

# 9th International Conference on Typhoid and Invasive non-typhoidal salmonellosis diseases 4/30 – 5/3 2016

## Invasive salmonellosis Epidemiological data from Africa Case fatality and association with drug resistance

Lisette Kalonji Mbuyi, Annelies Post, Marie-France Phoba,  
Jan Verhaegen, Dauly Ngbonda, Jean-Jacques Muyembe,  
Dadi Falay, Sophie Bertrand, Octavie Lunguya, Jan Jacobs



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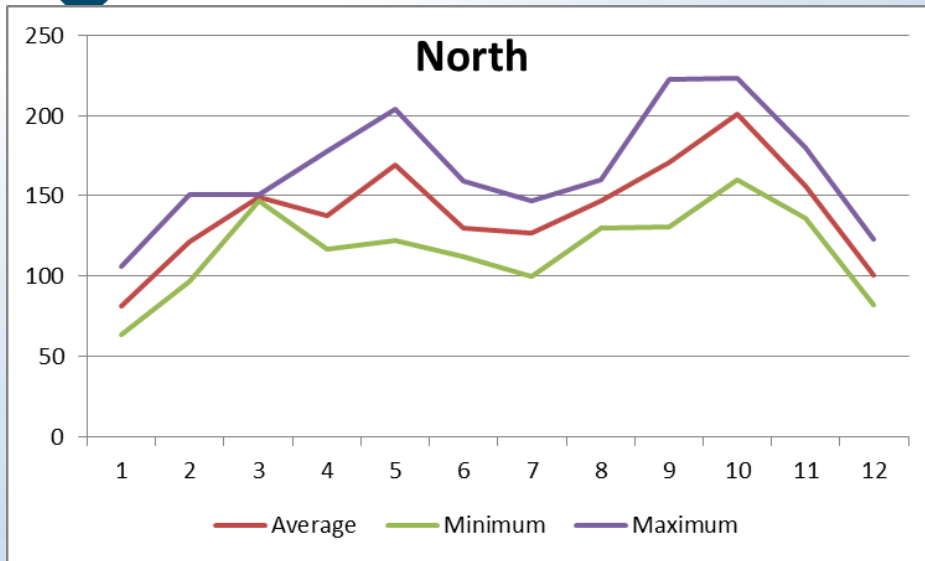
## Invasive salmonellosis Epidemiological data from Africa Case fatality and association with drug r



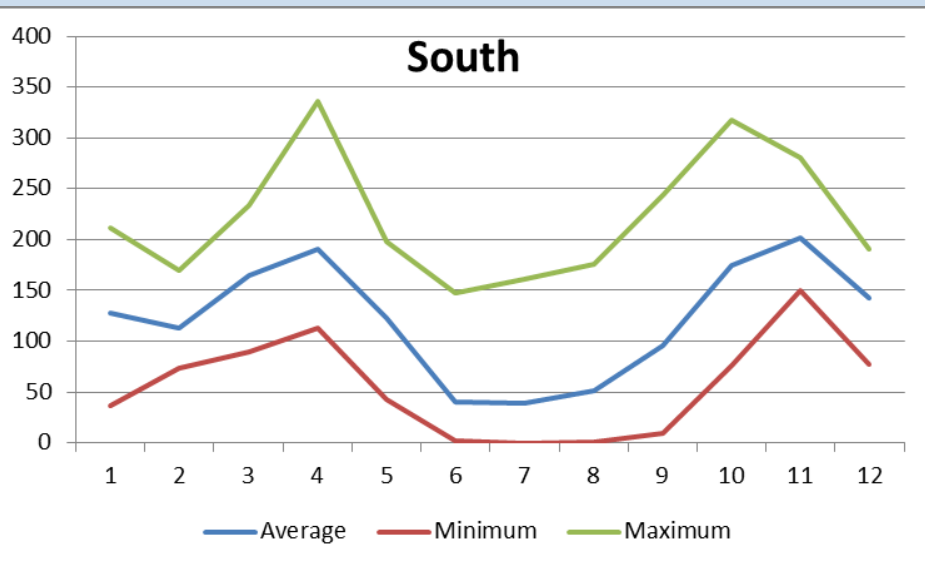
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# Democratic Republic of the Congo



8 months wet,  
4 months dry season



# Democratic Republic of the Congo

Demography 2 345.000 km<sup>2</sup>



75.508.308 inhabitants

2.5% increase/yr

48.5% < 15 yrs old, 4% > 60 yrs PAMM

Human Development Index = 186

HIV

Adults aged 15 to 49

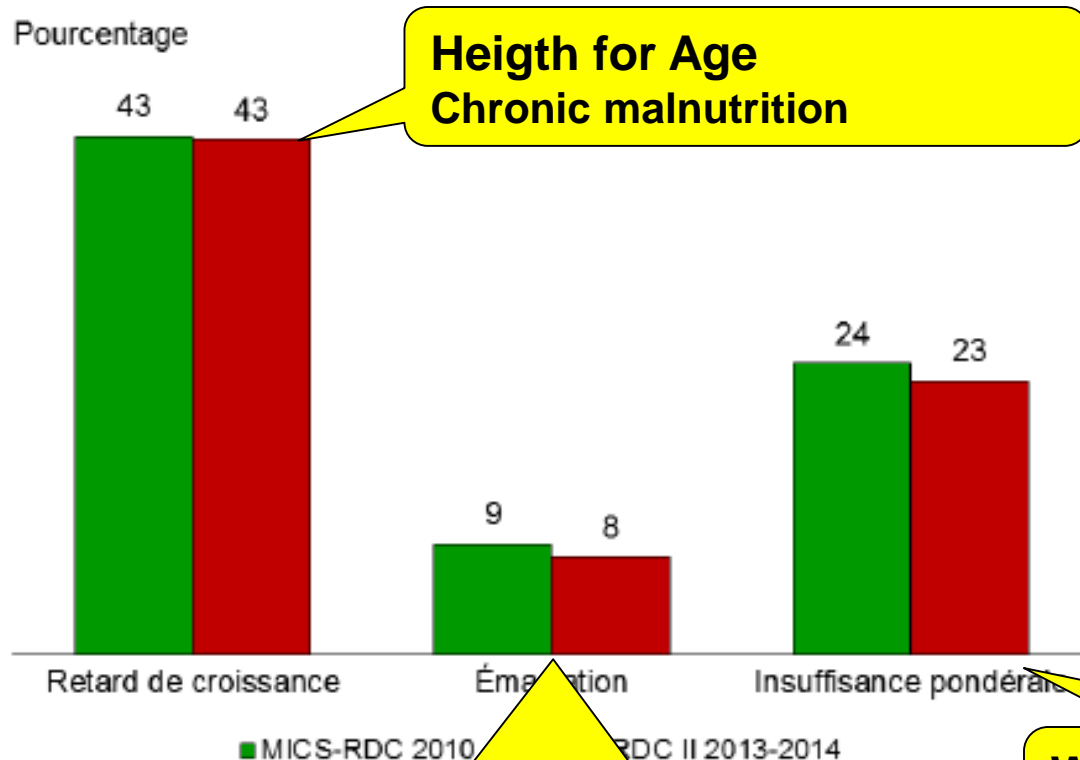
prevalence rate 1.1% [0.9% - 1.3%]

<http://www.unaids.org/en/regionscountries/countries/democraticrepublicofthecongo>



# Malnutrition:

**Graphique 6 Malnutrition des enfants de moins de cinq ans, MICS-RDC 2010 et EDS-RDC II 2013**



**Height for Age  
Chronic malnutrition**

**Weight for Height  
Acute malnutrition/iNTS associated**

**Weight for Age  
Combines acute and chronic**



RÉPUBLIQUE DÉMOCRATIQUE DU CONGO



DEUXIÈME ENQUÊTE DÉMOGRAPHIQUE  
ET DE SANTÉ  
(EDS-RDC II 2013-2014)

RAPPORT PRÉLIMINAIRE

Ministère du Plan et Suivi  
de la Mise en œuvre de la  
Révolution de la Modernité

Ministère de la Santé  
Publique

MEASURE DHS, ICF International  
Rockville, Maryland, U.S.A.



# Malaria: *P. falciparum* high endemicity, perennial, not declining

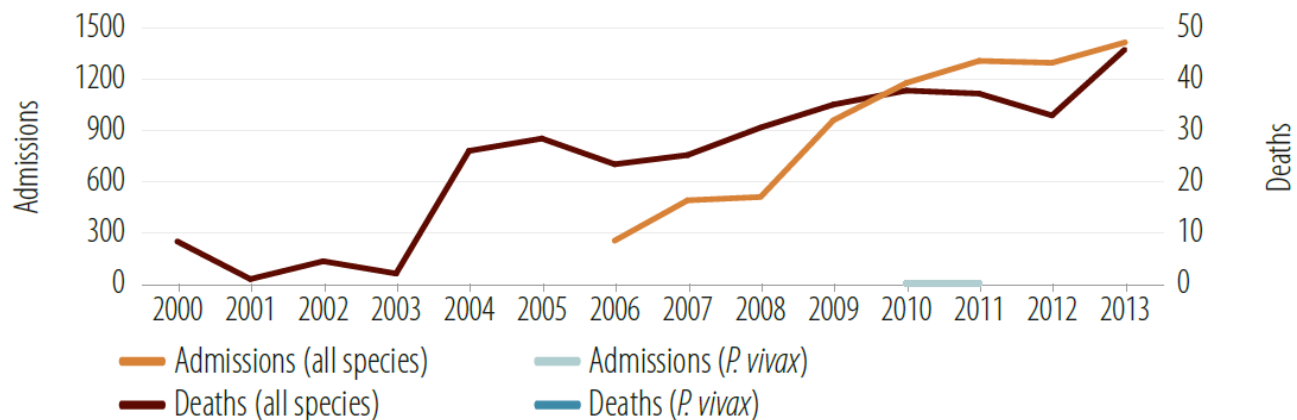


## I. Epidemiological profile

Population	2013	%
High transmission (> 1 case per 1000 population)	65 500 000	97
Low transmission (0–1 cases per 1000 population)	2 030 000	3
Malaria-free (0 cases)	0	0
Total	67 530 000	

