Escherichia coli population structure and antibiotic resistance at a buffalo/cattle interface in southern Africa

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Health issue at H/L/W interfaces

- Human activities → pressure on natural ecosystems
- Leading to conflicts for land & resources → H/L/W interfaces
- **The Health issue** is one of these conflicts

- The risk is compromising:
  - Animal production
  - Wildlife Conservation
  - Public Health (from local to global)
- Through
  - Pathogen transmission, spread and emergence
- But also
  - Spread of AB Resistance?
  - But little is known

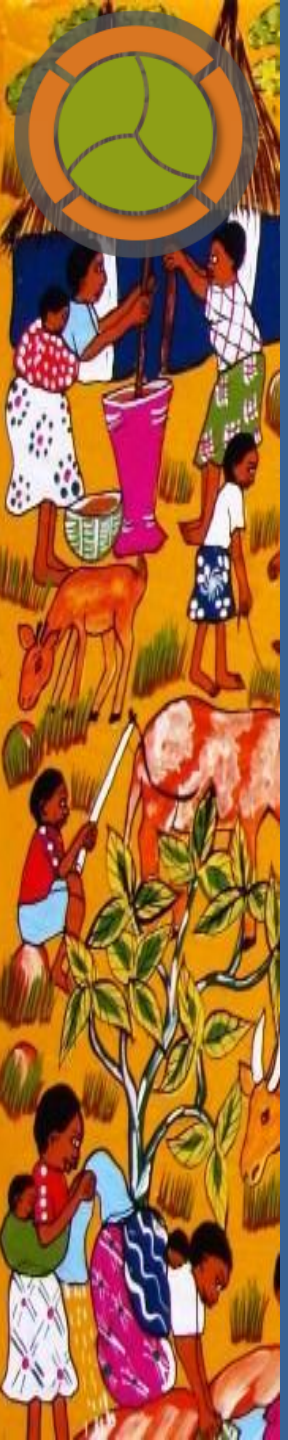
Antibiotic resistance spread and threats (2)

- Prophylaxis treatment in production animal
  - Will increase by 67% between 2010 and 2030 (Van Boeckel et al. 2015)
  - In the US = 80% of antimicrobial consumption (CVM updates)
- Select for antibiotic resistance of bacteria in domestic animal and human
  - That can spread in the wild
  - Anthropological pollution
  - Mutate, Recombined with natural antibiotic resistance

Need to understand patterns and processes of ABR spread

- Back in domestic animals and humans
  - Unknown threat
  - But could compromise the efficacy of AB, our main line of defence against infections.

FAO, OIE, WHO recognize ABR as a major threat



ABR At wildlife/livestock/human interfaces

- Only a few studies (Review: Allen et al. 2010)
- Need for more knowledge

Escherichia coli, a « gut choice »

