Genomic surveillance of *Salmonella* spp. circulating in surface waters used in agriculture and aquaculture in Mexico

Enrique J. Delgado Suárez, Francisco Barona Gómez, José Luis Steffani Vallejo, Tavata Meneses, Jacobo Sevillano Serrano

Project collaborators

Faculty of Veterinary Medicine, UNAM

- Enrique J. Delgado Suárez
- Orbelín Soberanis Ramos
- María Salud Rubio Lozano
- Luz del Carmen Sierra
- Tania Tavata Meneses
- Adrian Albano Vega
- BSc. and social service students (7)

Strain Biotech, LANGEBIO-CINVESTAV

- Francisco Barona Gómez
- José Luis Steffani Vallejo
- Jacobo Sevillano Serrano

Sampling design

324 samples from six (6) Mexican States:

- Rivers
- Dams
- Irrigation canals
- Lakes
- Ponds

Convenience sampling:

1. Accessible and close to agricultural, livestock or aquaculture activities
2. Close to the laboratory as to process samples within <24 h

Mexico City

Chinampera region - agriculture

Chinampas: ancient Aztec agricultural production system

https://lagazzettadf.com/noticia/2018/08/15/al-rescate-de-las-chinampas-en-xochimilco/

Hidalgo, Tlaxcala, Morelos, Guanajuato, and Mexico State:

Agriculture / aquaculture / livestock production

Examples of sampling locations

Sampling method (MMS-Sbodio et al. 2013)
doi: http://dx.doi.org/10.1016/j.foodres.2013.01.011

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Measuring water pH and turbidity

Salmonella identification & isolation protocols

Pre-encrichment – BPW

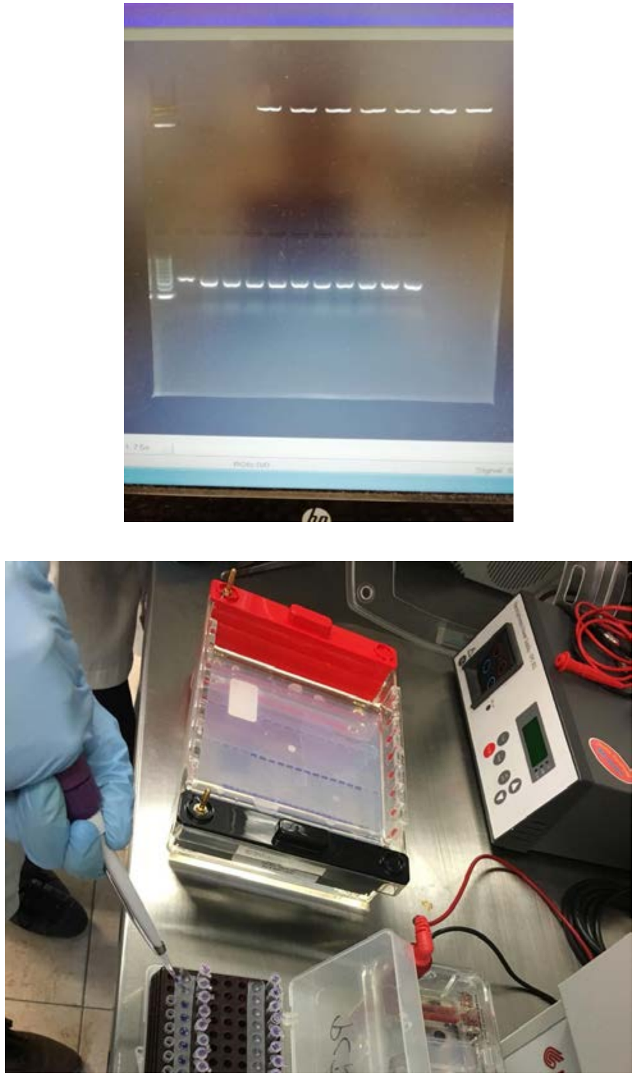
37 ºC / 18-24 h

Raw & enriched samples preserved for future metagenomic analyses

Selective enrichment
18-24 h

- Rappapor-Vasiliadis-Soya broth (RVS) – 42 ºC
- Tetrationate broth (TT) – 43 ºC

Raw & enriched samples preserved for future metagenomic analyses



PCR screening

- (0.5 RVS + 0.5 mL TT)
- Instagene for DNA extraction
- Target: *invA* gene (Rahn et al. 1992), 284 bp fragment

