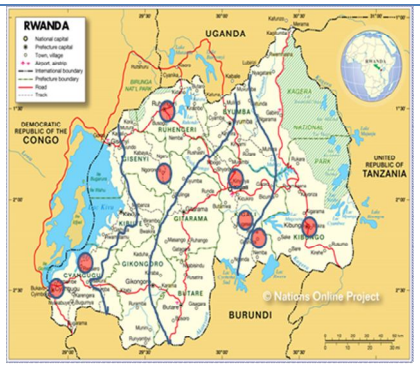
Republic of Rwanda

Risk Assessment of Yellow Fever Virus Circulation in Rwanda



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by

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World Health Organization

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Executive Summary

Despite the vulnerability of East Africa to the emergence of vector-borne diseases and the yellow fever (YF) activity in the neighboring countries, Rwanda has never reported any YF case. To clearly understand the viral activity and the potential risk of YF epidemic in the country, the government of Rwanda through MoH, with the support of WHO and partners conducted a comprehensive risk assessment of YF viral activity in the country during November-December 2012.

The main objective of the survey was to assess the YF naturally-acquired antibodies prevalence and distribution pattern of YFV in Rwanda in order to make recommendations concerning the national policy on implementation of prevention and control measures.

The study design was a multistage cluster. Distinct ecologic zones were first identified in the country based on rainfall, vegetation and altitude, which was used to account for differences in humidity, temperature, and land cover use. A random point generator in ArcGIS (ESRI, Redland, CA) was used to randomly pick two locations per zone. Using the latitude and longitude of each randomly selected point, the closest urban (city) and rural (village) localities to that point were identified. Seroprevalence estimates (accounting for the sample size estimates) were based on knowledge about the proximity of the zones to the neighboring countries (with history of YF activity or not) and potential trade routes. The number of samples per zone was estimated with a design effect of 2 to account for clustering and a 15% oversampling for lack of participation. Humans (>9 months) and mosquitoes (larvae and adults) were target populations but available non-human primates sera were also used. The number of households (and their average size) in the city and village was determined and the number of samples was stratified by their population. Sampled households were selected randomly using a random number generator. All serum specimens were tested for YFV-specific IgG antibodies using enzyme-linked immunoassay (ELISA) and the neutralizing antibody was assessed by plaque reduction neutralization testing (PRNT).

The results of the serosurvey show a very low prevalence (2 out of 1284 participants i.e. 0.2%) of naturally-acquired antibodies against YFV in human population. The serosurvey of 69 convenient samples (from Rwanda Development Board data base) of non-human primates (Olive Baboon and Vervet Monkey) does not show YFV circulation in this population.

Entomological investigations show that Breteau Index (BI) and Container Index (CI) are potentially elevated (more 5% and 3% respectively) in most of the ecologic zones (some areas). A total of 1971 adult mosquitoes were collected among which 190 (<10%) were *Aedes sp* (mainly *Ae. aegypti*, *Ae. africanus* and *Ae. simpsoni*), from all the ecologic zones (zones 1 and 4 having the highest density). None of these potential vectors were found infected by YFV using real time PCR.

In conclusion, although potential YFV vectors are present in Rwanda, they were not found infected and there is no evidence of virus circulation either in humans or non-human primates. Therefore, preventive mass campaign vaccination is not recommended in Rwanda but a good YF surveillance system is highly recommended so as to detect any imported case.

Background

Yellow fever virus (YFV) is an arthropod-borne virus (arbovirus) of the genus *Flavivirus* (family *Flaviviridae*). Its transmission is dependent upon vectors, principally *Aedes (Stegomyia)* mosquito species in Africa (Ellis & Barrett, 2008) where three main transmission cycles have been observed (Tomori, 2004; WHO, 2013):

(i) Sylvatic (or jungle) YF is usually a disease of non-human primates (NHP) and transmission is via several species of *Aedes* mosquitoes found in the forest canopy. Transmission to humans is incidental, via bites from mosquitoes that have fed on viremic NHP.

(ii) Intermediate YF transmission is seen in humid regions where *Aedes* species are able to breed both in the wild and around households, and to infect both NHP and humans. Intermediate transmission usually results in sporadic cases occurring simultaneously in different villages in the same area but large outbreaks of the disease have also been associated with this transmission cycle (Germain *et al*., 1981).

(iii) Urban YF transmission results in large epidemics which occur when infected people move to densely populated areas where the local population has little or no immunity to YF and where *Aedes (Stegomyia) aegypti* (*Ae. aegypti*) is active. Infected mosquitoes transmit the virus from person to person.

According to the World Health Organization (WHO) a recent analysis of African data sources from 1995-2013, estimates a burden of 84,000-170,000 severe cases and 29,000-60,000 deaths due to YF (WHO, 2013). Infected individuals manifesting with the severe form of the disease can have a case fatality rate as high as 50% (Tomori, 2004).

Following the isolation of the virus in West Africa in 1927, research in East Africa began auspiciously in 1936 and within 10 years resulted in a detailed description in Uganda of the first YFV transmission cycle for the continent, plus the identification of significant ecological and epidemiological details (Haddow, 1969). YF was also discovered in this part of Africa but in the absence of any known outbreaks or human disease (Sawyer and Whitman, 1936). It was not until 1940 that the first outbreak and isolated cases of disease were detected. However, it was evident from early on that in some forest edge localities, YFV seroconversion could occur in the absence of serious illness (Haddow, 1969). Silent transmission was again noted during the last outbreak in Kenya (Sanders *et al*., 1996).

In East Africa, the disease is maintained endemically in monkey-*Ae. (Stegomyia) africanus* jungle transmission cycles and may periodically emerge in intermediate cycles involving man-to-man transmission. These emergence events occur with limited frequency, or potentially remain

undetected, and are vectored primarily by *Ae. (Stegomyia) simpsoni* s.l. sp. complex (Huang, 1986). YF in this sub-region therefore remains characterized by unpredictable focal periodicity and a precarious potential for large epidemics. Outbreaks of YF in Kenya (1992–1993), Sudan (2003, 2005 and 2012) and Uganda (2010-2011) are important because each of them have involved the re-emergence of a YFV genotype (East Africa) that remained undetected for nearly 40-45 years. In addition, unlike West Africa and South America, YF has yet to emerge in urban areas of East Africa and be vectored by *Ae. aegypti*. This is a significant public health concern in a region where the majority of the population remains unvaccinated (Ellis & Barrett, 2008; WHO, 2013).