Parasites and vectors	
Major plasmodium species:	<i>P. falciparum</i> (0%), <i>P. vivax</i> (0%)
Major anopheles species:	<i>An. gambiae</i> , <i>An. funestus</i> , <i>An. nili</i> , <i>An. moucheti</i>
Programme phase:	Control
Reported confirmed cases:	6 715 223
Reported deaths:	30 918

Malaria admissions and deaths (per 100 000)





# Blood culture surveillance network

“Resume Microbiology” in DR Congo

Bloodstream & Meningitis

Antibiotic resistance

Capacity building

Patient care “routine” diagnostics

Epidemic preparedness

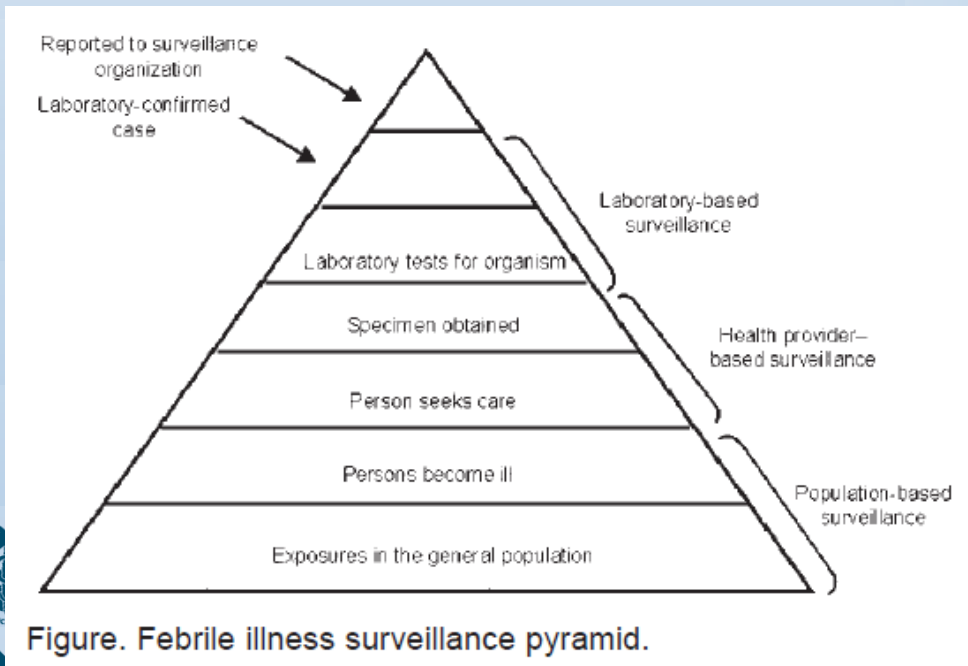


Figure. Febrile illness surveillance pyramid.



# Blood culture surveillance Methods

Suspected Case



Blood culture



Antibiogram



# Shipment and batch testing ITM - Reference lab IPH



® E
CI
32
24
16
12
8
6
4
3
2
1.5
1.0
.75
.50
.38
.25
.19
.125
.094
.064
.047
.032
.023
.016
.012
.008
.006
.004
.003
.002

® E
AZ
256
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.38
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.19
.125
.094
.064
.047
.032
.023
.016



# Limitations and Strengths

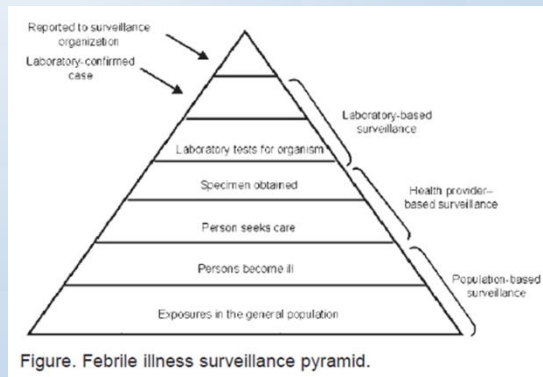
**Limited demographic/clinical data**  
**No population-based denominators**