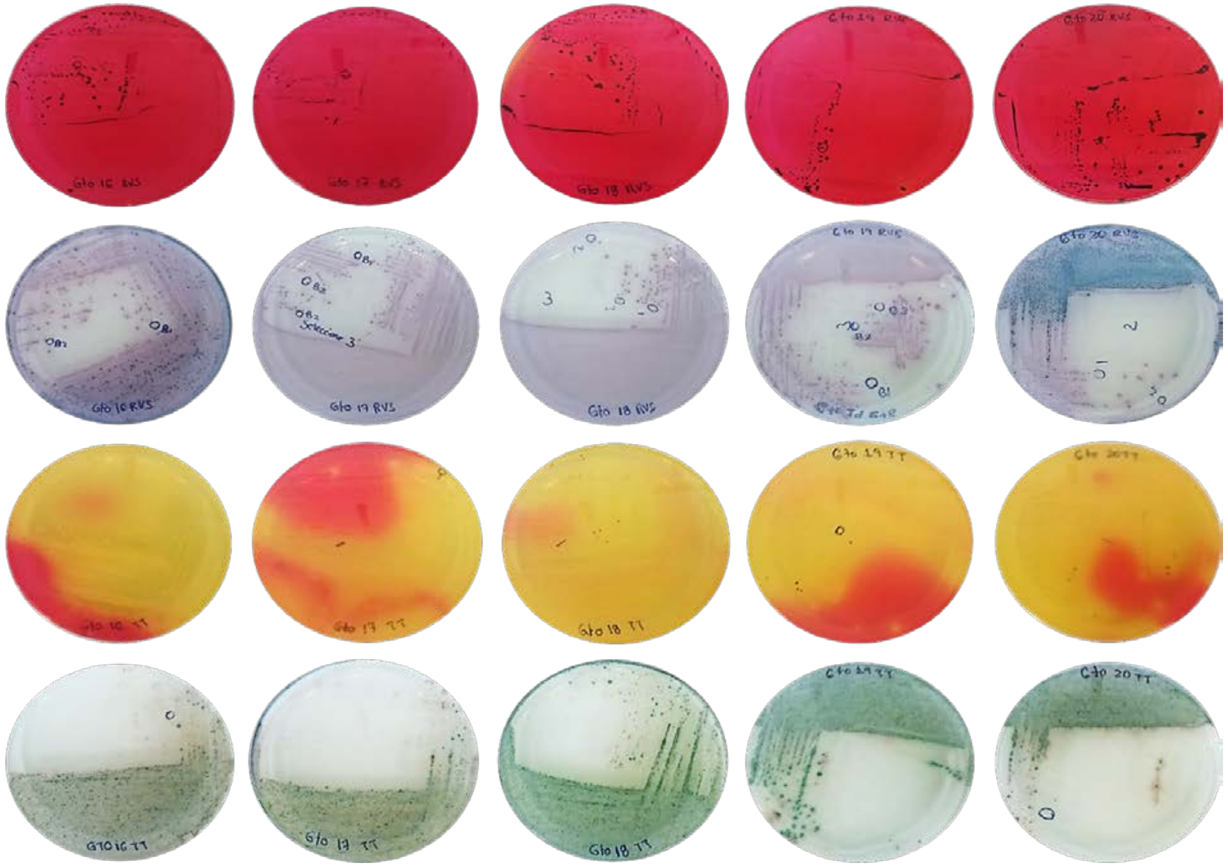
doi https://doi.org/10.1016/0890-8508(92)90002-F)

Positive samples → selective plating

Negative samples → discarded

Selective plating

(37 ºC/18-24 h)