Following all this, six countries (Rwanda, Tanzania, Ethiopia, Kenya, Sudan and South Sudan) of the sub-region started implementing national prevention and control measures of YF.

In Rwanda, the initial integrated disease surveillance and response (IDSR) strategy began in 2001 and targeted 19 priority diseases and syndromes (including yellow fever) recommended by WHO (African Region) because they are among the leading causes of illness, death and disability in African countries and are relevant to Rwanda based on epidemiologic criteria (WHO, 2002).

Nevertheless, implementation of the full IDSR program within the Ministry of Health (MoH) is ongoing and to date, there is no YF case based surveillance in the country and YF vaccination is not part of the routine national immunization programme (Kebede *et al.*, 2011).

Despite the vulnerability of East Africa to the emergence of vector-borne diseases and the yellow fever activity in the neighboring countries, Rwanda has never reported YF outbreak and literature search on arboviruses in this country yield no result except a paper on fever of unknown origin in a refugee camp in Democratic Republic of Congo (DRC), where arboviruses could be involved (Rey *et al.*, 1996).

The only category of the population that is routinely immunized against YF is international travelers, done in order to meet International Health Regulations (IHR) requirements. This implies that in Rwanda, the vast majority of the population may be non-immune to YF and the prevalence and incidence of YF is not known.

To clearly understand the viral activity and the potential risk of YF epidemic in the country, the government of Rwanda through MoH, with the support of WHO and partners conducted a comprehensive risk assessment of YF viral activity in the country in November-December 2012.

Objectives

General objective

The general objective of the survey was to assess the YF naturally-acquired antibodies prevalence and distribution pattern of YFV in Rwanda in order to make recommendations concerning the national policy on implementation of prevention and control measures.

Specific objectives

The risk assessment of YFV transmission specific objectives were to:

- Determine the seroprevalence of YFV infection in humans in different ecologic zones;
- Ascertain the availability, density and infectivity of YF vectors in different ecologic zones;
- Assess the role of NHP in YFV transmission in the forest areas;
- Permit the formulation of recommendations regarding the national YF vaccination policy for Rwanda;
- Utilize environmental and ecologic data to explore the potential source of YFV activity in the ecologic zones.

Organization and Approach

Training and pilot study

Under the supervision of the WHO team of experts, an *in situ* training was carried out in a pilot study site at Mageregere (a suburb situated 10 km south of Kigali) to orient team members to the specific techniques used for the YF epidemiological and entomological risk assessment. This training, was particularly focused on the methodological approaches, as well as the key elements of the evaluation of the YF risk epidemic such as: i) conditions to implement/organize the field investigation, ii) criteria used to determine the choice of the sites to be investigated and appropriate environment to be prospected in these sites, iii) most appropriate sampling procedures for the collection of the YF vectors, iv) treatment and storage of the collected specimens, and v) the methods to estimate the risk indices (e.g., Breteau Index, Container Index, etc.).

Study sites and sample site selection

For the assessment of current YFV activity, a multistage cluster design was utilized. The initial stage involved identifying distinct ecologic zones in the country (figure 1, left) based on

rainfall, vegetation and altitude, which was used to account for differences in humidity, temperature, and land cover use. This approach is being utilized as mosquito activity is likely closely linked to these factors and therefore is likely to impact YFV activity and circulation. The brief description of the four different ecologic zones of Rwanda is as follow:

Zone 1. Forested, high level of rainfall, border with DRC and Lake Kivu, altitude <2,300 m;

Zone 2. Forested (less in 2009 than 2001), high precipitation, altitude >2,300 m in few areas;

Zone 3. Drier, cropland, natural vegetation, altitude <2,300 m;

Zone 4. Drier, altitude <2,300 m, savannah, grasslands, possibly some croplands.

After mapping the distinct ecologic zones, they were outlined as a polygon and a random point generator in ArcGIS (ESRI, Redland, CA) was used to randomly pick two locations per zone to sample (figure 1, left). Using the coordinates (latitude and longitude) of each randomly selected point, the closest urban locality or town/city (and the nearest rural local locality or village) to that point was identified by WHO and MoH personnel (Figure 1, right).

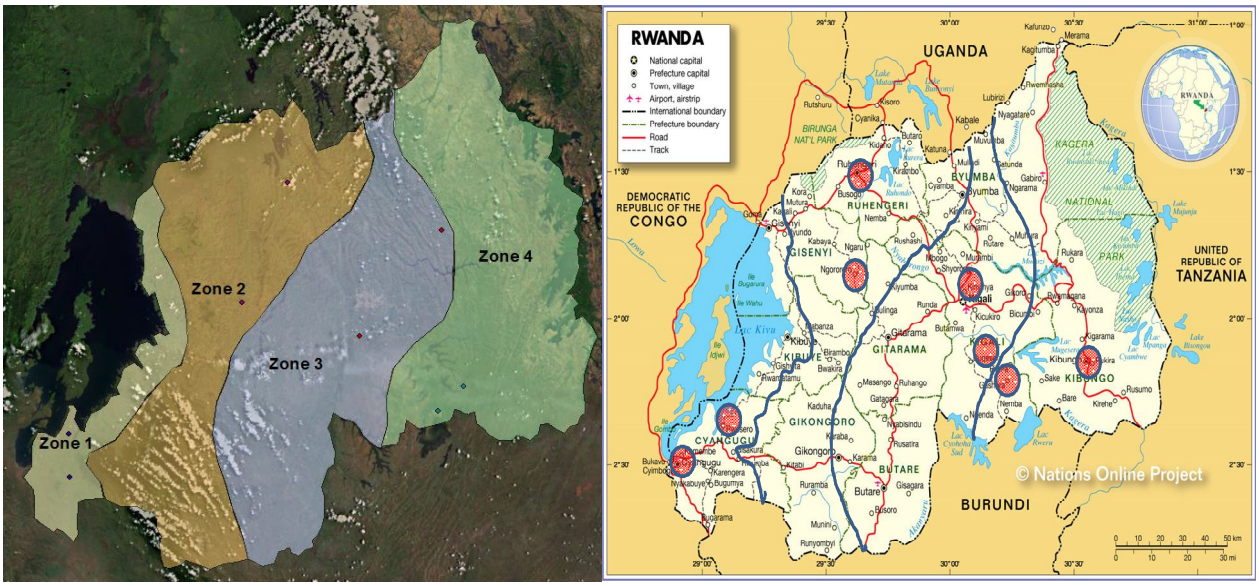

Figure 1. Map of Rwanda showing the ecologic zones (left) and the selected cities (right).