**Quality and Logistical issues**

**No GPS coordinates**

**No ...**

**No...**



**Constant performance over time**

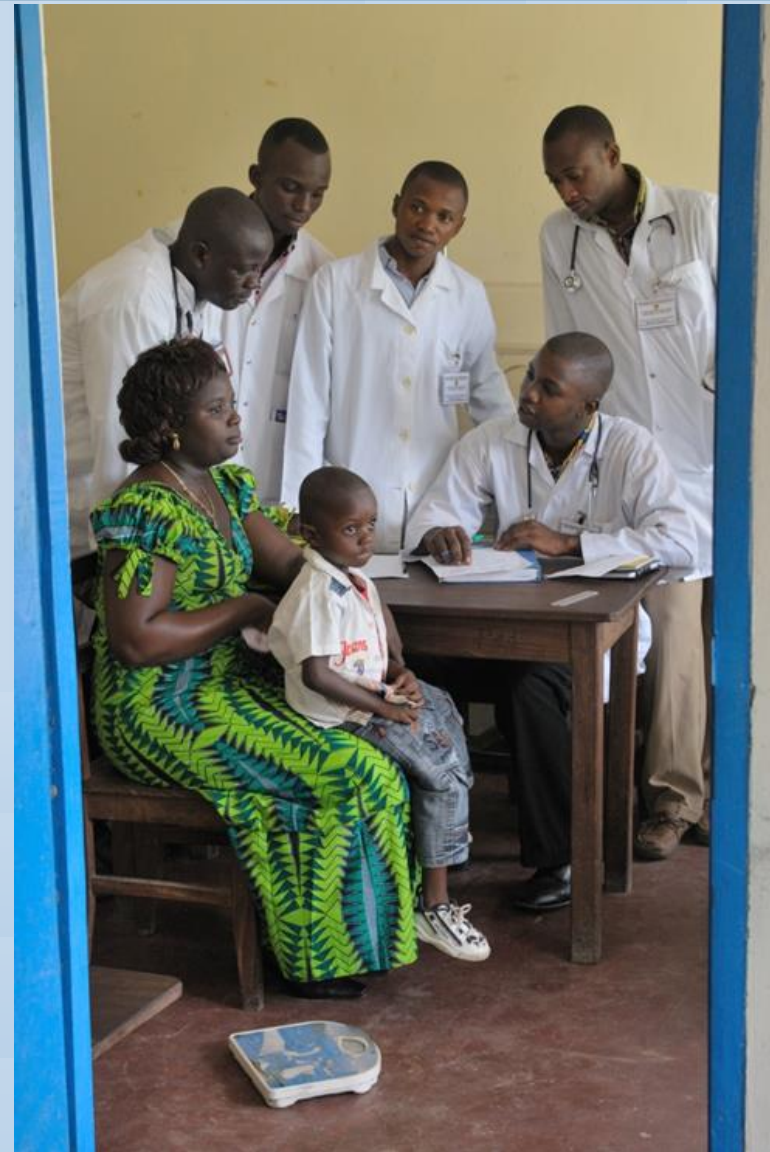
**High recovery of isolates**

**High(er) reactivity in case of alerts**

**Embedded in patient care**

**Service-for-free: non economic/urgency**

**bias**



# Criteria for sampling

In children 28 days and 14 years old

1. Body temperature of  $\geq 38^{\circ}\text{C}$  or  $\leq 35.5^{\circ}\text{C}$

2. Suspicion of severe localized infection.

pneumonia

meningitis

complicated urinary tract infection

osteoarthritis

skin and soft tissues infection

gynaecological infections

peritonitis

3. Clinical suspicion of sepsis

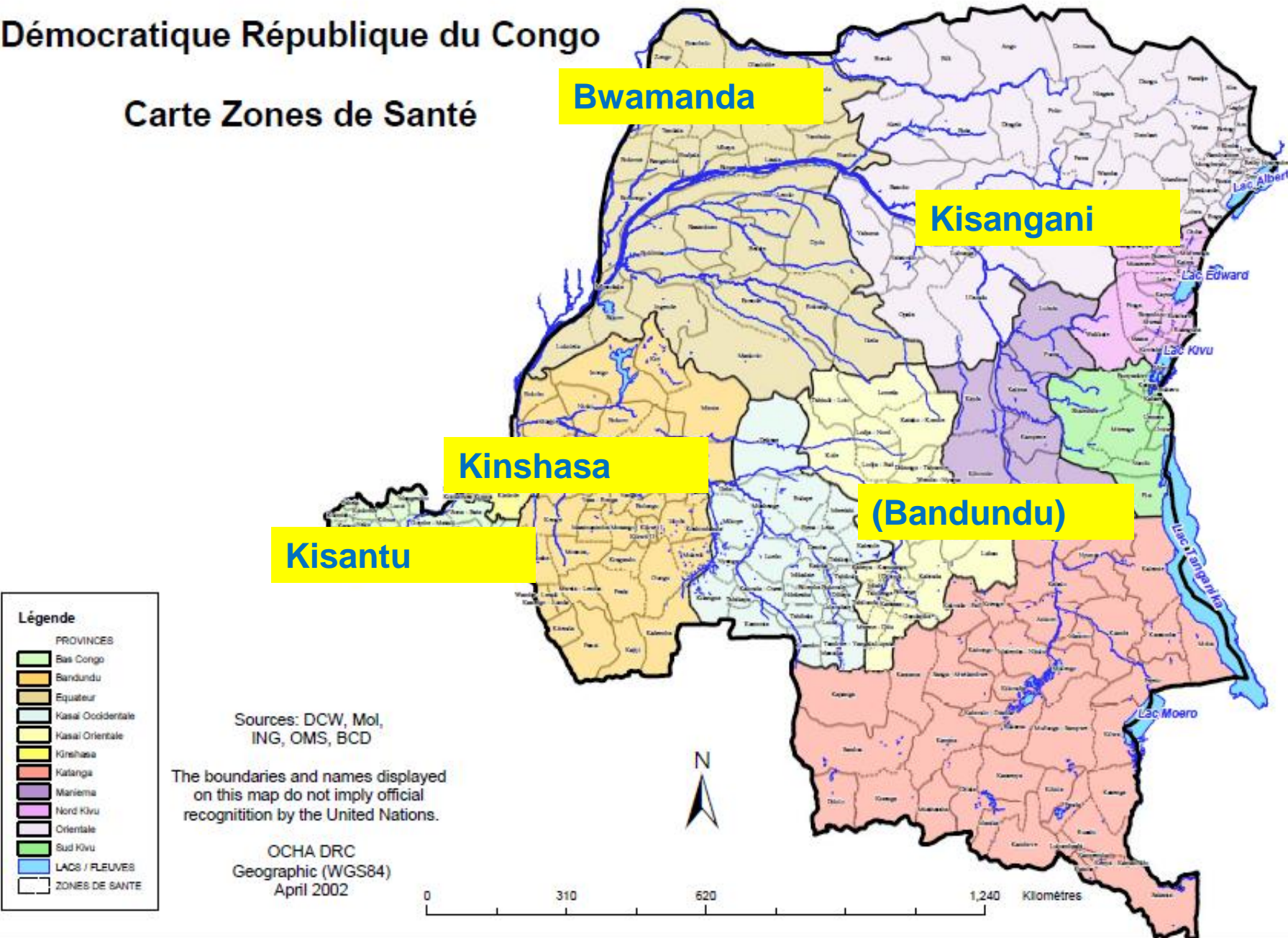
typhoid fever and severe malaria





# Démocratique République du Congo

## Carte Zones de Santé



# Logistical problems...





## Logistical problems...

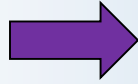


**Salmonella survives for > 2 months in a blood culture vial at room t°**

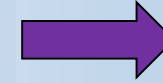
**Pneumococcus dies probably after 1 – 2 days**

# Breakdown of samples

**Blood cultures**  
n = 15.116



**Septic episodes**  
n = 14.150

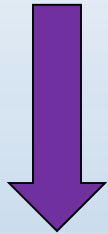


**Patients**  
n = 13.243

0.5 – 4ml in children  
2 x 10ml in adults

11.734 in children  
2.001 in adults

1 septic episode = 14 days



**Nr. of CSO n = 2.353 (15,6%)**  
Clinically significant organisms

**Nr. of CON n = 1.713 (11,3%)**  
Contaminants

Bacillus n = 375  
CNS n = 1320  
Coagulase negative staphylococcus



777 patients with 2 episodes  
91 patients with 3 episodes  
32 patients with ≥ 4 episodes

**Children** n = 11,002  
Median 2 yrs old (IQR 1 – 4 yrs)  
M/F ratio 1.16

**Adults** n = 1.835  
Median 35 yrs old (IQR 25 – 50 yrs)  
M/F ration = 1.04



# Contamination: Bacillus = gloves nants

**Nr. of CSO n = 2.353 (15,6%)**  
Clinically significant organisms

**Nr. of CON n = 1.713 (11,3%)**  
Contaminants

**Bacillus n = 375**

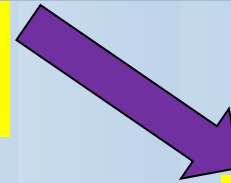
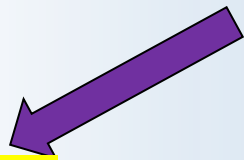
**CNS n = 1320**

**Coagulase negative staphylococcus**



# Breakdown of samples

**Septic episodes  
n = 14.150**



**11.734 in children  
CSO: n = 1901  
(first isolates)  
Clinically significant organisms)**

**2001 in adults  
CSO: n = 300  
(first isolates)  
Clinically significant organisms)**

## **Children CSO n = 1901**

- |                                |              |
|--------------------------------|--------------|
| <b>1. Salmonella non-Typhi</b> | <b>41.5%</b> |
| 2. Klebsiella spp.             | 9.0%         |
| 3. S. aureus                   | 7.9%         |
| <b>4. Salmonella Typhi</b>     | <b>6.5%</b>  |
| 5. Enterobacter spp.           | 6.3%         |
| 6. Escherichia coli            | 5.5%         |

## **Adults CSO n = 300**

- |                                |              |
|--------------------------------|--------------|
| <b>1. Salmonella Typhi</b>     | <b>21.3%</b> |
| 2. Escherichia coli            | 19.7%        |
| <b>3. Salmonella non-Typhi</b> | <b>11.3%</b> |
| 4. S. aureus                   | 10.3%        |
| 5. Klebsiella spp.             | 10.0%        |
| 6. Enterobacter                | 8.7%         |

# Salmonella single isolates

Single isolates (septic episodes)	Numbers	% of CSO (children/adults combined, n = 2201)	% of septic episodes (n = 11,734)	Numbers confirmed and available%
Salmonella	1,043	47.4	8.9	952
Typhi	194	8.8	1.7	163
Non-Typhi	840	38.2	7.2	789
Typhimurium	384	17.4	3.3	377
Enteritidis	398	18.1	3.4	391
Other	58	2.6	0.5	21





# Salmonella

**952/1037 (91.8%) first isolates available**

- 1. Serotype distribution**
- 2. Antibiotic resistance**
- 3. Some observations**



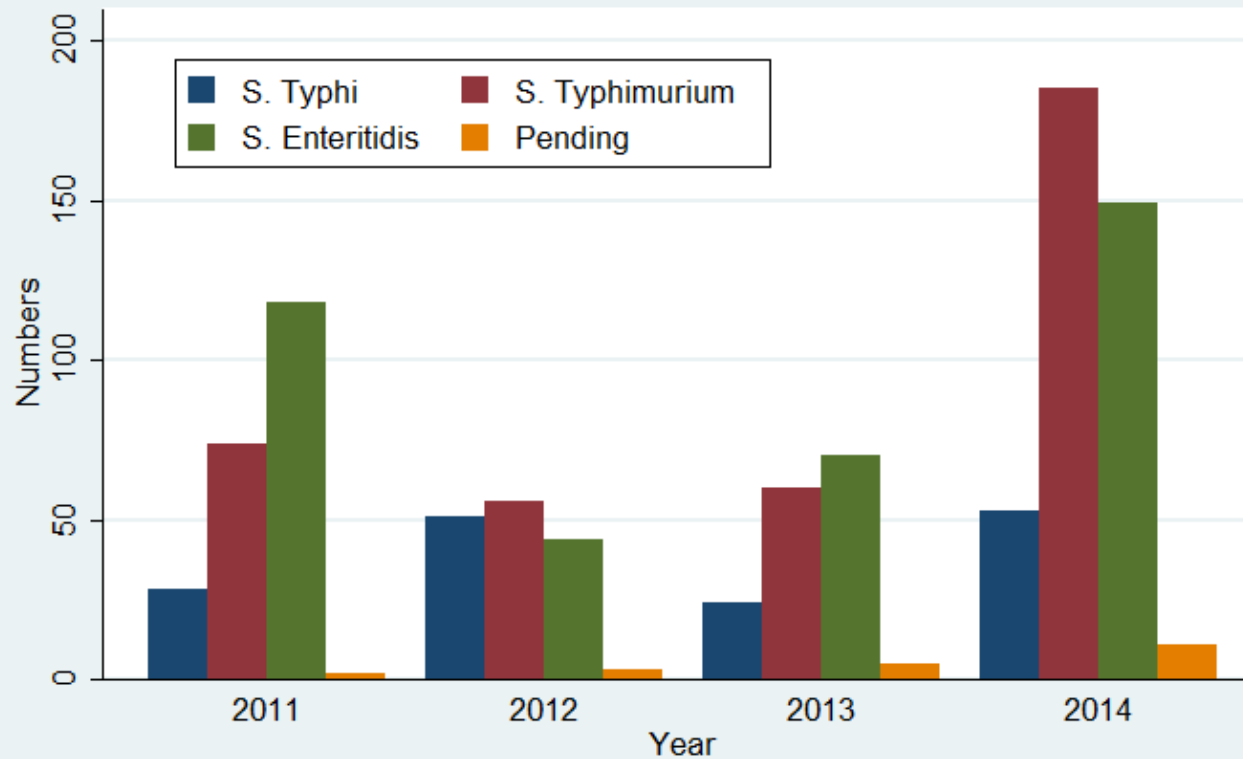


# Salmonella serotype distribution

Single isolates (septic episodes)	Numbers	%
Salmonella	952	100
Typhi	163	17.1%
Non-Typhi	789	82.9%
		% of NTS
Typhimurium	377	47.8%
Enteritidis	391	49.6%
Other	21	2.7%