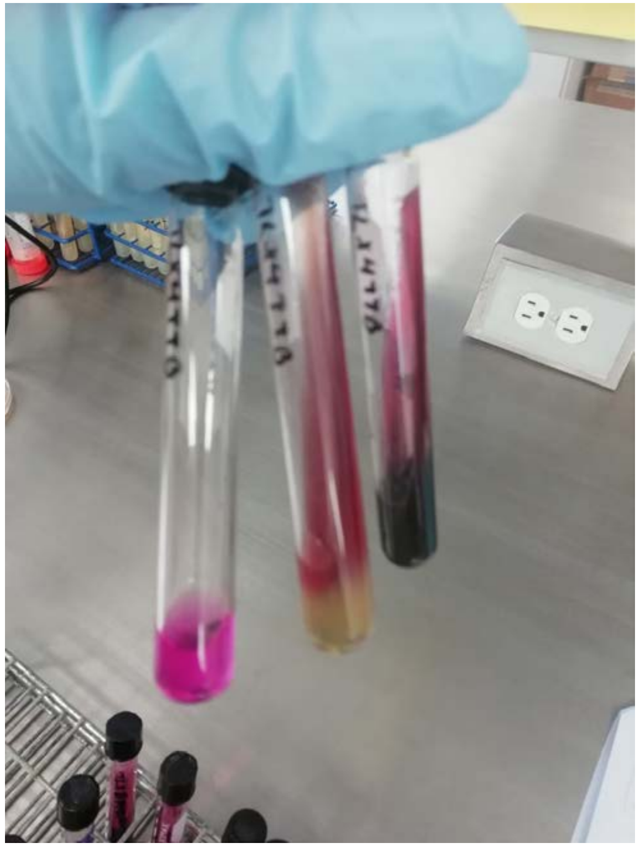
GTO 16 **GTO 17** **GTO 18** **GTO 19** **GTO 20**