Sample size calculations

As there was no data on vaccine coverage in the country (no reported preventive/reactive campaigns and no routine immunization against yellow fever) or historical data on YF in Rwanda, for sample size calculations, the seroprevalence of YF was estimated based on knowledge about the proximity of the zones to the neighboring countries with history of YF activity or not and potential trade routes. In fact, Mahaffy *et al.*, 1946 reported YF

seroprevalence of 5-12% for Uganda and DRC. It was therefore decided that the seroprevalence would be highest in Zone 2 given the large area shared on the border with Uganda. Zone 4 would have the lowest as most of its border is with Tanzania, a country that has never reported YF cases. Zone 3 would be closer to Zone 2 in its seroprevalence but not as high. This was decided based on the minimal amount of border shared with Uganda; however, it has the main road going from Uganda to Kigali. Furthermore, Kigali is likely to have a proportion of the population that has been vaccinated due to international travel. Finally Zone 1 is believed to fall in between Zone 3 and Zone 4 as it borders with Uganda and DRC, both countries with previous YF cases and activity. The seroprevalence per zone can be summarized as follow: Zone 2 > Zone 3 > Zone 1 > Zone 4.

Table 1 gives the seroprevalence estimates, estimated number of samples per zone (with a design effect of 2 to account for clustering), 15% oversampling for lack of participation, final number of samples per zone, and the number of samples at each A and B point.

Table 1. Sample size estimates for the YF risk assessment in Rwanda, Nov-Dec 2012

Zone #	Sites or Cities	Estimated seroprevalence (95% CI)	Estimated sample size	15% oversampling number	Number to sample per zone	Number to sample per site*
Zone 1	A: Kamembe B: Kagano	6% (1%-11%)	174	26	**200**	100 100
Zone 2	A: Ngororero B: Musanze	8% (3-13%)	226	34	**260**	130 130
Zone 3	A: Kimironko B: Nyamata	7% (2-12%)	200	30	**230**	115 115
Zone 4	A: Kibungo B: Gashora	3% (1-5%)	558	84	**642**	321 321

**This number is divided among the town and village based on the populations and the proportion this represents. Example: Zone 1A: Town population is 15,342 and village population is 783. Town makes up 95% and village 5% of the total population of those two areas combined. Therefore Zone1A town sample size is 95 and Zone 1A village sample size is 5.*

Target Population

Two primary populations were assessed in this rapid assessment, namely humans and mosquitoes. Nevertheless, available sera from NHP were used to assess potential YFV circulation in these vertebrate hosts. The human and mosquito data are used to determine YF viral activity in the country. The NHP data may augment the other data but due to its non-random nature it should not be used to extrapolate to either ecologic zone activity or country level YF viral activity.

Data sample collection

Human sampling and inclusion criteria

Once in a selected site the teams were escorted by a local guide preferably a community health worker who was first briefed on the planned activities in the locality (village or city).

An assessment was done to determine the location and average size of households in the city and village. The number of samples taken in the city and village was stratified by population. A random number generator was used to select specific households to sample. Sampling was done within the entire household which was selected and no household was replaced if the residents were not found to be at home. Thus a 15% degree of oversampling was used to ensure adequate sampling.

When possible, all ages, except those less than 9 months of age, member of a randomly selected household, and resident in the village for at least one week (and whose consent was obtained either directly for adults or through parents or guardians for minors) were sampled. Humans were not excluded if they have evidence of past yellow fever vaccination (example travellers).

Individuals were asked to provide a blood sample to measure IgM, IgG and neutralizing antibodies against YFV. Five milliliters (mls) of blood were collected from adults and children >10 years by venipuncture and one to three mls were collected from children ≥9 months and ≤10 years of age also by venipuncture. All specimens were collected by trained phlebotomists from the central level using standard sterile technique. In addition, basic demographic information was collected from each individual bled and recorded in a line list by a member of the field team and included: age, sex, history and time of yellow fever vaccination or disease. The epidemiologist of the field team was responsible for collecting the data in a standardized fashion and was trained on the data collection instrument prior to field deployment.

At the end of each working day, blood samples were processed in the admission facility and duplicate serum aliquots were made and stored in a deep freezer (-20°C). These samples were then transported to the National Reference Laboratory (NRL), where they were kept frozen at -70° C until testing.

Non-human primates sampling

Rwanda Development Board (RDB) provided NHP samples together with the data base from which a convenient sample (with complete demographic information) of 69 sera were selected comprising two susceptible species: 16 Olive Baboons (*Papio anubis*) and 53 Vervet Monkeys (*Chlorocebus aethiops*). There were 10 juveniles, 10 sub-adults, 43 adults and 6 with undetermined age. These samples were collected from different locations of the country (from Nyungwe to Akagera National parks).

Mosquito sampling

Attempts were made to collect mosquitoes in all the localities of the ecologic zones identified. Several collection methods were used to sample adult and aquatic stage mosquitoes. Therefore, all the teams proceeded as follow:

- Set up of ovitraps (~30) upon arrival in the Cities/villages for mosquito eggs prospection;
- Inspection of randomly selected houses indoor and outdoor of human habitation. Artificial and natural mosquito breeding sites were inspected. Each container holding both potable and non-potable water was inspected. Larvae and pupae were counted in each container found positive to estimate absolute population density of *Ae. aegypti* per habitation unit. Larval and pupae samples collected from infested container were kept in Kicukiro insectary (situated in a suburb of Kigali) for rearing and identification of the emerging adult stages;

- Human landing catches from 4 to 8 PM (by community health workers), for two consecutive days in all the areas (urban and rural). All the mosquitoes caught were identified in the field at the genus level and kept frozen for transportation to NRL Kigali, where they were frozen at -70° C before being shipped to *Institut Pasteur de Dakar* - Senegal (IPD) for species identification and detection of YFV by real time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Specimen testing

All serum specimens (humans and NHP) were tested for YFV-specific IgG antibodies using enzyme-linked immunoassay (ELISA) according to IPD protocol. Given the potential for cross-reactive antibodies within the *Flavivirus* genus, any sample tested positive for YFV-IgG was assessed for antibodies against other flaviviruses, including but not limited to West Nile virus,

dengue virus (serotypes 1-4) and Zika[1] virus. When any of these assays were positive, the neutralizing antibody was assessed by plaque reduction neutralization testing (PRNT) according to De Madrid & Porterfield (1969) as a confirmatory test to distinguish between Yellow fever and the other *Flavivirus.* This testing was performed at the IPD according to a laboratory testing algorithm and a guideline for interpreting laboratory results.