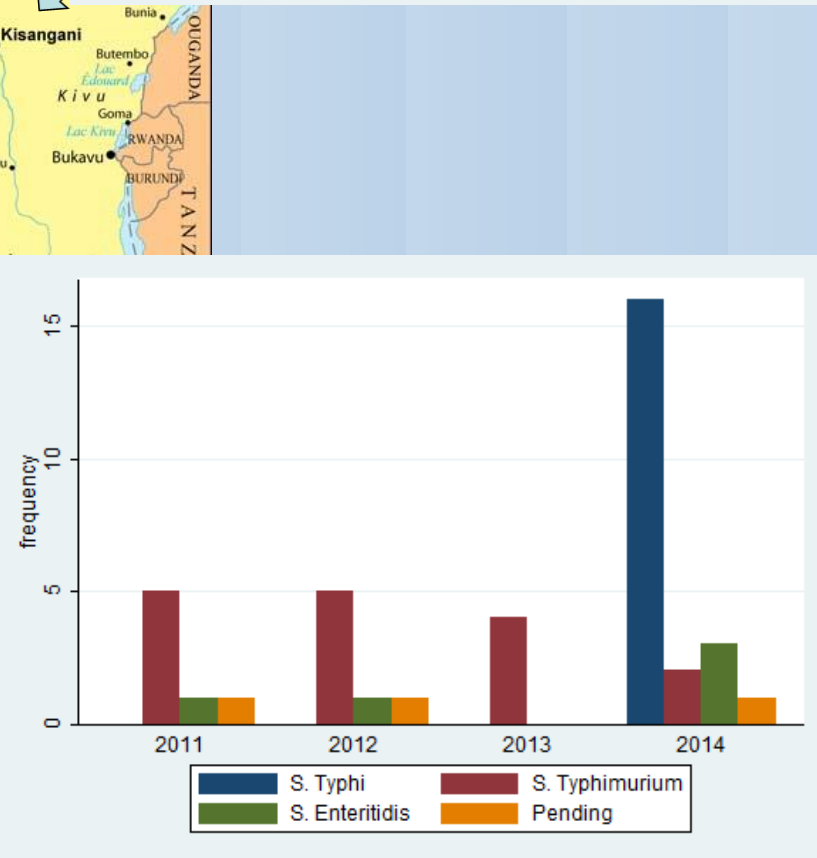
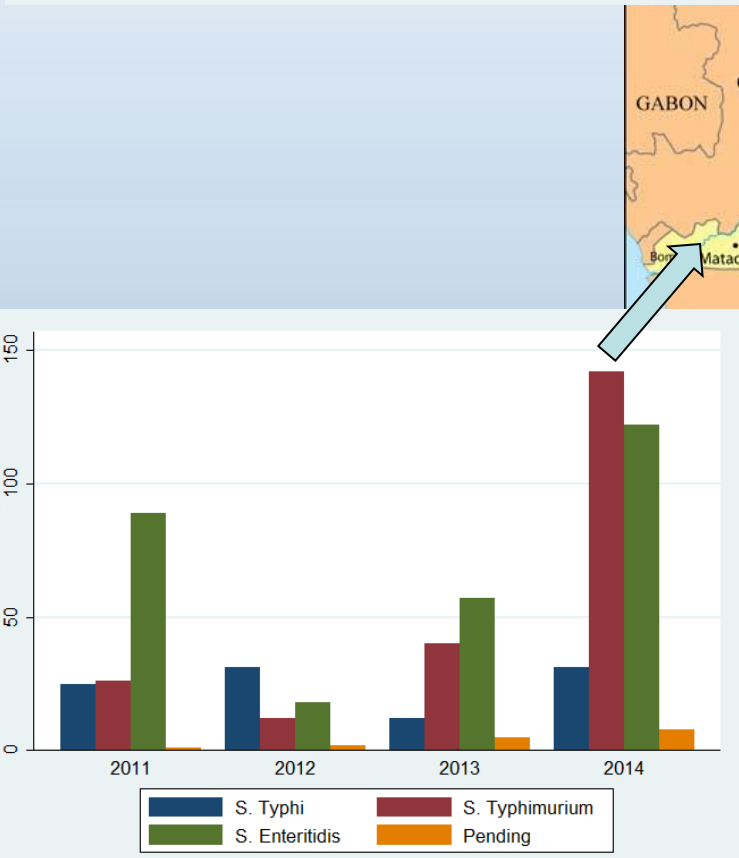
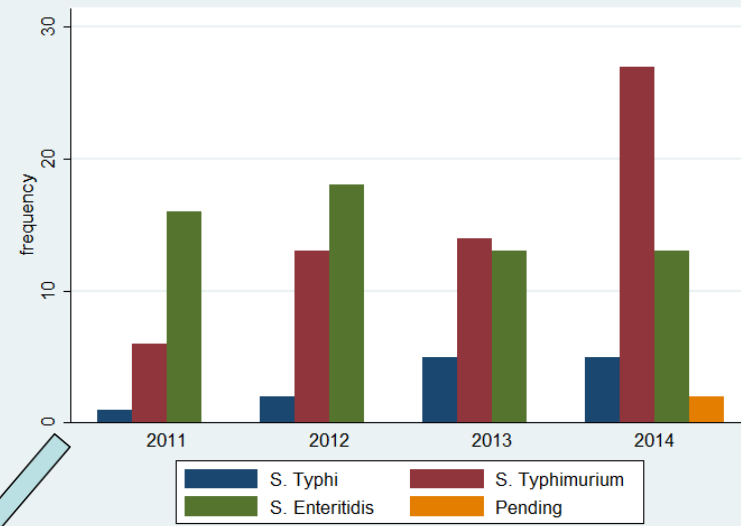
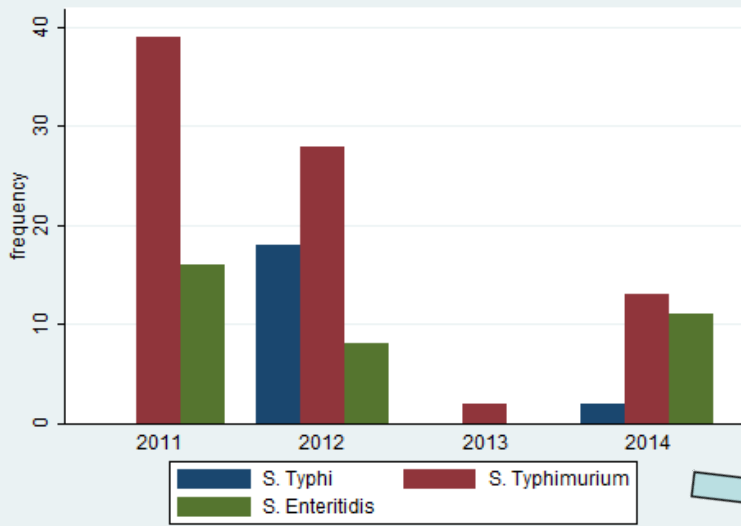


# Salmonella serotype distribution over time and place



**Salmonella Typhimurium varietas Copenhagen**  
**35/138 (25.3%) Typhimurium tested**





# Antibiotic resistance

Panel of antibiotics: ECDC 2014

ECDC TECHNICAL DOCUMENT

**EU protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates**

March 2014

Disk diffusion: CLSI M100-S25

Ciprofloxacin: E-test Macromethod (bioMérieux and Oxoid)

DCS Decreased ciprofloxacin susceptibility

DCS if MIC > 0.06(4)mg/l = 0.12

Resistant if MIC > 1 mg/l

Pefloxacin (EUCAST), Nalidixic acid (CLSI) disk

Azithromycin: EUCAST 2014 v4.0 MIC ≥ 16 mg/l

MDR: R to Amoxi/TMP-SMX and Chloramphenicol



Resistance rates	Typhi n= 162	Typhimurium n = 377	Enteritidis n = 391	Pending n = 21
ampicillin	68.7%	97.1%	85.4%	71.4%
cotrimoxazole	60.7%	95.5%	80.8%	66.7%
chloramphenicol	54.0%	91.8%	82.4%	61.9%
MDR	38.0%	90.2%	79.8%	57.1%
tetracyclin*	46.0%	23.1%	72.6%	33.3%
gentamicin*	0.6%	9.0%	0.5%	9.5%
DCS	36.8%	2.1%	1.8%	9.5%
azithromycin	0.6%	12.5%	0.3%	4.8%
ESBL	0.0%	12.7%	0.0%	4.8%
MDR + DCS	17.8%	1.9%	0.8%	4.8%
MRD + DCS + tetra	8.6%	0.3%	0.5%	0.0%
MDR + ESBL	0.0%	11.7%	0.0%	4.8%
MDR + ESBL + azithro	0.0%	10.9%	0.0%	4.8%



\* For surveillance purposes only, no resistance to meropenem

# MIC values ciprofloxacin (mg/l)



MIC value	< 0.01	0.016	0.023	0.038	0.047	0.064	0.125	0.19	0.25	0.38	0.5	Total
Typhi	18	66	3	9	6	2	8	1	40	6	4	163
Typhimurium	110	233	6	12	5	4	6	0	1	0	0	377
Enteritidis	189	176	7	9	2	2	3	0	1	0	2	391
Pending	8	9	0	2	0	0	1	0	1	0	0	21

MIC 50 = 0.016 mg/l

MIC 90 = 0.038 mg/l

DCS in Salmonella Typhi = 38.0%, half of which are MDR





# MIC values azithromycin (mg/l)

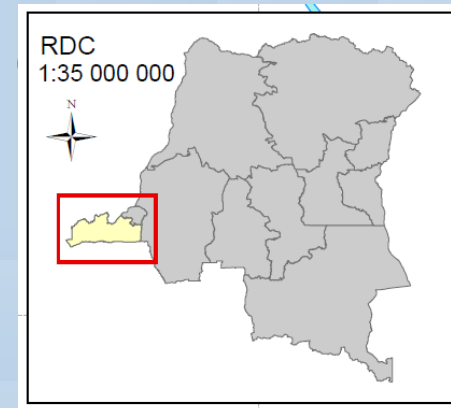
MIC value	< 2	2	3	4	6	8	12	15	32	48	64	128	256	Total
Typhi	12	37	40	50	20	3	0	0	1	0	0	0	0	163
Typhimurium	28	112	104	66	19	1	0	0	21	9	1	1	15	377
Enteritidis	74	114	133	59	9	0	0	1	0	0	0	0	1	391
Pending	4	8	5	1	1	0	1	0	0	0	0	0	1	21

MIC 50 = 3 mg/l

MIC 90 = 6 mg/l

**MIC azithromycin  $\geq$  16 mg/l : mainly Typhimurium**

- nearly half are high-level resistant
- all but one combined with ESBL
- all but one Bas-Congo, since 03/2013

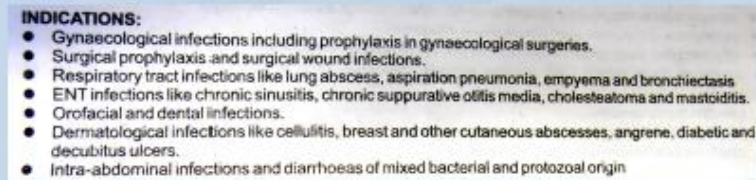


# Azithromycin is a reserve antibiotic but is heavily promoted...

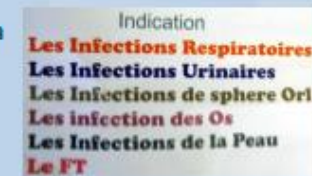
**Figure 1** Package insert for levofloxacin (intravenous) mentioning sinusitis as an indication



**Figure 3** Package insert for ofloxacin with doubtful and ill-defined indications



**Figure 4** Flyer for azithromycin with non-detailed syndrome based indications



**Figure 2** Flyer for ciprofloxacin and tinidazole combination with ill-defined indications



**Figure 5** Flyer for azithromycin with misleading figure



# Azithromycin is a reserve antibiotic but is heavily promoted...

Figure 1 Package insert for levofloxacin (intravenous) mentioning sinusitis as an indication



Jan, do not forget to reveal the need for treatment guidelines!