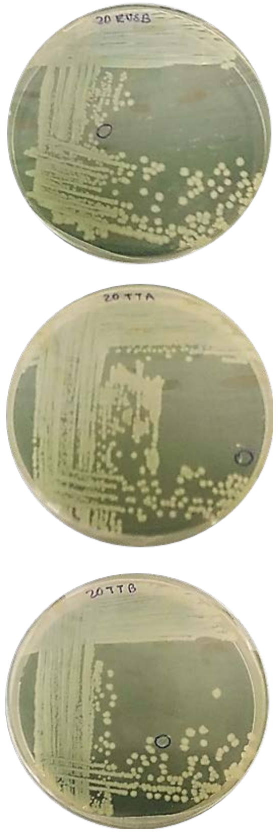
RVS
XLT4

RVS
CHROMagar

TT
XLT4

TT
CHROMagar



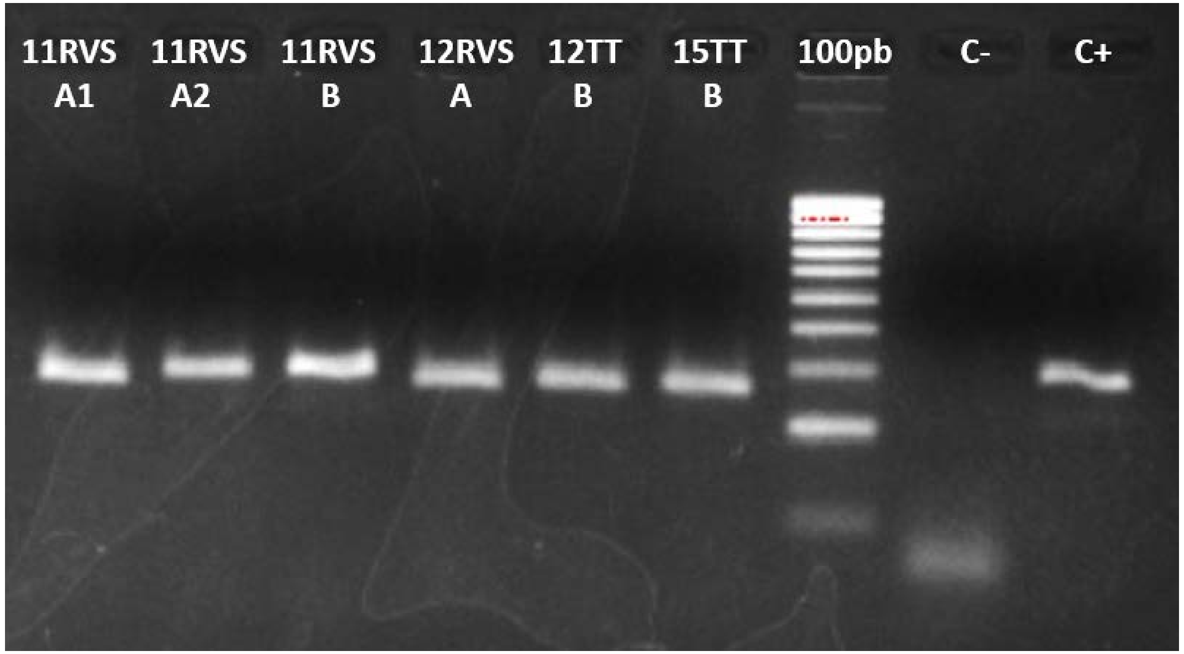


Biochemical confirmation

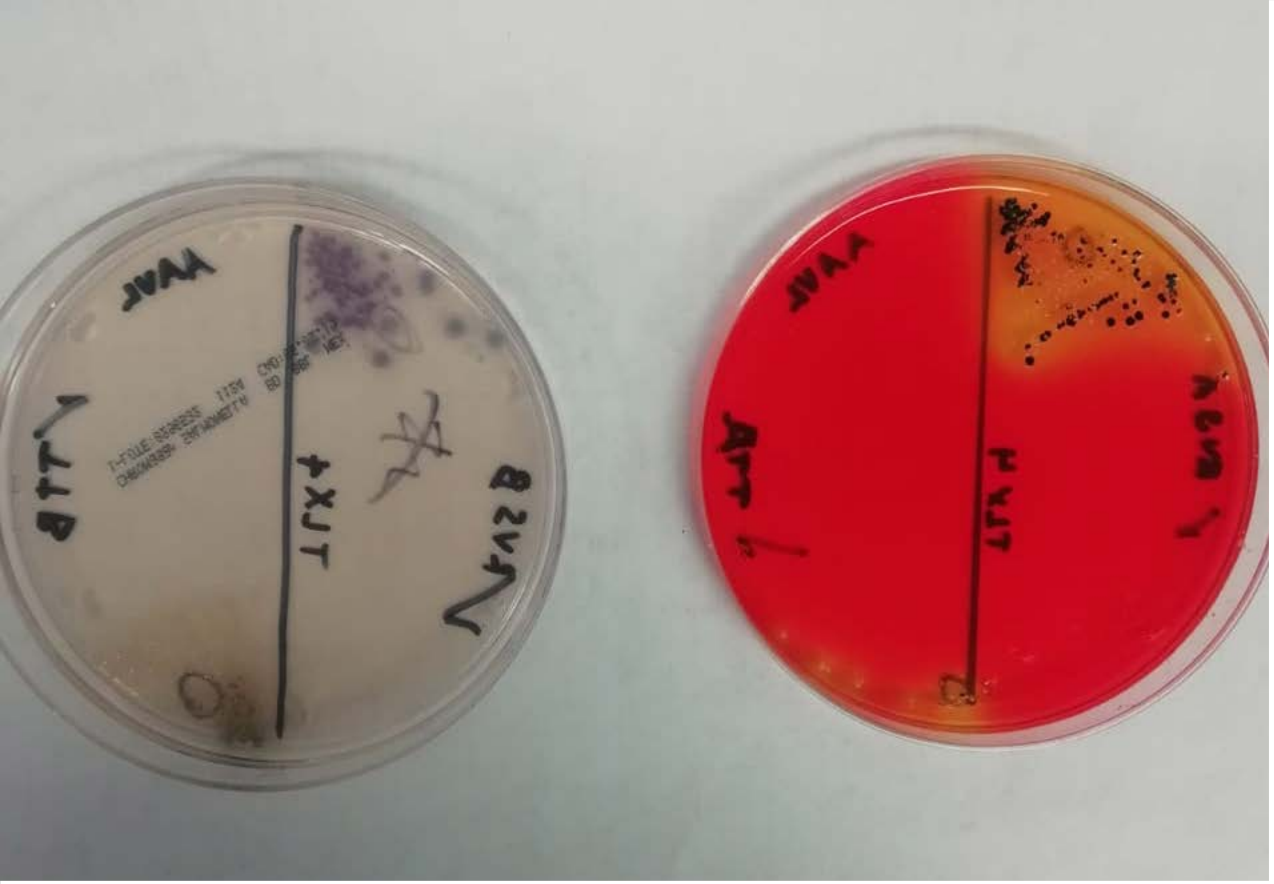
(LIA, TSI, Urea)