Samples having PRNT titres ≥ 1:10 were considered to be seropositive to YF while those having PRNT titres ≥ 1:20 were considered to have seroprotective levels of antibodies against YFV either by having had the disease or receiving the YF vaccine.

The results of the serologic testing for YF was not provided to individual participants as the samples did not have any identifiers to link it back to the person from which it was obtained.

Adult mosquitoes were identified using a chill table microscope and morphological keys available (Edwards, 1941; Huang, 1986). For the detection of YFV in mosquitoes, similar species were pooled in a maximum of 10 mosquitoes and then ground together and centrifuged. Supernatant collected was used to extract viral Ribonucleic Acid (RNA) that was then detected by real time RT-PCR (Weidmann *et al.*, 2010).

Ethical considerations

The methods of the assessment were reviewed and approved by following committees or institutions:

- Rwanda National Institute of Statistics;
- National Health Research Committee (Approval reference No. NHRC/2012/PROT/0004 of November 1, 2012);
- Rwanda National Ethics Committee (Letter No. 382/RNEC/2012 of November 29, 2012);
- Yellow Fever risk assessment Expert Committee.

[1] Zika virus was first isolated in 1947 from a rhesus monkey in the Zika Forest of Uganda. From 1951 through 1981, evidence of human infection was reported from other African countries such as Uganda, Tanzania, Egypt, Central African Republic, Sierra Leone and Gabon, as well as in parts of Asia including India, Malaysia, the Philippines, Thailand, Vietnam and Indonesia. Common symptoms of infection with the virus include mild headaches, maculopapular rash, fever, malaise, conjunctivitis, and arthralgia.

Analysis and Findings

Human serosurvey results

A total of 1287 participants from 327 households (approximately 4 per house) consented to be part of the assessment. The gender of participants did not differ significantly between the zones (Table 2). There were differences between zones 2, 3, and 4 regarding their distribution within the age groups, with zone 2 having more middle-aged adults (i.e., aged 40-64 years) than zone 3 and 4, and zone 3 having more younger adults (i.e., aged 15-39 years) than zone 2 and 4. Serum samples were obtained from all persons who consented to have blood taken.

Table 2. Gender and age of serosurvey participants by ecologic zone, Nov-Dec 2012

Zone *Participants/zone*	Zone 1 *n=200* *No. (%)*	Zone 2 *n=260* *No. (%)*	Zone 3 *n=215* *No. (%)*	Zone 4 *n=612* *No. (%)*	Total *n=1287* *No. (%)*
Sex					
Male	80 (40)	104 (40)	82 (38)	256 (42)	522 (41)
Female	119 (60)	156 (60)	125 (58)	356 (58)	756 (59)
Missing	1 (<1)	0 (0)	8 (4)	0 (0)	9 (<1)
Age group					
< 15 years	94 (47)	113 (43)	84 (39)	270 (44)	561 (44)
15-39 years	76 (38)	79 (30)	105 (49)	227 (37)	487 (38)
40-64 years	25 (13)	57 (22)	23 (10)	85 (14)	190 (15)
65+ years	4 (2)	11 (4)	3 (1)	30 (5)	48 (4)
Missing	1 (<1)	0 (0)	0 (0)	0 (0)	1 (<1)

Human Serosurvey Vaccination History

Of the 1287 participants who had a serum sample successfully obtained, vaccination history was available for 1285 (99.84%) of them. Of these, only one (0.08%) person reported having received the vaccine. The vaccinated person was a 45-year- old woman living in zone 2, who reported having received the vaccine in 1992 but did not have proof of vaccination.

Overview of the testing Results

Of the 1284 participants who did not report having received vaccination and had a blood sample available for testing, a total of 52 (4.05%) samples were positive on ELISA for YFV antibodies (Figure 2). Following confirmatory testing with PRNTs of the 52 ELISA positive,

only 2 (3.85%) were YFV confirmed; 3 (5.77%) were positive for YF but differential PRNT were not performed (not confirmed) and PRNT for YF was < 1/40 (Figure 2).

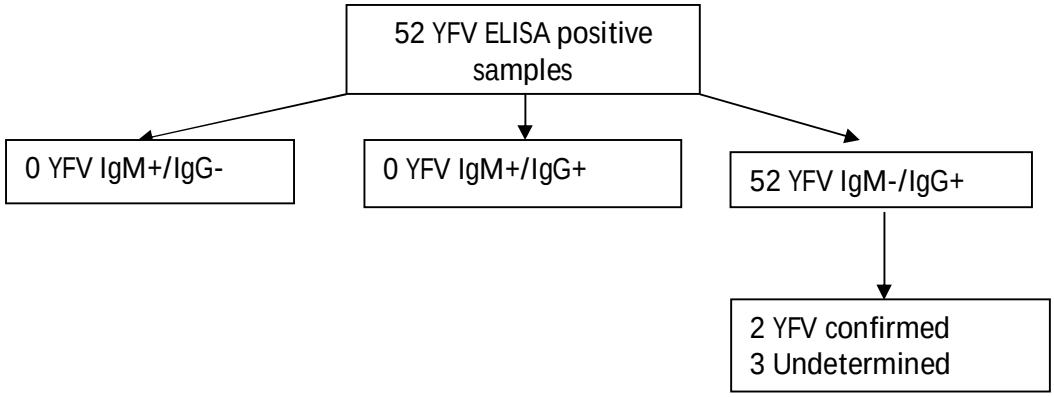

Figure 2. Confirmatory testing results of YFV ELISA positive samples

Naturally-acquired YFV Results

Two (0.16%) among the 1284 participants surveyed had naturally-acquired antibodies (antibodies confirmed against YFV without a history of vaccination). The positive persons were:

(i) A 54-year-old male from zone 4a (Gashora city) with a titer of 20 against YFV,

(ii) A male, age unknown, who lived in zone 1a (Kamembe city) and had a titer of 40 against YFV.