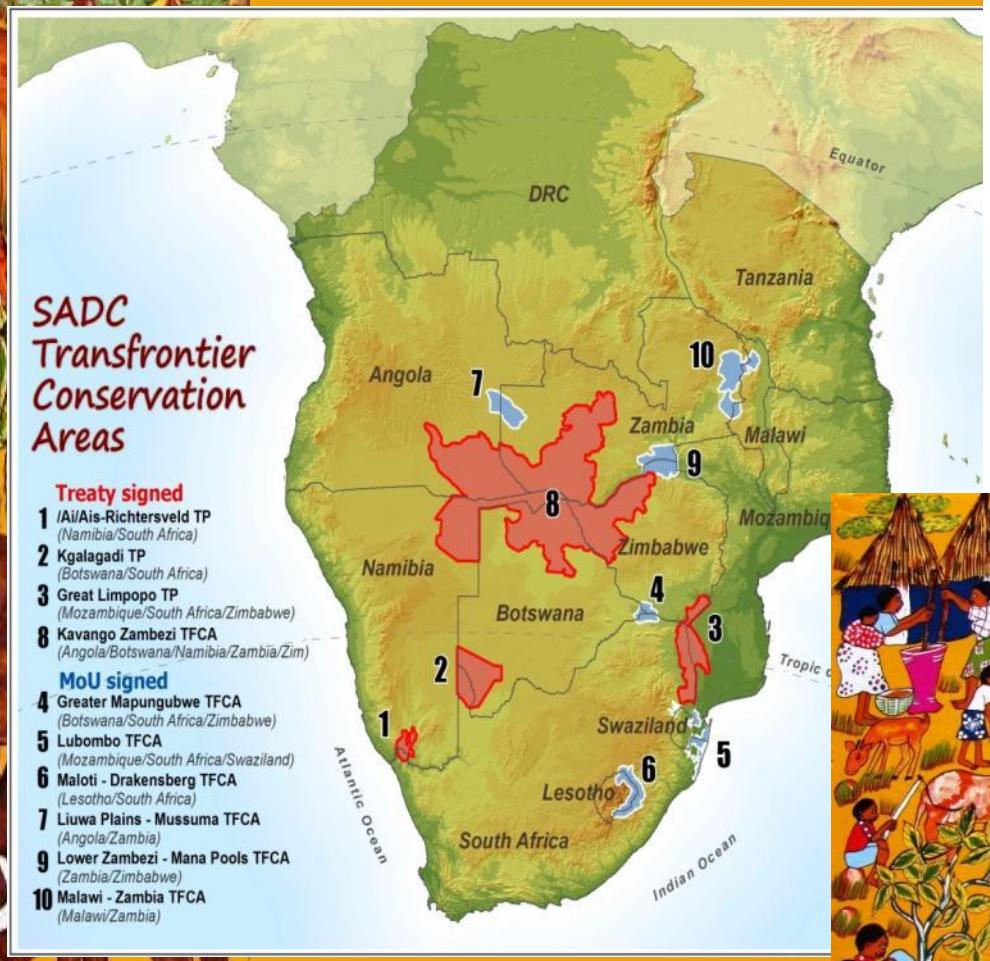
- Ubiquist,
- One of the best known bacteria (genetically)
- Share the same niche as enteric pathogens