PCR confirmation

- Isolated colonies
- Instagene for DNA extraction
- Target: *invA* gene



JIFSAN_190619_GTO11RVSA1a15TT




Preservation of pure isolates

- TSA slants were prepared ≈ 48 h prior to shipping
- 20% glycerol /BHI back ups are preserved at – 80ºC

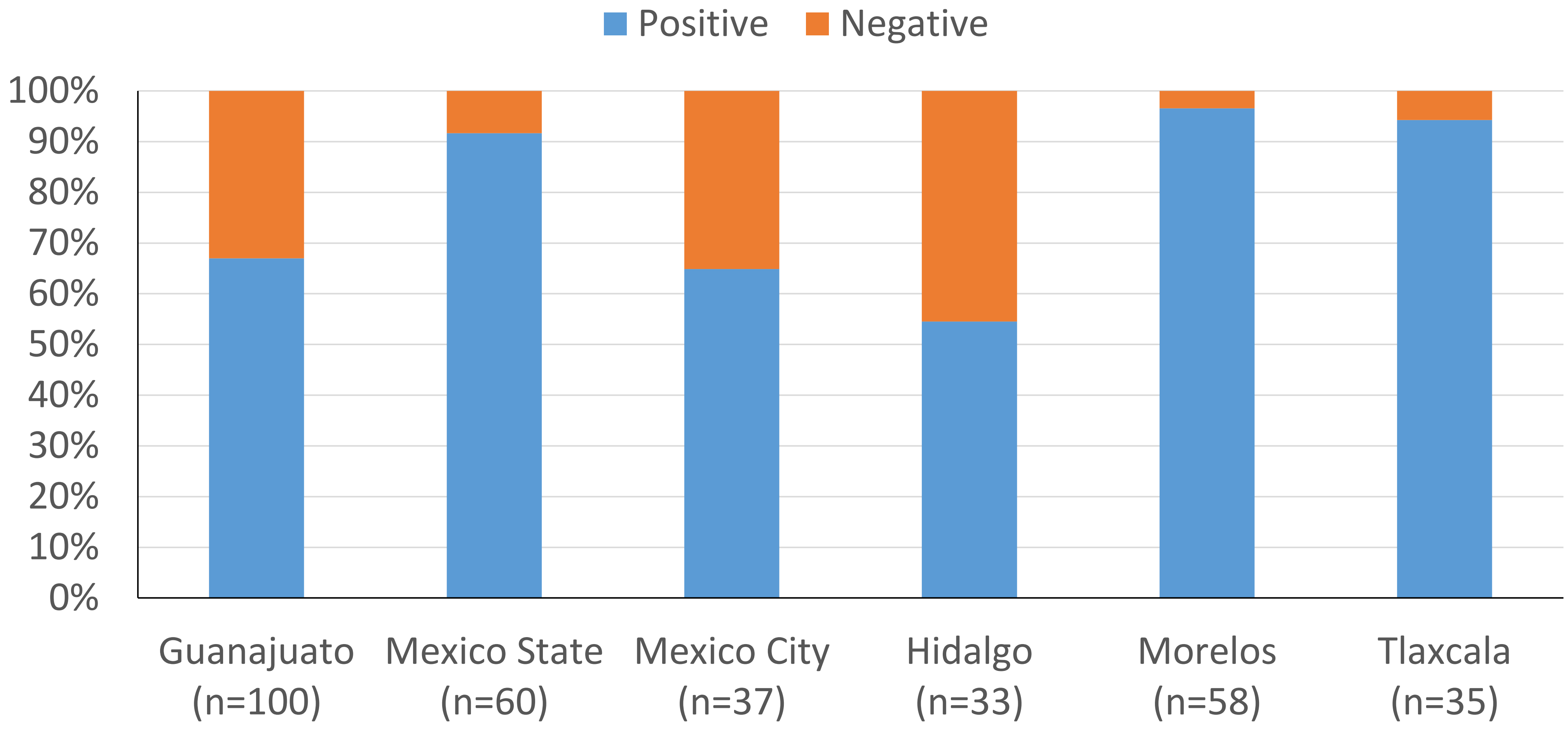
Remarks on methodology

- MMS is excellent for water sampling
- Instagene works great for DNA extraction
- Salmonella CHROMagar performance is poor as compared to XLT4 (higher rate of false positives and is expensive)