Figure 3 Package insert for ofloxacin with doubtful and ill-mentioning sinusitis as an indication



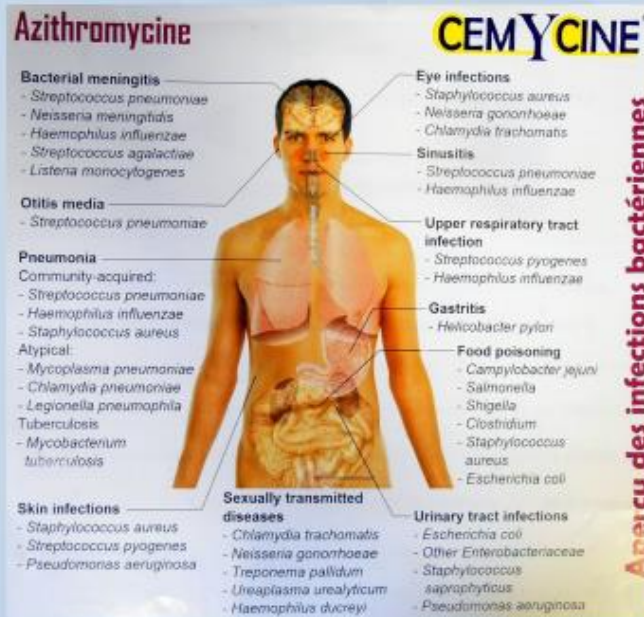
Figure 4 Flyer for azithromycin with non-detailed syndrome based indications



Figure 2 Flyer for ciprofloxacin and tinidazole combination with ill-defined indications



Figure 5 Flyer for azithromycin with misleading figure





# Effect of antibiotic use prior to sampling on recovery of CSO

	Numbers of septic episodes				
Antibiotics	CSO (% of total)	Typhi (% of total)	NTS (% of total)	No growth of CSO	Total
Yes	550 14.0%	55 0.15%	201 6.0%	2433 73.3%	3376
No	693 14.0%	73 1.5%	238 4.8%	3603 73.0%	4933
Total	1243	128	439	6036	8085

**41.7% of patients were on antibiotics  $\leq$  48h before sampling**

**Proportion of growth similar in both groups**



# Demographics/Clinical presentation – some epidemiological data

1. Age/Gender
2. Outbreaks
3. Symptoms
4. Outcome

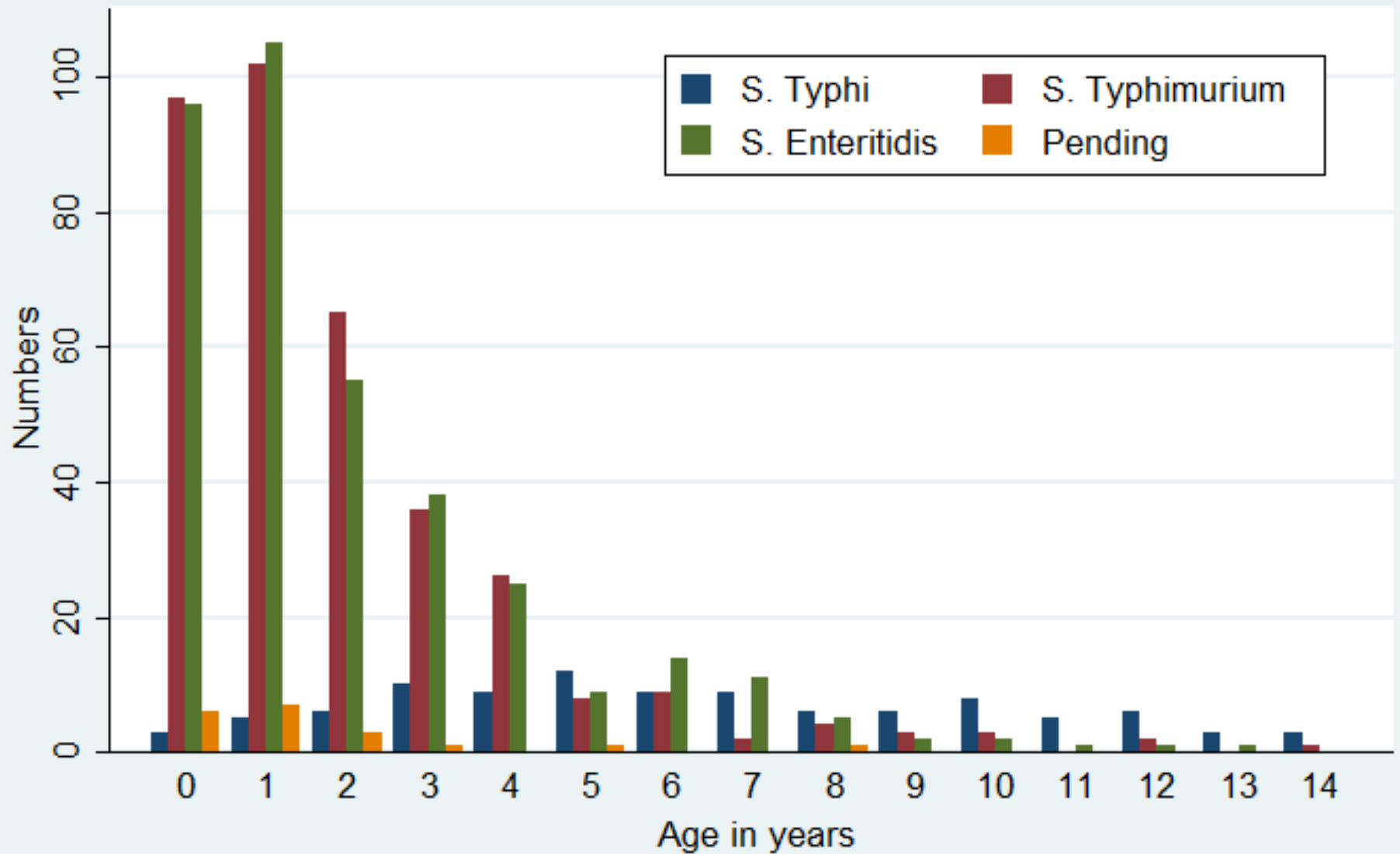


# Age and gender distribution

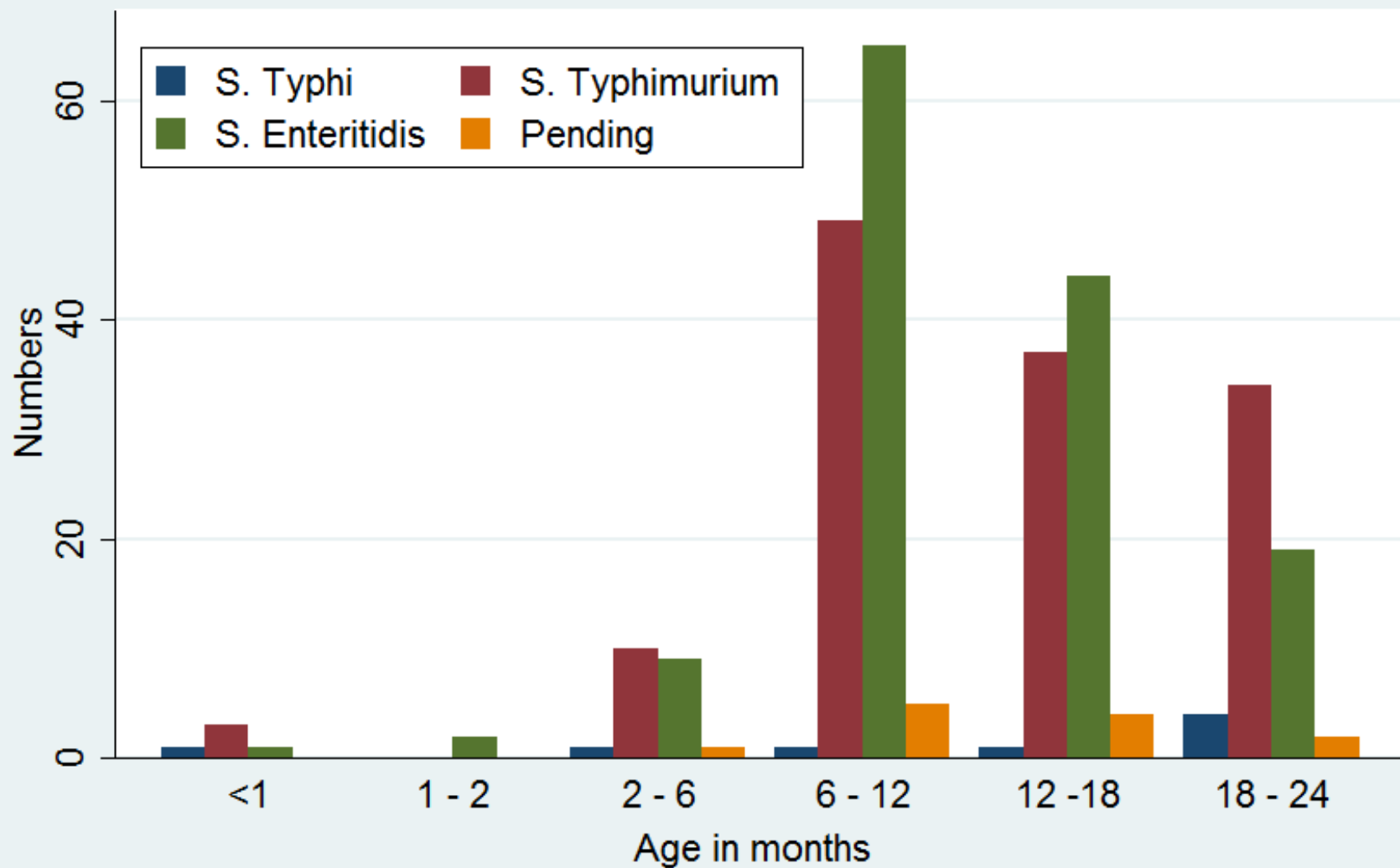
	Typhi	Non-typhi	Typhimurium	Enteritidis
<b>Total numbers</b>	<b>163</b>	<b>789</b>	<b>377</b>	<b>391</b>
<b>Mean age ± SD</b>	<b>16.1 ± 15.4</b>	<b>3.5 ± 8.4</b>	<b>3.2 ± 7.9</b>	<b>3.8 ± 8.7</b>
<b>Median age (range)</b>	<b>10 (0 – 75)</b>	<b>1 (0 – 76)</b>	<b>1 (0 – 64)</b>	<b>1 (0 – 76)</b>
<b>M:F ratio</b>	<b>1.22</b>	<b>1.28</b>	<b>1.22</b>	<b>1.34</b>
<b>% below 15 yrs</b>	<b>63.7%</b>	<b>95.7%</b>	<b>97.0%</b>	<b>94.8%</b>
<b>% below 5 yrs</b>	<b>21.0%</b>	<b>85.4%</b>	<b>88.3%</b>	<b>82.9%</b>
<b>% below 2 yrs</b>	<b>4.9%</b>	<b>52.3%</b>	<b>52.8%</b>	<b>51.4%</b>