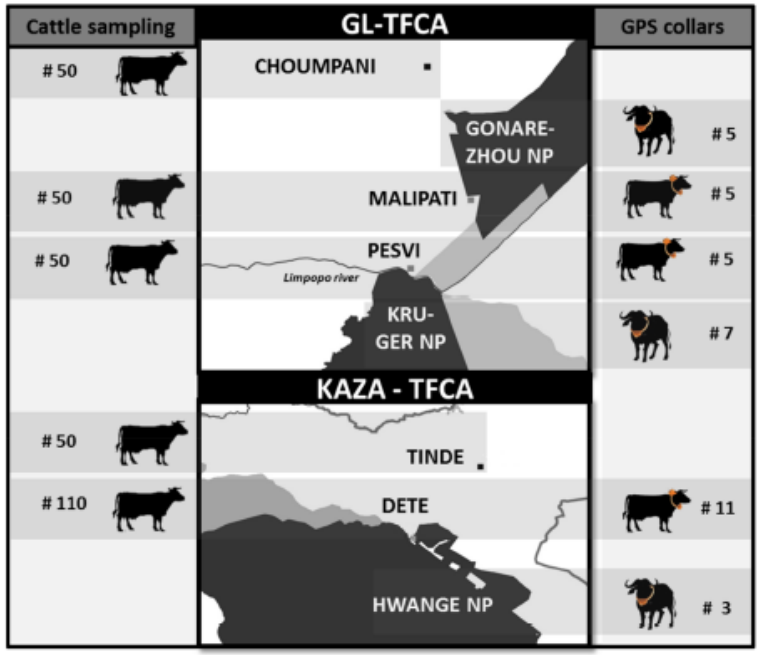
Study on

→ *Escherichia coli* population structure and antibiotic resistance

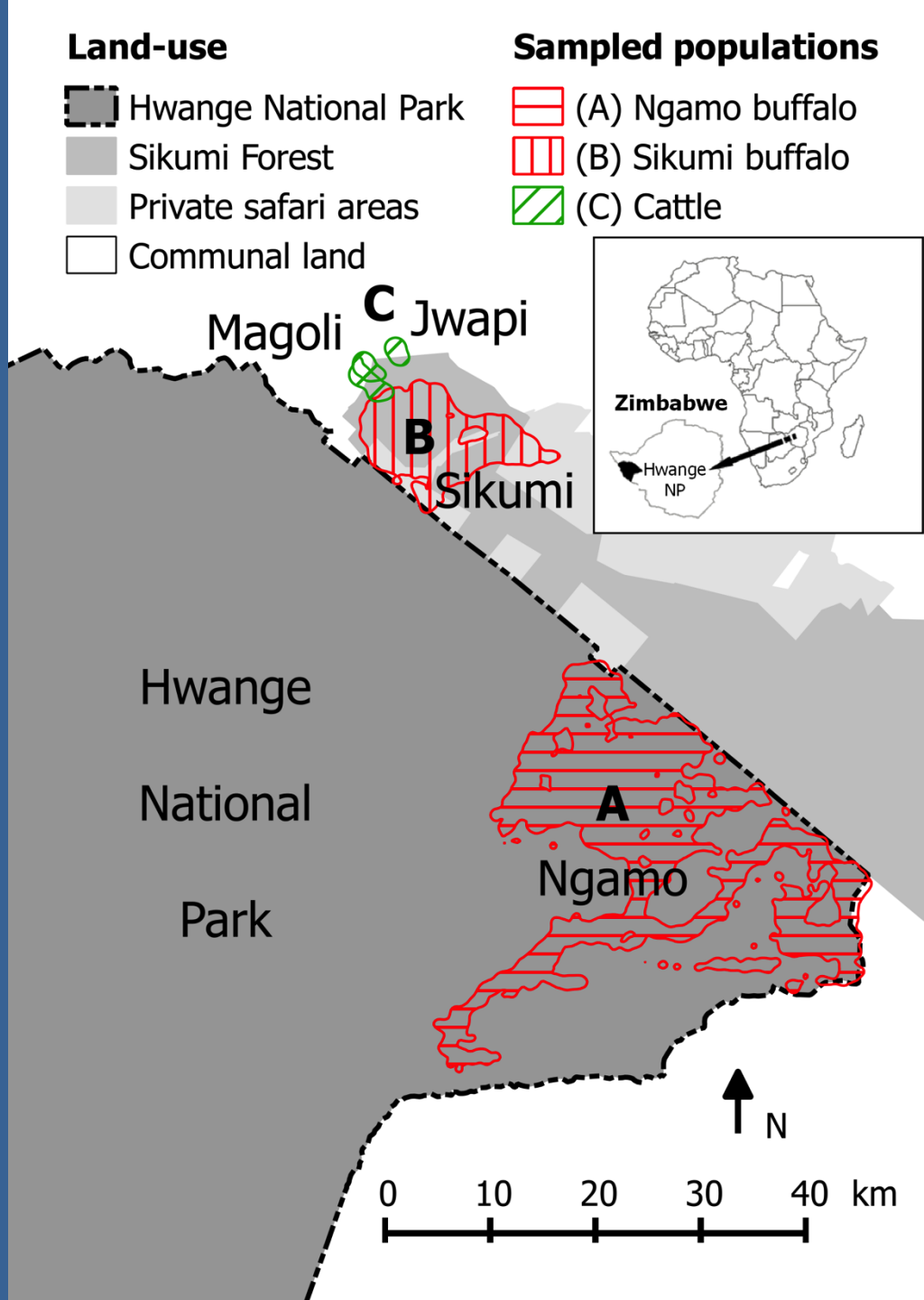
→ at a buffalo/cattle interface in southern Africa