For both of them the testing of their sample was negative for Dengue, West Nile, or Zika virus IgG antibodies.

The three persons with undetermined *Flavivirus* status were all around 50 years and older, and 2 of them were male. Each of them lived in a different zone i.e., zone 4a (Gashora city), zone 4b (Karwema village), and Zone 2b (Kabeza village). These individuals were tested IgG positive for Dengue and Zika viruses. Another individual, was a 14- year-old male living in zone 4b (Karwema village) “who reported having YF disease in the past”. Zone 3 had no one with any of the antibodies tested.

Non-human primates serosurvey results

Out of the 69 NHP sera tested, seven were Zika virus positive for IgG by ELISA test, no samples were IgG positive for YFV, WNV or DENV. Yellow Fever PRNT was performed in all the 69 serum samples IgG negative but no case was found positive.

Entomological results

For the larval mosquito sampling, 1440 containers were inspected from 565 households. A total of 55 containers were infested among which 46 (83.63%) were found outdoor. The typology of the infested containers was as follow: 23 (41.81%) plant leaves, 9 (16.36%) water storage containers (6 plastics and 3 metals), 8 (14.54%) clay jars, 6 (10.9%) old tires, 5 (9.09%) flower pots, etc.

Table 3 shows that Breteau Index (BI) and Container Index (CI) were potentially elevated (more 5% and 3% respectively) in most of the ecologic zones (some areas), particularly in zone 1 (rural area of Rwesero only), zone 2 (Musanze city for both BI and CI, and the rural area of Ntaganzwa for CI), zone 3 (city of Nyamata only) and zone 4 (city of Gashora and the village of Karwema for both BI and CI, and the village of Ramiro for CI).

The rearing of larvae collected from the field yielded 12 adults which were identified as *Ae. aegypti*. No larvae were recovered from the eggs prospection probably because (1) ovitraps were set only for 2 days and (2) heavy rains in certain areas during the survey.

Table 3. Mosquito aquatic stages and epidemic risk indices in each locality in Rwanda, Nov-Dec 2012

Zone	Locality	No Houses surveyed	No Habitation Unit (HU)	No Container inspected	No Positive Container	Breteau index (BI)	Container Index (CI)
1A	**Kamembe**	48	152	113	1	0,66	0,88
1A	Ruganda	32	77	106	0	0,00	0,00
1B	**Kagano**	33	90	102	0	0,00	0,00
1B	Rwesero	63	171	171	13	**7,60**	**7,60**
2A	**Ngororero**	21	43	44	1	2,33	2,27
2A	Ntaganzwa	55	117	109	4	3,42	**3,67**
2B	**Musanze**	18	50	43	4	**8,00**	**9,30**
2B	Kabeza	55	133	114	0	0,00	0,00
3A	**Nyamata**	20	57	48	8	**14,04**	**16,67**
3A	Ntarama	0	0	0	0	-	-
3B	**Kimironko**	20	53	49	0	0,00	0,00
3B	Kibagabaga	58	153	187	5	3,27	2,67
4A	**Gashora**	30	74	110	5	**6,76**	**4,55**
4A	Ramiro	37	78	88	3	3,85	**3,41**
4B	**Kibungo**	26	78	48	0	0,00	0,00
4B	Karwema	49	121	108	11	**9,09**	**10,19**

NB. BI is the ratio of the number of containers found positive for larvae and pupae for 100 houses surveyed; CI is the percentage of the number of container found positive out of the total number of containers inspected; HU is the number of rooms where at least one individual is sleeping.

Human landing catches were carried out from 4 to 8 pm, for two consecutive days in all the areas (urban and rural) except for Ngororero city (zone 2A) where, due to logistical reasons, catches were only carried out for one day.

A total of 1971 adult mosquitoes (table 4) belonging to 5 genera were collected: 190 (9.6%) *Aedes*, 19 (1.0%) *Anopheles*, 1626 (82.5%) *Culex*, 41 (2.1%) *Mansonia* and 95 (4.8%) *Coquillettidia*. The genus *Aedes* was caught in all the ecologic zones with zone 4 having the highest number of specimen (>54%), followed by zone 1 (32%). *Culex sp* was the most abundant genus in all the ecologic zones.

Table 4. Number of mosquitoes collected by ecologic zone in Rwanda, Nov-Dec 2012

Species	Number of specimen collected by ecologic zone				Total
	Zone 1	Zone 2	Zone 3	Zone 4	
Aedes sp	**61**	3	22	**104**	**190**
Anopheles sp	1	0	6	12	**19**
Culex sp	362	262	427	575	**1626**
Mansonia sp	1	0	40	0	**41**
Coquilletidia	0	2	4	89	**95**
Total	**425**	**267**	**499**	**780**	**1971**

NB. Human landing catches were carried out for two consecutive days except for Ngororero city (zone 2)

The distribution of the 190 *Aedes sp* collected per ecologic zone and per site (rural and urban) is presented in table 5. The genus *Aedes* was caught in all the urban and rural localities except for zone 2B (Musanze and Kabeza) and zone 2A (Ntaganzwa rural locality).

The identification of the adult *Aedes sp* collected shows that 6 potential YFV vectors are present: *Ae. aegypti (25.4%), Ae. africanus (10.3%), Ae. Simpsoni (3.2%), Ae. neoafricanus (0.5%), Ae. metallicus (0.5%), Ae. opock (0.5%). Aedes (Finlaya) sp*, a potential vector of arboviruses (except YFV) and filariae in Asia was the most abundant *Aedes* species (42.2%).

Real time RT-PCR for the detection of YFV RNA was performed on pools of potential YF vectors collected but none of them was positive.