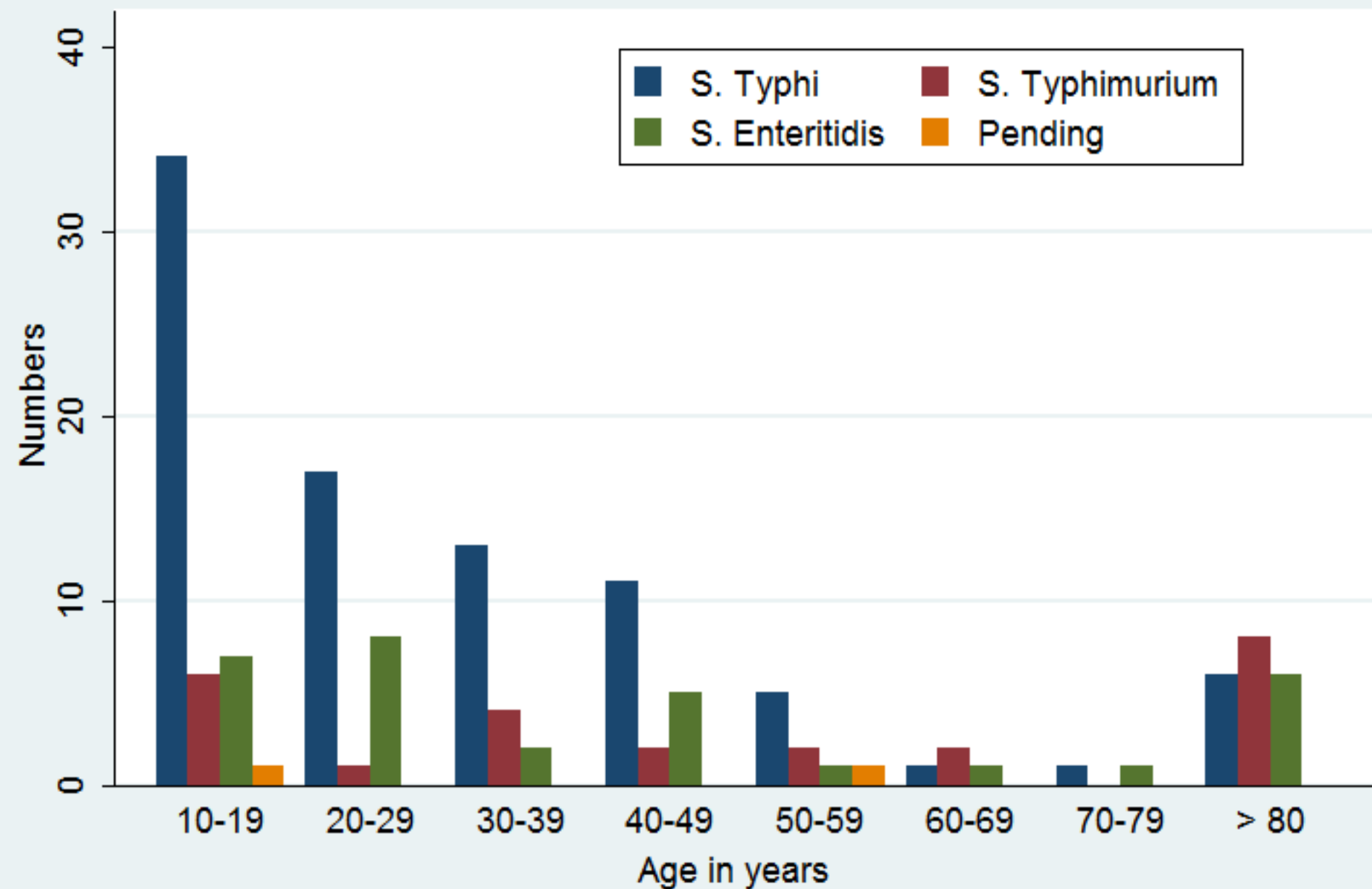
# Age and gender distribution children



# No clusters of healthcare-based infections



# No “second peak” of NTS in adults



# Economy at the household level: 5 day hospital admission

Child 10kgs private structure, 5 days Hospital Admission	Francs congolais	US \$
<b>Transport</b> (child+ care-taker to and back from hospital, median 13.5km; range 1 - 130km)	4000	4,3
<b>Regeistation</b>	5000	5,3
<b>Antibiotics/5.6 days</b> (Ceftriaxone ou cefotaxime (spécialité) 38000 FC, Gentamicin 800FC, Solvant)	38000	40,4
<b>Antipyretics</b> (Dipyronne ou Paracetamol 1000FC)	1000	1,1
<b>Antimalaials</b> (Quinine 750FC, Artemether/Luméfántrine 1500FC)	1500	1,6
<b>IV-Perfusions</b> (Sérum glucosé 5% ou Physiologique 1500FC)	1500	1,6
<b>Food and meals</b> (child and caretaker)	5000	5,3
<b>Hospital Stay</b>	5000	5,3
<b>Nursing</b>	5000	5,3
<b>Blood Transfusion</b>		
<b>Laboratoy analysis</b> (Hb 700FC, TBF 1000FC, WBC 1500FC, Differential count 1500FC ESR 1000FC)	6500	6,9
<b>Total</b>	<b>72500</b>	<b>77,1</b>



# Economy at the household level: 5 day hospital admission



**Private sector: 77.1 \$**

**Public sector: 44.6 \$**

**74% use private sector**

**71.3% lives with < \$/day** (PNAM)

**5 days IV treatment is (too?) short**



# Outbreaks: “flambées de fièvre”

Minus 5 years old

Fever + Severe anemia

Non-response to antimalarials

Microscopy or RDT\* malaria positive

Increase in Hospital Admissions

Increase in Case fatality rates

Increase in Transfusions

\* RDT = malaria rapid diagnostic test

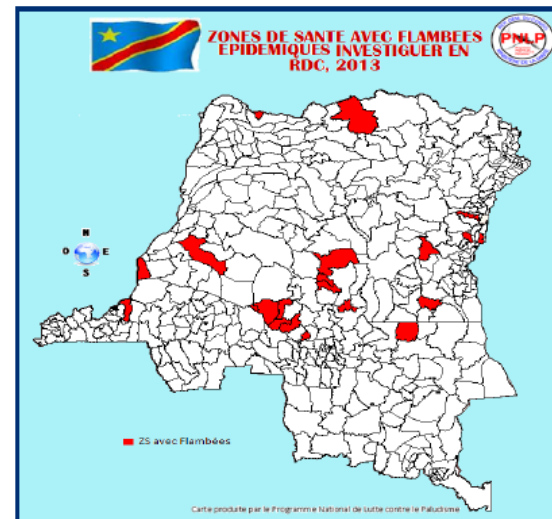


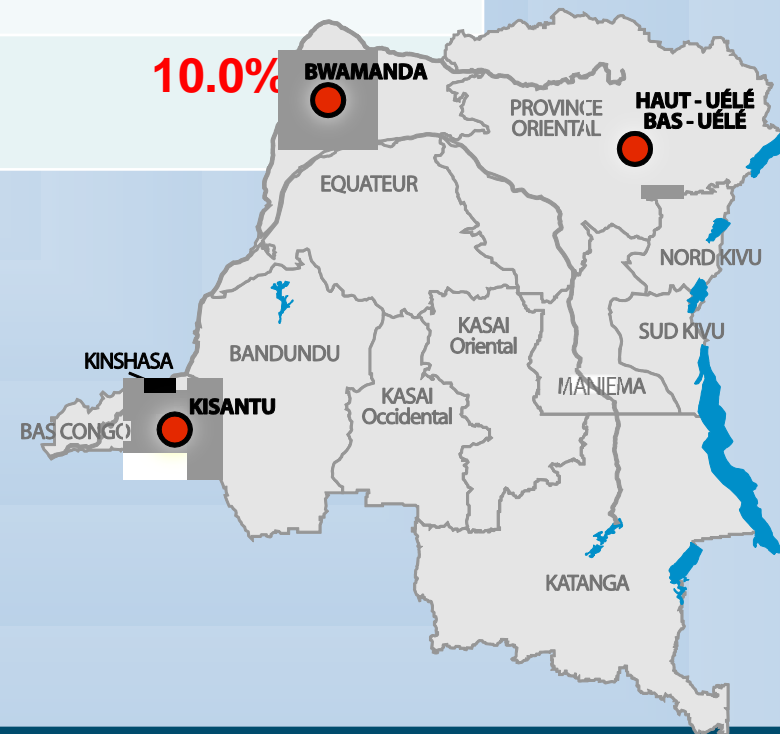
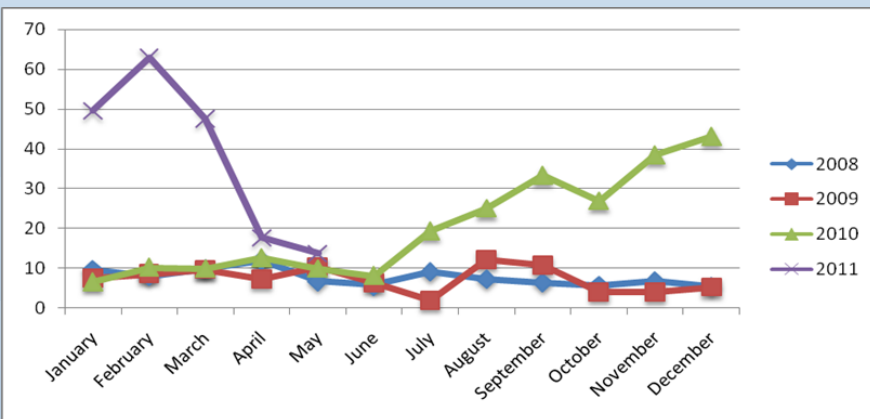
Figure 14. La carte montre les ZS qui ont connu des cas de flambées de fièvre en 2013 lesquels ont fait l'objet d'une investigation