Long-term study in TFCAs



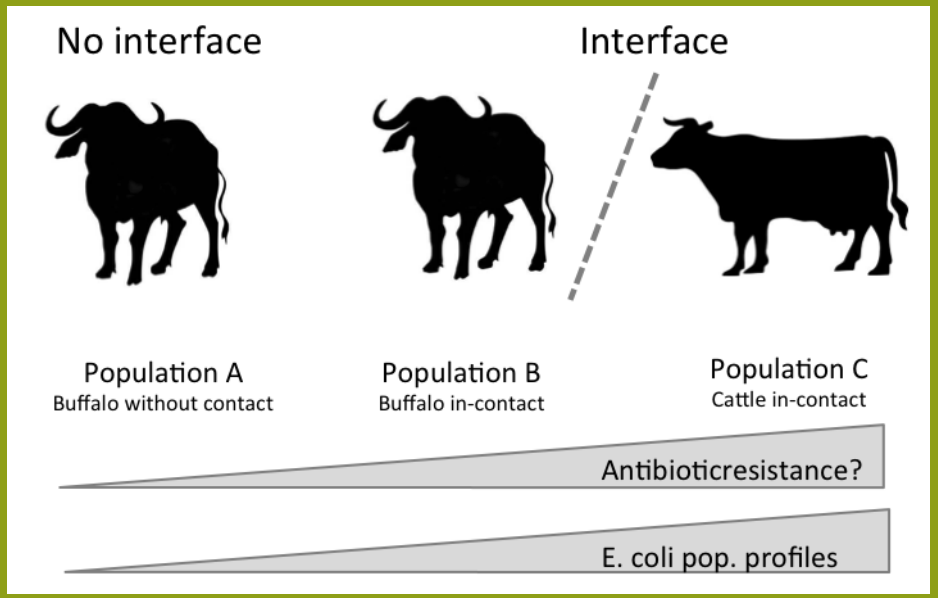


Study site


Land-use
Hwange National Park
Sikumi Forest
Private safari areas
Communal land
Sampled populations
(A) Ngamo buffalo
(B) Sikumi buffalo
(C) Cattle
Magoli
C
Jwapi
B
Sikumi
Zimbabwe
Hwange NP
Hwange
National
Park
A
Ngamo
N
0
10
20
30
40
km

Protocol design & hypotheses

- *E. coli* population profiles
  - Drivers: phylogeny, diet and environmental transmission/contacts



- Diet-Controlled
- Phylogeny?
- Contact?

- Antibiotic resistance
  - Descriptive approach
  - What type of ABR?

Material & Methods

- Snap-shot fresh fecal ground collection
  - in 5 days (October-November 2012) in the 3 populations
  - Following cattle when coming back at kraal
  - Locating 2 known buffalo herds with VHF collars
- Questionnaires to investigate
  - main AB used by human (clinics) & cattle (farmers/DVS) in the area
- Global antibiotic resistance for each sample
  - Against 7 ABs
  - Murray Score (Nb ABR/NB possible ABR)
- Isolation & characterisation of
  - 1 dominant &
  - 1 sub-dominant ABR *E. coli* strain per sample

→ Phylogroups of *E. coli* (Clermont quadruplex method)
→ ABR profile
→ Molecular profile (PCR) of each strain (for phylogeny) and type of ABR

- Sample size

- C - Cattle N = 50
- B - In-contact Buffalo N = 52
- A – No contact Buffalo N=53

- Interviews:
  - AB for cattle (unrestricted use)
    - Tetracycline> Oxytetracycline >Penicillin>Streptomycin
  - AB for human (mainly against TB)
    - Trimethoprim>Co-trimoxazole, >Amoxicillin>Doxycycline

Global ABR of samples

- In terms of Murray Score:
  - Cattle C >> Buffalo A+B
  - In contact population (B+C) >> No contact population A

Sample level

TABLE 1 Global antibiotic resistance prevalence of fecal *Enterobacteriaceae* for each ungulate population

Antibiotic	No. of resistant samples (%)[a]		
	Host population A ($n = 53$)	Host population B ($n = 52$)	Host population C ($n = 50$)
Streptomycin	2 (3.8)	9 (17.3)	8 (16.0)
Tetracycline	0	4 (7.7)	17 (34.0)
Amoxicillin	20 (37.7)	45 (86.5)	34 (68.0)
Trimethoprim	9 (17.0)	11 (21.2)	23 (46.0)
Sulfonamide	20 (37.7)	20 (38.5)	25 (50.0)
Kanamycin	2 (3.8)	2 (3.8)	5 (10.0)
Chloramphenicol	1 (1.9)	3 (5.8)	7 (14.0)