Table 5. Distribution of adult *Aedes sp* collected during the YF RA in Rwanda, Nov-Dec. 2012

Ecologic Zone	Random Point	Longitude and Latitude	Selected Urban (U) Area (Cell sampled)	Selected Rural (R) Area (Village "Umudugudu" sampled)	*Aedes* sp. Collected U + R = total
1	A	29° 1'47.30"E 2°31'33.77"S	Kamembe (Gihundwe)	Ruganda (Ruhimbi)	12 + 1 = **13**
	B	29° 1'30.57"E 2°23'15.11"S	Kagano (Ninzi)	Rwesero (Mutusa)	35 + 13 = **48**
2	A	29°38'2.06"E 1°57'41.93"S	Ngororero (Nyange)	Ntaganzwa (Miyiha)	3 + 0 = **3**
	B	29°47'52.03"E 1°34'30.16"S	Musanze (Cyabararika)	Kabeza (Karunyra)	0 + 0 = **0**
3	A	30° 3'10.57"E 2° 4'16.38"S	Nyamata (Nyamata ville)	Ntarama (Rwangara)	3 + 7 = **10**
	B	30°20'43.93"E 1°43'48.36"S	Kimironko (Nyagatovu)	Kibagabaga (Gasharu & Karongi)	11 + 1 = **12**
4	A	30°20' 1.95"E 2°18'47.18"S	Gashora (Biryogo)	Ramiro (Kagasa I & II)	4 + 10 = **14**
	B	30°25'13.76"E 2°13'56.91"S	Kibungo (Cyasemakamba)	Karwema (Musenyi)	13 + 77 = **90**
TOTAL					**81 +109= 190**

NB. Human landing catches were carried out for two consecutive days except for Ngororero city (zone 2)

Discussion

Despite a safe and effective human vaccine (17D) developed in 1937, there are approximately 84,000-170,000 severe cases and 29,000-60,000 deaths, due to YFV each year, of which 90% are in Africa (WHO, 2013).

Nearly 70 years have passed since the discovery of YF in East Africa but the disease has remained enigmatic because of unpredictable focal periodicity, lengthy inter-epidemic periods and a precarious potential for large epidemics. In recent decades, active or sustained surveillance has been largely nonexistent or of only limited capacity. Consequently, endemic and/or enzootic transmission is poorly documented and, as such, typically remains of little value in predicting the potential for future outbreaks. The most recent occurrences of YF, in Kenya during 1992-1993 (Sanders *et al*., 1998), in Uganda during 2010-2011 (WHO, 2011; Wamala *et al*. 2012) and Sudan in 2003, 2005 and 2012 (Onyango *et al.*, 2004;; Soghaier *et al.*, 2013), emerged unexpectedly in areas with unvaccinated populations. Prior to these outbreaks, YF had not been detected in Sudan and Uganda for over 40-45 years and had never previously been isolated in Kenya. Of additional interest, each of these outbreaks involved a YFV genotype that had remained undetected for more than 45 years and had never previously been involved in a clinically apparent outbreak.

Although Rwanda belongs geographically to East Africa, no case of YF or any other arboviral diseases have been reported. This could perhaps be partly explained by the absence of any surveillance and/or seroprevalence studies on arboviral diseases in this country. The present risk assessment of yellow fever virus circulation was carried out during November-December 2012 in the four ecologic zones of Rwanda to assess the YF naturally-acquired antibody prevalence and the distribution pattern of YFV in the country.

The results of the human assessment show that only two (0.16%) of the 1284 sampled participants (with no history of YF vaccination) had evidence of naturally-acquired and low YFV antibodies titers (1/20 and 1/40) by PRNT, the most specific test for the detection of YFV antibodies. Besides YF, three participants had undetermined flavivirus status; all of them being around 50 years and older which means that they could have been infected elsewhere (in an endemic country although history of travel is unknown). Such a very low YFV antibody prevalence was recorded some 70 years ago in the neighboring Tanzania (and its Island of Zanzibar), although with a different test (mouse protection test) on 800 human samples.

Interestingly, this neighboring country has never reported any YF cases to date. On the other hand, countries that exhibited YFV antibody prevalence of more than 4% in 1930s such as Uganda (5.4%), Kenya (10.5%), DRC (11.9%) and Sudan (16.5%) (Sawyer and Whitman, 1936; Mahaffy *et al.*, 1946), are countries where resurgence or re-emergence of YFV has been observed in recent decades.

The two confirmed YF naturally-infected participants were not from zone 2 which include the capital city of Rwanda (Kigali) despite the fact that there is a main road connecting this city to the capital city of Uganda (with non-negligible movements of people), a country with known YF cases and activity.

For the non-human primates assessment, none of the 69 samples (16 Olive Baboons and 53 Vervet monkeys) had evidence of naturally-acquired YFV antibodies. Absence of YFV infections in NHP has also been recorded in the neighboring Tanzania more than five decades ago after surveying 7 monkeys (Lumsden and Hewitt, 1954). It is noteworthy that high YFV seroprevalences (>13%) have been recorded in monkeys in Kenya (Haddow, 1952), Uganda (Haddow *et al.*, 1951), and in DRC (Findlay *et al.*, 1936), which are countries where YFV cases and activity are common. Besides the absence of YFV antibodies, 7 NHP were considered to be Zika virus immune by ELISA IgG. ZIKAV is a flavivirus (*Flaviviridae: Flavivirus*) closely related to YFV. It was first isolated from a sentinel monkey in the Zika forest of Uganda in 1947 and a year later it was also isolated from *Ae. africanus* at the same location (Dick *et al.*, 1952). Since that time, ZIKAV has been sporadically isolated, occasionally associated with human disease, and serologic evidence of circulation has been found in Africa (Uganda, Nigeria, Senegal, Egypt) and Asia (Lanciotti *et al.*, 2008).

The entomological epidemic risk indices (Breteau Index and Container Index) were relatively elevated in most of the ecologic zones (but only in certain localities). According to WHO (1986) there is an epidemic risk when these indices are up to the threshold of 5% for the Breteau and 3% for Container index. Adults of *Aedes* sp were caught in all the ecologic zones with the great majority being collected in zone 4 (>54%) and zone 1 (32%). The identification of these *Aedes* yield potential vectors for YFV, *Ae. aegypti*, *Ae. africanus* and *Ae. simpsoni* being the most abundant although detection of YFV by real time RT-PCR (the most specific test) was negative for all of them. Despite the presence of *Ae. aegypti*, its known strong anthropophilic behavior and laboratory evidence of its competency, urban epidemics of YFV, vectored by this species has been completely absent in East Africa (Ellis & Barrett, 2008). *Ae. africanus* is the

most important vector of YF in forested areas (jungle transmission cycle) of Africa (Smithburn *et al.*, 1949). *Ae. simpsoni*, is consider an important bridge vector in areas of Uganda where it is found prolifically in banana plantations that may border forested areas with proximal YF activity (Haddow, 1969).

