients in US	C	449	12	2.7%				
Germany[34]	1991	Various groups of healthy individuals, blood donors and routine diagnostic samples, plus 56 individuals who had had contact with Marburg patients in 1972.	C	1288	11 44	EBOV 0.85% RESTV 3.4%	ELISA IFA or WB	ELISA: 1:100 IFA: 1:40 WB: +ve if stained ≥ 2 viral proteins)	Antigens: EBOV (May), RESTV, Marv(Mus) Validation: Considered positive if ELISA confirmed by IFA or WB. Confirmation: ELISA vs IFA 75%; ELISA vs Western Blot 77%.	Authors state that the sample groups showed no significant differences in the prevalence of antibody against the 3 filoviruses and so they treated as one group for analysis and only overall results reported. WB results: “most” sera reacted with the NP protein, “less” with VP40, VP35 & VP30, and ‘few’ with VP24. None reacted to GP or L proteins.

Kikwit, DRC[35]	1995	Four forest site populations near Kikwit town, site of outbreak	B	230	5	EBOV 2.2%	ELISA (k)	≥ 1:400 & OD sum ≥ 1.25	Antigen: unspecified but Kuhn[52] reports EBOV; sera tested in CDC (US) Special Pathogens Lab.	Differentiation between forest and city workers was difficult: publicity brought people out of their areas: self identified occupations. 95% of participants including all 9 positives said they knew someone with Ebola.
		City workers, Kikwit	B	184	4	EBOV 2.2%				
		Asymptomatic volunteers from unaffected villages near Kikwit	C	161	15	EBOV 9.3%				5/15 positives knew someone who had had Ebola
Kikwit, DRC[36]	1995	Household contacts aged 3m-58y	A	101	4	Ebolavirus 4.0%	ELISA (k)	≥ 1:400 & OD sum ≥ 1.25	Antigen: unspecified but probably EBOV; sera tested in CDC (US) Special Pathogens Lab.	Paper cites 5 positive sera but 1 miscarried 3 days before giving her positive specimen so fits case definition for Ebola. One of the remaining 4 may have acquired Ebola by sexual transmission from a convalescent. Out of 81 sero-negative household contacts, 15 had episodes of illness fitting case definition at some point during follow-up.
Central African Republic [37]	1992-97	Pygmy general population: southern regions of CAR (Lobaye, Belemboke)	C	684	48	EBOV 7.0%	ELISA (n)	≥ 1: 400	Antigens: EBOV, MARV, RVFV, LASV, Yellow fever (YF) Hantaviruses (Seoul, Puumala and Thottapalayam) Validation: 244 sera taken in Lobaye in 1995 (11.6% ELISA positive to EBOV) were retested with IFA to EBOV (May) & SUDV (Bon): 34% were positive.	Prevalence of EBOV seropositivity varied between 2% and 13% in different participant groups.
		Bantu villagers : southern region of CAR (Lobaye, Belemboke, Nola, Bangassou)	C	860	44	EBOV 5.1%				
Central African Republic[38]	1992-95	Pygmy subgroup (Lobaye, Belemboke: all sites no known outbreaks)	C	683	48	EBOV 7.0%	ELISA (k)	Mean + 2 SD of negative controls ≥1:400 & OD sum of 4 dilutions > 1.0	Antigens: EBOV (May) Marv(Mus); tests performed by Institut Pasteur, Bangui. Validation: 14 positive & 54 negative samples sent to CDC (US) to be tested against strain antigens: all results confirmed.	Primary or secondary forest areas with some agricultural activities
		Non-pygmy subgroup (Lobaye, Belemboke, Bangassou, Nola: all sites no known outbreaks)	C	648	23	EBOV 3.5%				
Ogooue Ivindo, Gabon[39]	1995-96	Residents of 3 encampments (Andock, Minkebe, Mekoua) in the area where the epidemic occurred (including some contacts)	B	236	23	EBOV/SUDV /RESTV 9.7%	ELISA (k)	mean + 3 SD of negative controls	Antigens: EBOV, SUDV, RESTV with known positive/negative controls	1 positive serum from a survivor excluded from encampment group; unclear how many known case contacts are included in this group.
		Residents of 3 outbreak villages (Mayibout 1 & 2, Mvadi) where cases were reported during the outbreak	B	205	34	EBOV/SUDV /RESTV 16.6%				