Global ABR of samples

- Murray Score different: C > B >>*A (*=sign.dif.)
- Gradient C>B>A for trimethoprim, sulfonamide, chloremphenicol)
- Gradient B>C>>A for streptomycin & amoxicillin
- Gradient C>B=A for kanamycin

Sample level

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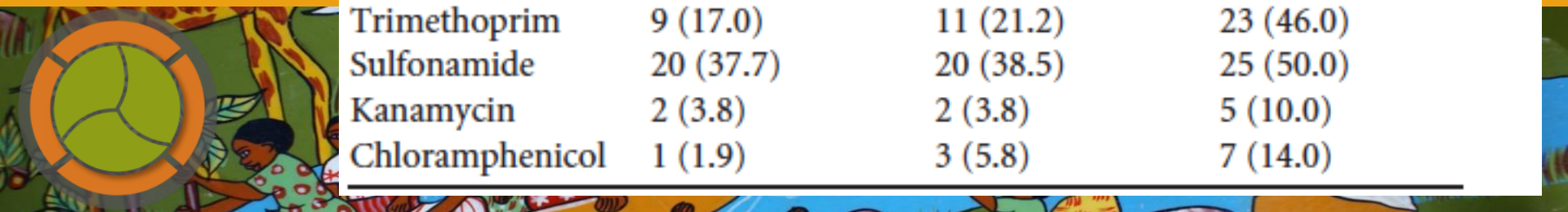
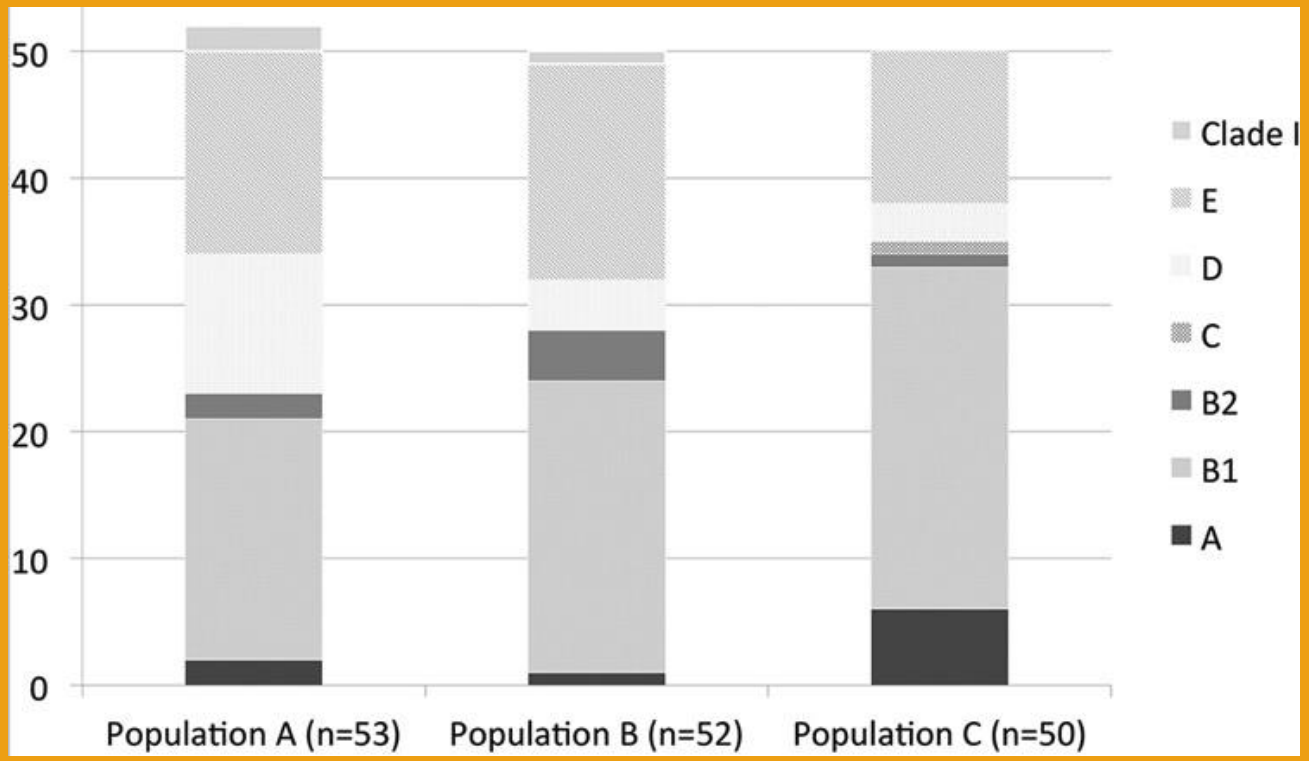