(réaménagement des routes avec des bassins de rétention d'eau tout le long), l'urbanisation, certaines activités économiques (fabrication artisanale des briques, culture de riz irrigués, aménagement d'étangs piscicoles,...), des mouvements de population pour des raisons économiques ou pour des raisons sécuritaires, etc. Il y a donc nécessité de

La RDC a dû faire face ces 3 dernières années à des flambées épidémiques de fièvres présumées palustres dont les conséquences en termes de létalité ont été désastreuses. Si certaines de ces flambées étaient liées à des ruptures de stock de médicaments antipaludiques, d'autres sont liées à des facteurs de co-morbidité (salmonellose par ex.). De plus, certaines populations de la RDC sont dans des zones à faciès épidémiologique « montagnard » et sont donc exposés également aux flambées épidémiques. A cela s'ajoutent les facteurs anthropiques tels que la déforestation, les grands chantiers

# Outcome during outbreaks of iNTS

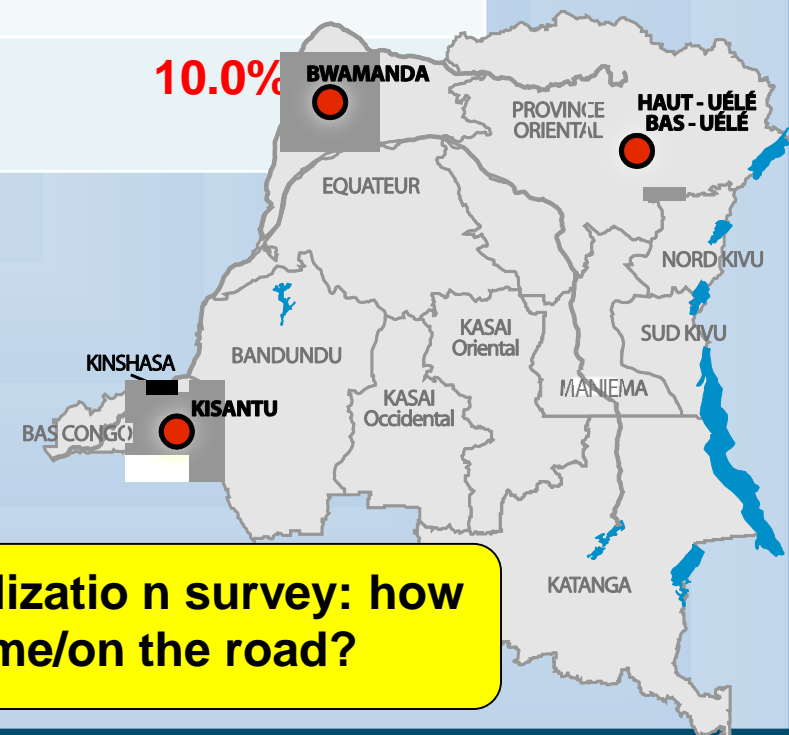
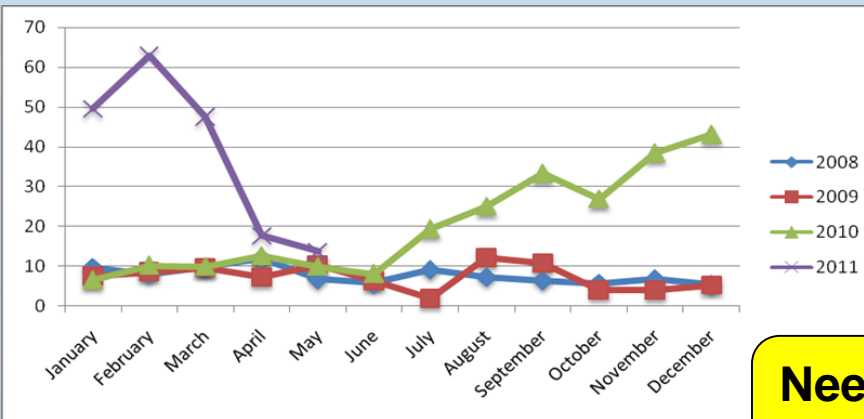
	Onset	Culture-proven
<b>Kisantu</b> Sept 2010 – May 2011	<b>2/3 of admissions</b>	<b>23.2%</b>
<b>Bwamanda</b> Nov 2011 – May 2012	<b>15.4%</b>	<b>11.1%</b>
<b>Pawa 2012</b>	<b>14.0%</b> <b>70% on day 1</b>	<b>10.0%</b>



# Outcome during outbreaks of iNTS

Early installed treatment seems to improve outcome !?

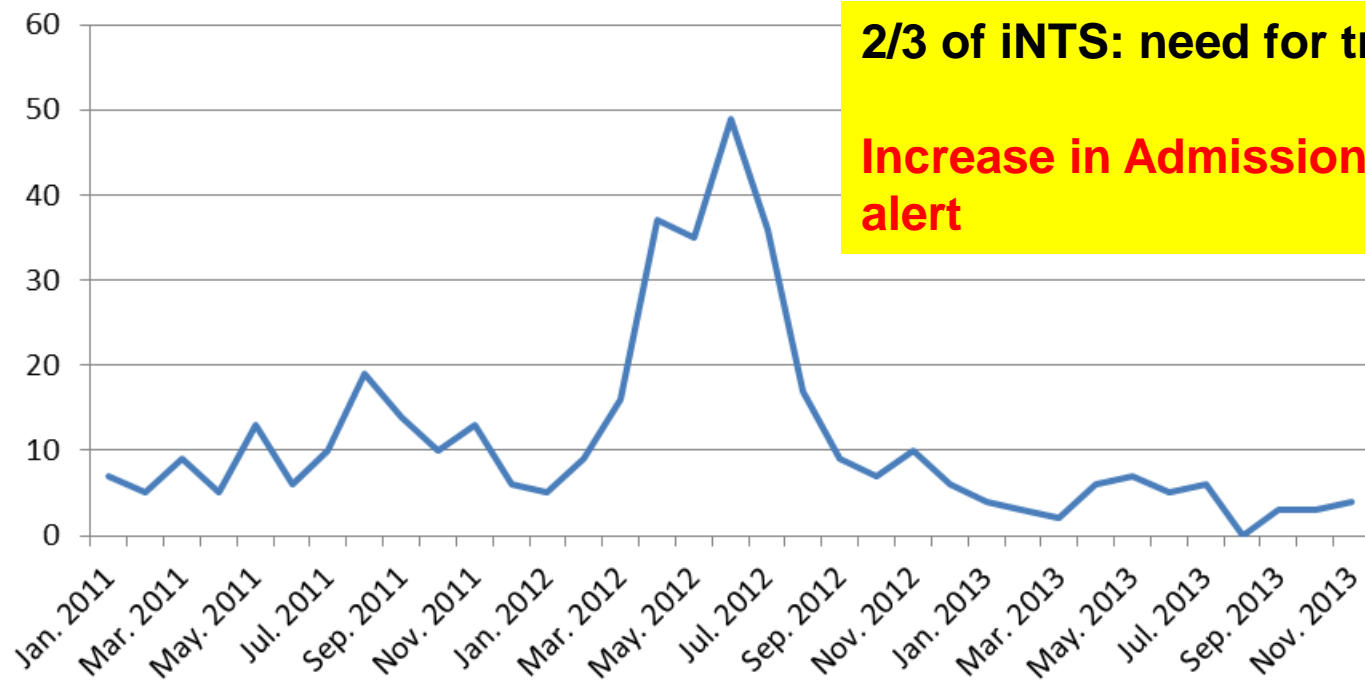
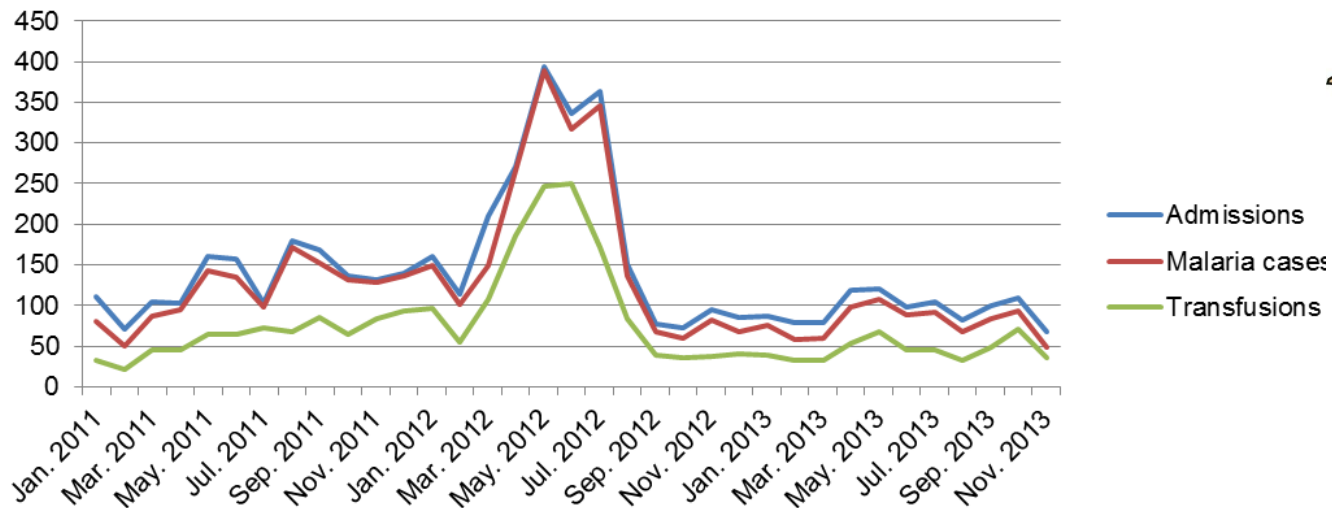
	Onset	Culture
<b>Kisantu</b> Sept 2010 – May 2011	<b>2/3 of admissions</b>	<b>23.2%</b>
<b>Bwamanda</b> Nov 2011 – May 2012	<b>15.4%</b>	<b>11.1%</b>
<b>Pawa 2012</b>	<b>14.0%</b> <b>70% on day 1</b>	<b>10.0%</b>



Need health utilization survey: how many die at home/on the road?