Kikwit[40]	1995	Healthcare workers in outbreak area (70% hospital; 30% health centre) who did not have known EVD	B	400	8	EBOV 2.0%	ELISA (k)	Sum of adjusted OD >1.25	Antigen: EBOV	The 8 positives were from a group of 12 samples which were "borderline positive" on 1st test. Only 4 of these samples were retested: all were negative and have been excluded. 129 of the 402 subjects reported being ill during Ebola period. Two with fever and haemorrhage (tested EBOV negative) have been excluded.
Gabon [41]	1996	Selected asymptomatic family members directly exposed to body fluids during outbreaks in 1996	A	24	11	EBOV 45.9%	ELISA (k)	Mean adjusted OD for 10 control samples	Antigen: EBOV Validation : confirmed with western blot on NP and VP40 proteins	Subjects were asymptomatic throughout and were sampled several times. 1st samples showed no antibodies suggesting no prior immunity; IgG appeared 15-18 days after first possible exposure. Paper also describes results of viral RNA detection after 2 rounds of RT-PCR, finding positive results in 7/11 antibody-positive individuals tested and 0/13 antibody-negative individuals.
Nouna River, Ogooue-Ivindo, Gabon[42]	1996	Residents in gold-mining villages with contact exposure in 1995 epidemic	A	56	12	EBOV 21.4%	ELISA (?)	OD > mean +2 SD of 3 known negative controls	Antigen: ebolavirus Gabon 95-39/3 (Centre International de Recherches Medicales de Franceville)	All subjects reported fever and diarrhoea at least once in 1-year period of study, but not haemorrhagic symptoms. IgG positive titre range (OD 310-2,666). Age, sex, ethnic group not associated with seropositivity. Non- significant difference in seropositivity in people on site during 1995 epidemic (8.2%) and not on site (3.7%), among those with no reported contact
		Residents in same villages without contact exposure	B	180	12	EBOV 6.7%				
Upper Ivindo River, Ogooue-Ivindo, Gabon[43]	1997	Individuals from 8 permanent villages in outbreak-prone region (4 survivors excluded)	B	975	10	EBOV 1.0%	ELISA (k)	Mean OD negative controls +3 SD	Antigen: EBOV, performed in National Institute for Communicable Diseases South Africa. Validation: All positives plus a random selection of 28 negatives were retested with same protocol in CDC (US) – all were confirmed with response mainly directed to NP, VP40, VP35 and sGP viral proteins.	Serosurvey done in 1997; questionnaires done in 1999 on 10 positives: only 1 had contact, none were ill.