Phylogroup distribution



Dominant strains level

- *E. coli* present in 98% of samples
- **Similar phylogroup profile**: B1 dominates (common for ungulates), then E followed by A & D
- Little ABR (only in 1 B1 in the population buffalo B)

Characterisation of resistance to

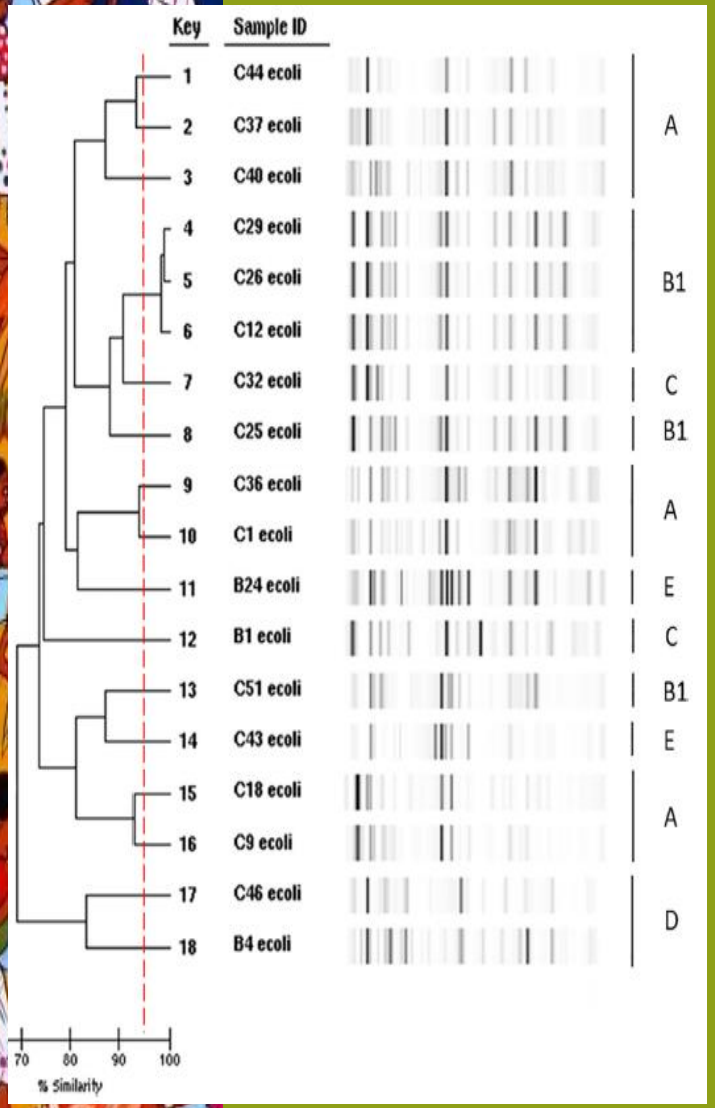
Tetracycline (most used AB in cattle)
Amoxicillin (most used AB in human)
Trimethoprim (most used AB in human)