Overall results

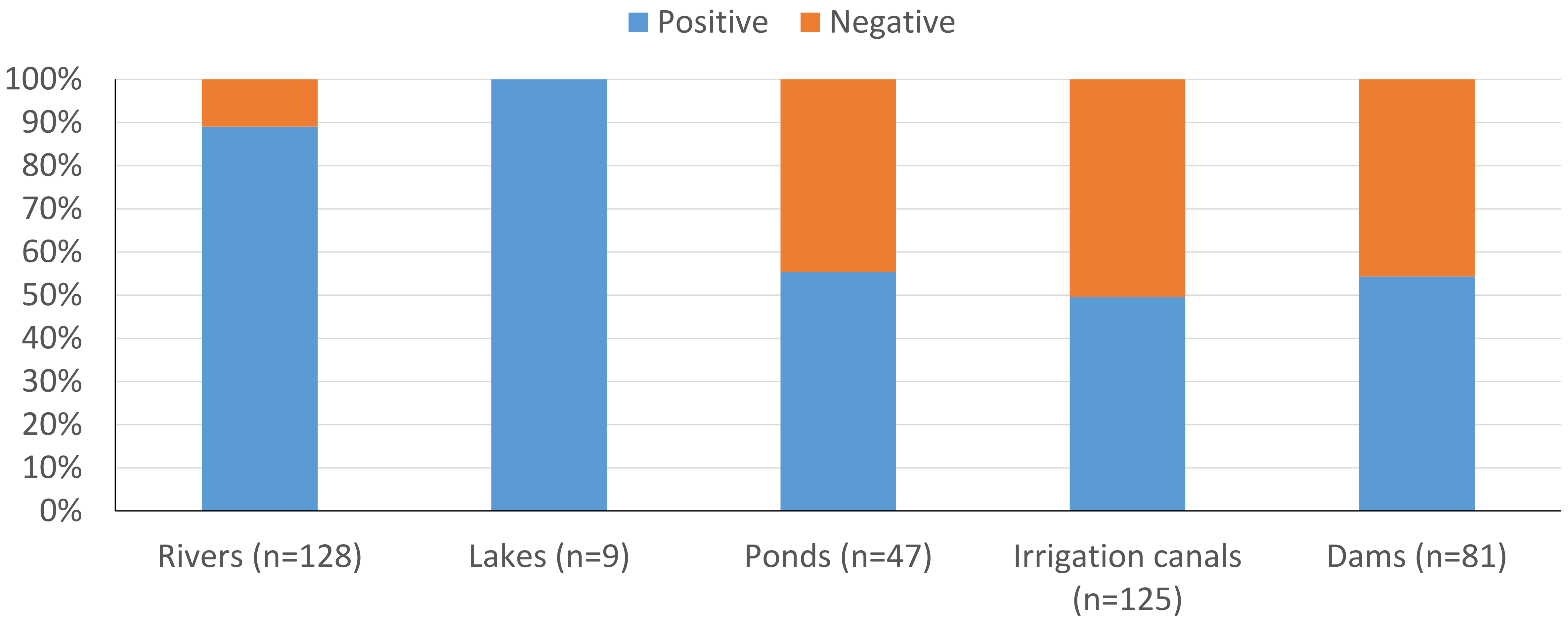
- 78.7 % *Salmonella* positive (255/324)
- 589 isolates in total
- Isolates shipped to CFSAN to date = 211
- Still to be shipped = 378
- Estimated number of future shipments = 6-7

Salmonella prevalence by region



Strong association between region and frequency of Salmonella positiveness:
χ^2=45.4, P<0.0001

Salmonella prevalence by water source



Strong association between water source and frequency of Salmonella positiveness: χ^2=56.7, P<0.0001

Next steps

- Download genomic data from NCBI once available
- Perform bioinformatic analysis
- Write publication(s)
- Perform metagenomic analysis with stored samples (raw, non-selective enriched, and selective enriched)

Thank you for your attention

Enrique J. Delgado Suarez

Faculty of Veterinary Medicine, UNAM

enriquedelgado.suarez@gmail.com

Francisco Barona Gómez

Evolution of Metabolic Diversity Laboratory

LANGEBIO-CINVESTAV

francisco.barona@cinvestav.mx