Belarus & Ukraine[44]	1997	"Foreign visitors" mostly from Africa: unclear if any had history of EVD symptoms	C	562	30	EBOV 5.3%	IFA (w)	Not specified	Antigens: EBOV (May), MARV (Voege) LASV (Jos)	Authors suggest positive results among foreign visitors reflect historic infection/ recovered cases, and unexpected results reflect cross-reactivity with infections such as malaria, HIV and influenza. Other observers suggest the results are just as likely to be artifact.[63]
		Belarus/Ukraine residents "at risk of HIV"	C	506	20	EBOV 4.0%				
		Blood donors from the Blood Transfusion Institute, MoH Belarus & workers at the Belorussian Scientific Research Institute of Epidemiology & Microbiology	C	131	21	EBOV 16.0%				
Watsa region, DRC[45]	2002	Efe tribe pygmies exposed to a possible case at some time in their lives in household, occupation or funeral setting; no history of haemorrhagic fever symptoms	A	38	4	EBOV 10.5%	ELISA (k)	2 × mean +3 SD of negative controls value	Antigen: EBOV ODs were expressed as percent positivity of a confirmed EBOV-positive sample; negative controls were from 60 South African subjects 'almost certain' to be seronegative.	A total of 300 people were sampled from 39 communities. 137 who reported experiencing haemorrhagic fever symptoms sometime in their life are excluded from this summary. 22% of those reporting symptoms were IgG positive.
		Efe pygmies no reported exposure to possible cases; no history of haemorrhagic fever symptoms	C	125	22	EBOV 17.6%				
Gabon[46]	2005-08	Random sample of asymptomatic people aged >16 years without exposure, over all 9 provinces of Gabon	C	4349	667	EBOV 15.3%	ELISA (k) & WB	Cut-off based on negative controls from a French population	Antigen: EBOV Validation: Random sample of 138 positives were tested by western blot in 2008 and all were positive to at least one EBOV antigen.[53]	Gabon experienced 7 outbreaks between 1994 & 2002 affecting >20 villages and towns; in total there were 208 cases and 151 deaths.
		Random sample of asymptomatic children from 6 villages in outbreak-prone province (Ogooue-Ivindo)	B	362	47	EBOV 12.9%				
Bundibugyo, Uganda[47]	2007	Adult contacts of survivors >18 y. Samples taken ~29 months after outbreak	A	210	2	EBOV/SUDV/ TAFV/BDBV 1.0%	ELISA (s)	Mean OD of negative controls plus 3 SD	Antigens: EBOV, SUDV, Tai Forest ebolavirus (TAFV), Bundibugyo ebolavirus (BDBV), MARV(Mus) Validation: 28/36 confirmed cases tested positive to BDBV, 20/36 to EBOV, 10/36 to SUDV, 12/36 to TAFV & 29/39 to any of the 4 strains.	15/223 contacts positive but 13 were symptomatic at the time of the outbreak, therefore only the 2 asymptomatic contacts are included in the table.
Republic of Congo[48]	2011	Healthy blood donors 18-65y, no known case exposure	C	809	20	EBOV 2.5%	Double IFA	Reciprocal endpoint titres ≥20	Antigens: EBOV (ATCC 1978), MARV (Popp 1967) Authors state: 'double IFA' technique has higher specificity than 'regular' IFA because only antibodies that detect filoviral antigens in co-localisation with a monoclonal antibody are considered.	Seropositivity ranged from 1.6% - 4% depending on city/rural location; 4% in Pointe Noire.
Liberia [PREVAIL][49]	2015	Close contacts of cases. NB 126 of the contacts were sexual partners of survivors after discharge.	A	760	98	EBOV 12.9%	ELISA (Alpha)	unspecified	Antigen: EBOV	Preliminary results. Study excluded from meta-analysis of known case contact group (A) because unclear what proportion of participants were symptomatic.

Kono, Sierra Leone[50]	2015-16	Asymptomatic close contacts of cases aged ≥ 4 years who had been resident in quarantined houses during the period of active Ebola transmission	A	185s	12	EBOV 6.4%	ELISA (Alpha)	4.7 U/ml	Antigen: EBOV GP Validation: 29/30 PCR-confirmed EVD survivors, and 3/132 community controls were positive: 96.7% sensitivity, 97.7% specificity	2 other positives had fever. Not clear if negatives were asked about symptoms
		Individuals from 3 villages without reported cases	C	132	3	2.3				
Western Area, Sierra Leone[51]	2015	Household contacts of cases, asymptomatic at the time EVD was in the household	A	388	10	EBOV 2.6%	ELISA (PHE)	Mean OD of negative controls + fixed OD measure (0.1)	Antigen: EBOV GP. "Positive" only if repeat test was positive Validation: 93/97 PCR-confirmed EVD survivors and 0/339 community controls were positive: sensitivity 95.9% (95%CI 89.9 – 98.9%); specificity 100% (95%CI 98.9 – 100%)	Tests were done on oral fluid.
		Individuals from 3 villages in Western Area without reported EVD cases	C	339	0	EBOV 0%				

Abbreviations

CDC (US): Centers for Disease Control and Prevention, Atlanta, USA; PHE: Public Health England; EBOV: Zaire ebolavirus; SUDV: Sudan ebolavirus; BDBV: Bundibugyo ebolavirus; TAFV: Tai Forest Fever Virus; MARV: Marburg Fever Virus; CCHFV: Crimean Congo Haemorrhagic Fever Virus; RVFV: Rift Valley Fever Virus; LASV: Lassa Fever Virus; IFA: immunofluorescence assays; ELISA: Enzyme-linked immunosorbent assay; WB: Western Blot; OD: optical density; SD: standard deviation

Assay technique notation

IFA (w): Wulff & Lange[62]
IFA(j): Johnson[63]
IFA (g): Gardner[64]
IFA (b) Baskirtsev[65]
Double IFA: Emmerich[66]
IFA (?) ELISA (?): technique not referenced
ELISA (k): Ksiazek[67]
ELISA (s): Schoepp[68]
ELISA (v): Viral Haemorrhagic Fever Consortium (SL)[69]
ELISA (n): Nicklasson[70]
ELISA(r) : Rezapkin[71]
ELISA(PHE): Lambe[54]
ELISA(Alpha): ADI[75]