Subdominant strain level

- No resistance in Buffalo population A (no contact)
- 3 resistant strains in Buffalo population B (in-contact)
- 23 resistant strains (in 19 samples) in Cattle C
- One of the Buffalo B resistant strain was identical to a Cattle C resistant strain (confirmed by RAPD analysis)

Within resistant subdominant strains

Subdominant strain level



- High diversity of phylogroups
- Main resistant phylogroups A & B1 (39% each) (contrasted with for dominant strain was B1 & E)
- Often multiple resistance (to several AB)
- Multiplex PCR assays (on *tet* & *dfr* and $bla_{\text{tem-1}}$ genes) reveal shared ABR genes between in-contact Buffalo B and Cattle C

Discussion (1)

- Buffalo & Cattle had similar phylogroup profiles (dominant strains) as hypothesised because
  - Closely phylogenetically related, same diet, share water points and pasture
  - Phylogroup “A” (associated to humans) is more prevalent in C >> B > A : transfer from human to cattle (39% of resistant strains in cattle were of phylogroup A)

Discussion (2)

- ABR gradient
  - At the Global sample level (enterobacteriaceae)
    - Murray Score is decreasing C > B > A
  - More subdominant strains with ABR
    - C > B > A (A= 0)
  - Only one dominant strains with ABR
    - Fitness cost of ABR strains in natural environment?
- This gradient was
  - Structured by host phylogeny
  - Structured by pattern of contact

Discussion (3)

- Hypothesise the role of Human/animal interface in the spread of bacteria and its ABR.

- Gradient identified is due to ABR diffusion from (human+cattle) towards buffalo and not by “natural ABR emergence”
  - Because ABR in bufffalo is from AB used in cattle and humans
  - Because ABR in cattle was for AB used in humans
  - Because the resistance genes identified are known to emerge rarely in the wild

More genes (e.g. transposons) than strains that are transmitted between individuals and populations

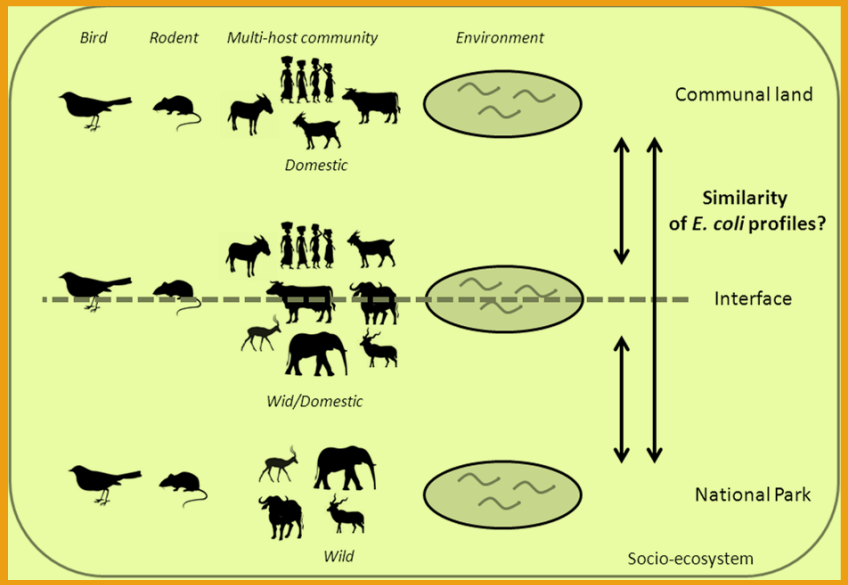
Conclusion (1)

- Confirm in a sub-Saharan savannah ecosystem with a low AB pressure,
  - that human/livestock/wildlife interfaces contribute to an anthropological pollution of protected areas and their wild populations
    - with unknown consequences for all components of the interface

→ Need to understand the patterns of ABR spread in multi-host systems and evolutionary processes in the wild

Conclusion (2)

- Can be also used in complex multi-host systems:
  - to track transmission pathways within multi-hosts systems and help
  - Identifying future routes of pathogen transmission and emergence



→ Tool for disease surveillance and control

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