Arguably, as there seems to be no apparent circulation of YFV (especially no jungle or intermediate YF) in Rwanda, the greatest public health threat in regard to YF in this country could be its potential emergence in urban areas because of the largely non-immune (and unvaccinated) human populations and the presence of potential vectors. But it remains unclear why, unlike West Africa, large urban epidemics vectored by *Ae. aegypti* have not occurred in East Africa. It is interesting that the situation in East Africa has similarities to that in South America where there has been an absence of reported *Ae. aegypti* transmitted human infections since 1928. Possible explanations may include that the virus has simply not been introduced in these areas; the genotype of YFV is not well adapted to local domestic *Ae. aegypti* populations, or cross-flavivirus immunity and/or human demographics contribute to this phenomenon (Ellis & Barrett, 2008).

Of additional concern is the strategic location of East Africa along major trade routes that connect the region with the Indian Ocean and Southeast Asia and one of the great unanswered questions is why YF has never previously been detected in those areas in spite of the efficient movement of vectors and arboviral diseases across the Indian Ocean. For example an outbreak of Chikungunya, which was first reported in Kenya, spread throughout the Indian Ocean to parts of Asia and resulted in greater than 500 000 cases (WHO, 2006).

This risk assessment as most of the studies could have some limitations or sources of bias such as: the inaccurate estimates of population and estimated seroprevalence due to the absence of YF historical and vaccine coverage data which accounted for the sample size estimates, but this was minimized by the random sampling of statistically significant number of participants. Also, measures of seroconversion could be biased by laboratory error and possible cross-reactive flavivirus antibodies, but this was minimal with the use of standard assays in an experienced reference laboratory. Finally, for the NHP, as a convenient sample was tested from only two susceptible monkey species, the findings could not be generalized but it is interesting to note that these samples were from 7 different localities of Rwanda (from Nyungwe to Akagera National parks).

Conclusions and recommendations

On the whole, the risk assessment of yellow fever virus (YFV) circulation in Rwanda was successfully carried out during November-December 2012. It is to date the first complete - it covers all the ecologic zones of Rwanda and the entomology, sero-epidemiology or even non-human primates aspects - and well documented survey ever carried out in the country.

The results of the serosurvey show a very low prevalence (2 out of 1284 participants i.e. 0.2%) of naturally-acquired antibodies against YFV in human population.

The serosurvey of 69 convenient samples (from RDB data base) of non-human primates (Olive Baboon and Vervet Monkey) does not show YFV circulation in this population despite the spatial distribution of the samples tested.

Entomological investigations show that Breteau Index (BI) and Container Index (CI) are potentially elevated (more 5% and 3% respectively) in most of the ecologic zones (some areas). A total of 1971 adult mosquitoes were collected among which 190 (<10%) were *Aedes sp* (mainly *Ae. aegypti, Ae. africanus* and *Ae. simpsoni*) from all the ecologic zones (zones 1 and 4 having the highest density). No potential vector was found infected by YFV using real time PCR.

In conclusion, although potential YFV vectors are present in Rwanda, they were not found infected and there is no evidence of virus circulation either in humans or non-human primates.

In the framework of the implementation of prevention and control measures in Rwanda, the following recommendations are made:

- According to these results, preventive mass campaign vaccination is not recommended in Rwanda;
- Because of the presence of potential vectors, educating the population on the elimination of mosquito breeding sites is recommended;
- It is highly recommended to start a good YF surveillance system and test YF suspected cases especially in the Western and the Eastern provinces. Suspected cases of YF can be confirmed in the regional reference laboratory for YF in Dakar, Senegal;
- These results could also serve as arguments to request for a reclassification of Rwanda by the International Health regulations Review committee.

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References

Dick GW, Kitchen SF, Haddow AJ Zika virus isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952; 46: 509-20.

De Madrid AT, Porterfield JS. A simple micro-culture method for the study of group B arboviruses. *Bull World Health Organ* 1969; 40: 113-121.

Ellis ER, Barrett ADT. The enigma of yellow fever in East Africa. *Rev Med Virol*. 2008; 18: 331-346.

Edwards FW. Mosquitoes of the Ethiopian region: III Culicine adults and pupae. *London: British Museum* (Natural History) 1941.

Findlay GM, Kirk R, MacCallum FO. Yellow fever and the Anglo-Egyptian Sudan: distribution of immune bodies to yellow fever. *Ann Trop Med Parasitol* 1941; 35: 121-139.

Germain M, Cornet M, Mouchet J, et al. La Fievre Jaune Selvatique en Afrique: données récentes et conceptions actuelles. *Med Trop*. 1981; 41: 31-43.

Haddow AJ. A review of the results of yellow fever protection-tests on the sera of primates from Kenya. Ann Trop Med Parasitol 1952; 46: 135-143.

Haddow AJ. The natural history of yellow fever in Africa. *Proc R Soc Edinb* 1969; 70: 191-227.

Haddow AJ, Dick GWA, Lumsden WHR, Smithburn KC. Monkeys in relation to the epidemiology of Yellow fever in Uganda. *Trans R Soc Trop Med Hyg* 1951; 45: 189-224.

Kebede S, Gatabazi JB, Rugimbanya P, Mukankwiro T, Perry HN, Wondimagegnehu Alemu, Ndihokubwayo JB, Kramer MH and Mukabayire O. Strengthening systems for communicable disease surveillance: creating a laboratory network in Rwanda. *Health Research Policy and Systems* 2011; 9: 27.

Huang YM. *Aedes (Stegomyia) bromeliae* (Diptera: Culicidae), the yellow fever virus vector in East Africa. *J Med Entomol* 1986; 23: 196-200.

Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 2008; 14: 1232-9.

Lumsden WHR, Hewitt LE. Yellow fever protection tests on sera from wild primates, Newala District, Tanganyika. *Rep E Afr Virus Res Inst* 1954; 4: 5.

Mahaffy AF, Smithburn KC, Hughes TP. The distribution of immunity to yellow fever in Central and East Africa. *Trans R Soc Trop Med Hyg* 1946; 40(1): 57-82.