# Outbreak in Pawa, Isiro, Poko & Wamba



**2/3 of iNTS: need for transfusion**

**Increase in Admissions/Transfusion = alert**

— Case fatality

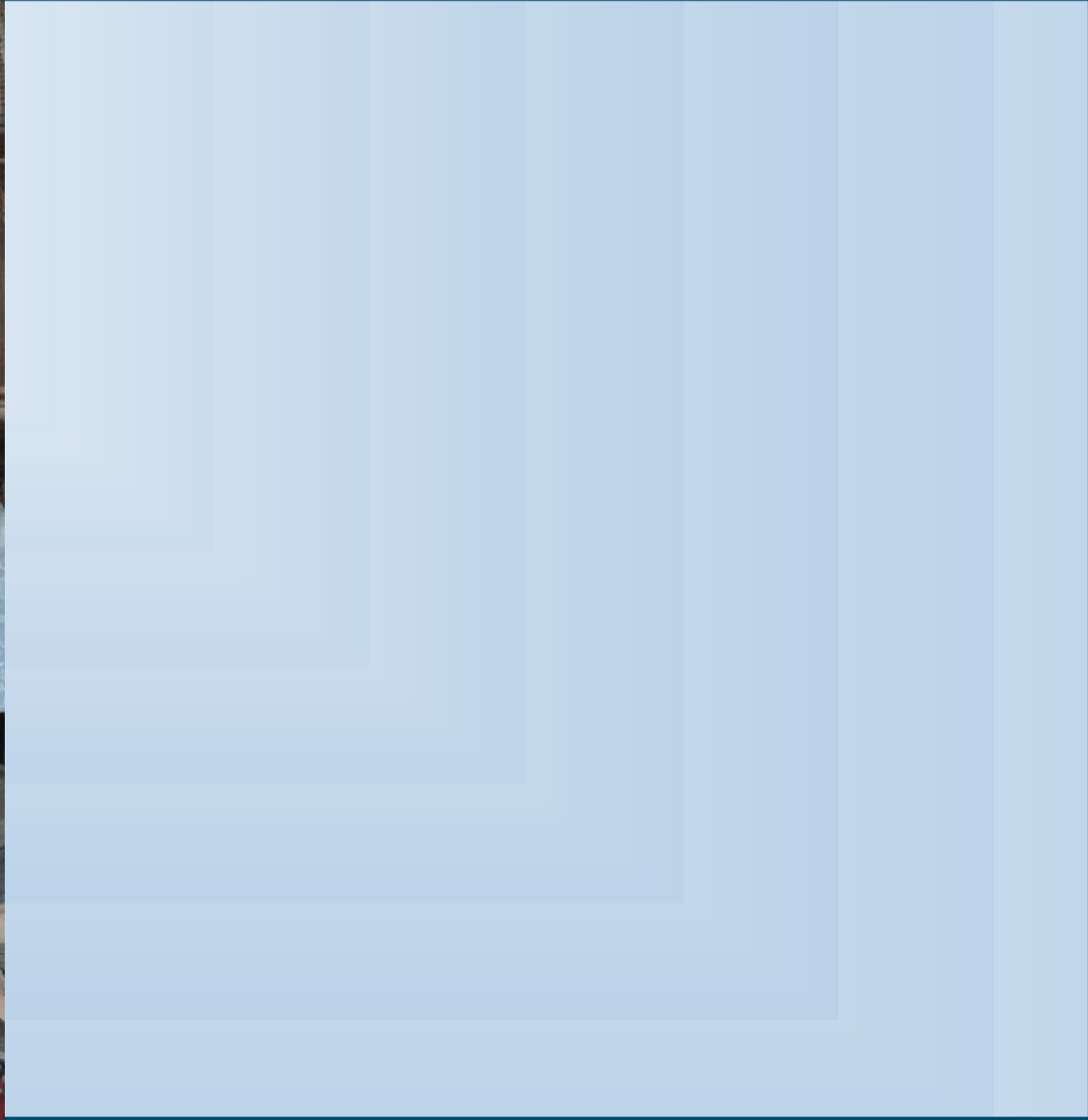
# iNTS Oriental Province, DRC 2009 - 2014

	Typhi n = 13	Non-Typhi n = 75	Typhimurium n = 39	Enteritidis n = 34
Low weight-for-age	0 (0)	14 (20.3)	8 (21.6)	6 (19.4)
Very Low WfA	0 (0)	11 (15.9)	6 (16.2)	5 (16.1)
Malaria	4 (30.8)	52 (69.3)	24 (61.5)	28 (82.4)
Hemoglobin(g/dl) median (range)	9.4 (4.0 – 11.8)	6.0 (3.0 – 12.0)	6.3 (4.0 - 12.0)	5.0 (3.0 – 9.8)
Anemia (Hb < 11 g/dl)	10 (76.9)	73 (98.6)	37 (97.4)	34 (100)
Severe anemia (Hb < 5 g/dl)	1 (7.7)	22 (29.7)	10 (26.3)	12 (35.3)
Blood transfusion	4 (30.8)	49 (65.3)	24 (61.5)	25 (73.5)
Died in hospital	1 (7.7)	10 (13.3)	7 (17.9)	3 (8.8)





# Comparison with previous findings



# Comparison with findings 2007 – 2010 Salmonella Typhi

2007 - 2010 (n = 201)	2011 – 2014 (n = 194)
20.3% of clinically significant organisms	8.8% (more children, higher % CSO)
2.1% of blood cultures	1.7%
MDR 30.3% DCS 15.4.% AZI 1.0%	MDR 38.0% DCS 36.8%, half of which are MDR, AZI 0.6%
Median age 15 yrs, IQR 8 -25 yr, 33% in first and 60% in second decade	Median age 16 yrs, range 0 - 75 yr), nearly two-thirds below 15 years old



# Comparison with findings 2007 - 2010 , non-Typhi Salmonella

2007 - 2012	2011 - 2014
<b>Typhimurium 79% Enteritidis 18%</b>	<b>Typhimurium 47.8% Enteritidis 49.9%</b>
<b>23.0% of clinically significant organisms</b>	<b>38.2% of clinically significant organisms</b>
<b>2.4% of blood cultures</b>	<b>7.9% of blood cultures</b>
<b>MDR 80.7% DCS 4.3% AZI 3.0% ESBL 1.3%</b>	<b>MDR &gt; 80% DCS 2% combined AZI-R and ESBL in 10.7% of Typhimurium</b>
<b>M/F 1.24, Median age 2 yrs, IQR 1 – 11 yr</b>	<b>M/F 1.22 – 1.34, Median age 1 yr, IQR 0 – 75 yr</b>
<b>Rainy season</b>	
	<b>Outbreaks, transfusion needs, particularly with Enteritidis</b>



# On-going & Future projects

**Ethical clearance for study of demographic and clinical data**

**Molecular typing/resistance**

**Salmonella carrier study**

**Salmonella Schistosoma association study**



**Diagnosis : proteomics**

**Stijn Deborggraeve**

**Sara Saleh**

**Sandra Van Puyvelde**

**Saskia Decuypere**



**metabolomics**

**Public Health: Koen Peeters**

**Burkina Faso guest:**



**Guiraud Issa**



**Clinical Microbiology**

**Laura Kuijpers**

**Annelies Post**

**Barbara Barbé**



# Collaborators, acknowledgements, funders

## INRB, DRC

Lisette Kalonji Mbuyi  
Marie-France Phoba  
Edmonde Bonebe  
Jean-Jacques Muyembe  
**Octavie Lunguya**



## UNIKIS, DRC

Dadi Falay  
Dauly Ngbonda  
Brigitte Mapendo



## ITM Belgium

Annelies Post  
Barbara Barbé  
Marleen Verlinden  
Kim van Bambost

## KU Leuven

Jan Verhaegen  
Hugo Devlieger  
Chris Van Geet



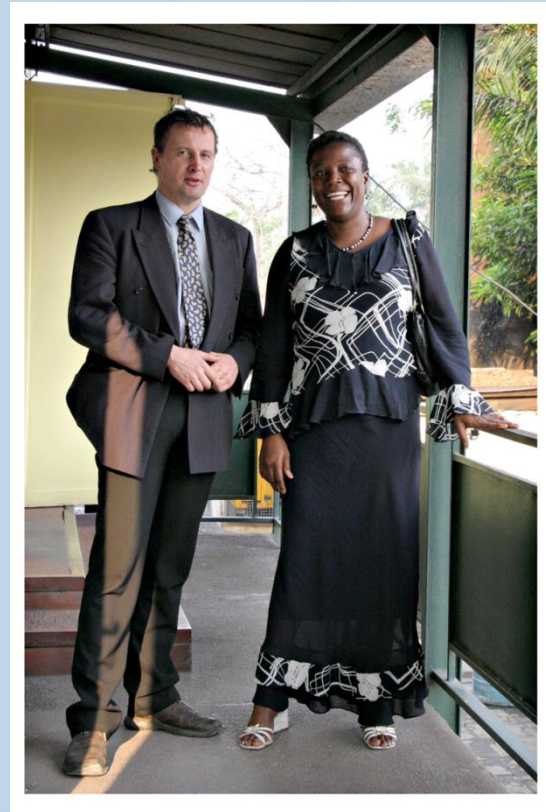
## ISP Belgium

Sophie Bertrand



## Belgian Development Cooperation

THE BELGIAN  
DEVELOPMENT COOPERATION **.be**





# Thank you for your attention!