Onyango CO, Ofula VO, Sang RC, et al. Yellow fever outbreak, Imatong, southern Sudan. Emerg Infect Dis 2004; 10: 1063-1068.

Rey JL, Cavallo JD, et al. Les fièvres d'origine indéterminées (FOI) dans les camps de réfugiés rwandais de la région de Goma au Zaïre. *Bull Soc Path Ex* 1996; 89: 204-208.

Sawyer WA, Whitman L. The yellow fever immunity survey of North, East, and South Africa. *Trans R Soc Trop Med Hyg* 1936; 29(4): 397-412.

Sanders EJ, Marfin AA, Tukei PM, et al. First recorded outbreak of yellow fever in Kenya, 1992–1993. I. Epidemiologic investigations. *Am J Trop Med Hyg* 1998; 59: 644-649.

Smithburn KC, Haddow AJ, Lumsden WHR. An outbreak of sylvan yellow fever in Uganda with *Aedes (Stegomyia) africanus* Theobald as principal vector and insect host of the virus. *Ann Trop Med Parasitol* 1949; 43: 74-89.

Soghaier MA, Hagar A, Abbas MA, Elmangory MM, Eltahir KM, Sall AA. Yellow Fever outbreak in Darfur, Sudan in October 2012; the initial outbreak investigation report. *J Infect Public Health* 2013; 6(5): 370-6.

Tomori O. Yellow fever: The recurring plague. *Crit Rev Clin Lab Sci.* 2004; 41: 391-427.

Wamala JF, Malimbo M, Okot CL, Atai-Omoruto AD, Tenywa E, Miller JR, Balinandi S, Shoemaker T, Oyoo C, Omony EO, Kagirita A, Musenero MM, Makumbi I, Nanyunja M, Lutwama JJ, Downing R, Mbonye AK. Epidemiological and laboratory characterization of a yellow fever outbreak in northern Uganda, October 2010-January 2011. *Int J Infect Dis*. 2012; 16(7): 536-42.

Weidmann M, Faye O, Faye O, Kranaster R, Marx A, Nunes M, Vasconcelos P, Hufert F, Sall A. Improved LNA probe-based assay for the detection of African and South American yellow fever virus strains. *Journal of Clinical Virology* 2010; 48: 187-192.

World Health Organization, Regional Office for Africa: Technical guidelines for Integrated Disease Surveillance and Response in the African Region Harare; 2002. [*http://www.cdc.gov/idsr/IDSR_resources/detection/detect_respond_eng.pdf*].

World Health Organization. Chikungunya and dengue, south-west Indian Ocean. *Wkly Epidemiol Rec* 2006; 81: 105-116.

World Health Organization. Assessment of yellow fever epidemic risk- a decision making tool for preventive immunization campaigns. *Wkly Epidemiol Rep* 2007; 82: 153-160.

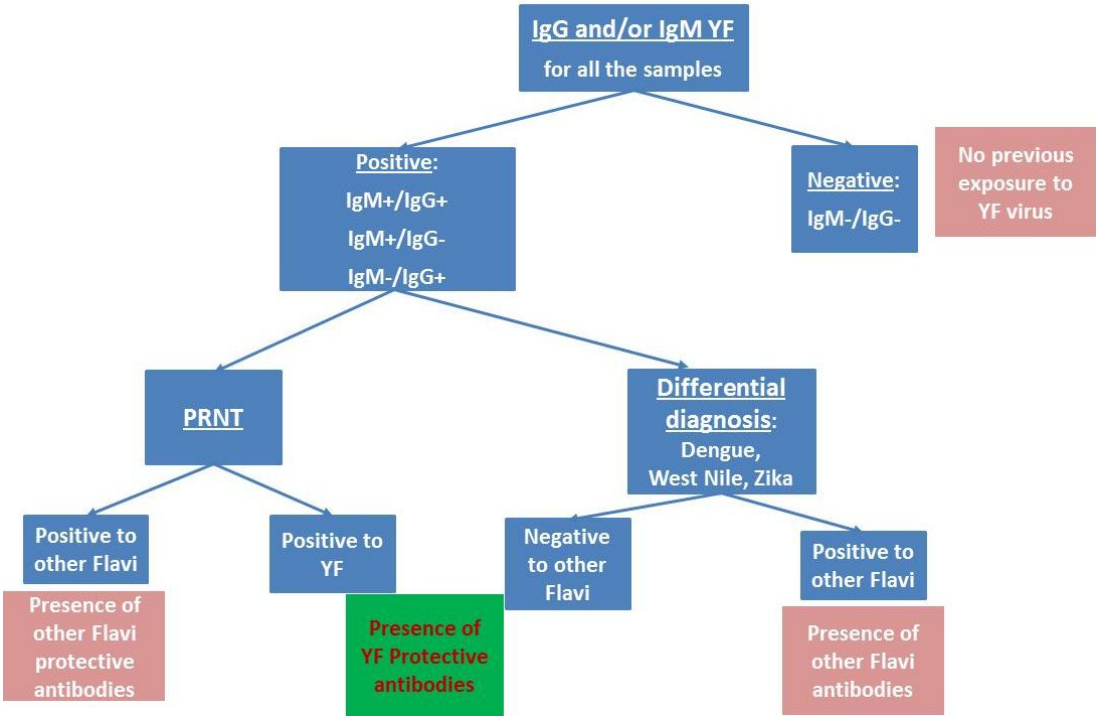
World Health Organization. Outbreak news. Yellow fever, Uganda. *Wkly Epidemiol Rep* 2011; 86(5):37-8.

World Health Organization. Vaccines and vaccination against yellow fever. WHO Position Paper June 2013. *Wkly Epidemiol Rep* 2013; 88: 269-284.

World Health Organization. Prevention and Control of Yellow Fever in Africa. *WHO Geneva*, 1986; 94pp.

Appendix

Laboratory testing algorithm for serum samples



Some pictures of the yellow fever risk assessment in Rwanda

Training in RBC Kigali prior to field deployment
(© Demanou, Nov. 2012)

Human serosurvey in Gashora (zone 4), Rwanda
(© Demanou, Nov. 2012)

Plant leaves with *Aedes* sp larvae in a village (pilot study)
(© Demanou, Nov. 2012)