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Bacteriocin-rich extract from engineered lactic acid bacteria as an antibiotic alternative in ruminants and aquaculture (BAC4RumA)

Project No: 11037

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The AMR Challenge in Africa

- **AMR threatens food security, livelihoods, and public health.**
- **Over-reliance on antibiotics in livestock and aquaculture.**
- **Limited, non-accessible and non-affordable diagnostics, lack of implementation of regulations, poor awareness.**

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Mastitis in Ruminants (Nigeria)

- **30% prevalence across Africa; up to 85% in Nigerian herds (Adamu et al. 2020)**
- **Socioeconomic losses from decreased milk yield, treatment cost and food safety and security.**
- **70% of farms use antibiotics without a prescription (Alhaji et al., 2019)**

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Tilapia farming in Nigeria

- **Nigeria is the largest aquaculture producer in SSA (300,000 tons/yr)**
- **Changing production system has led to overreliance on antibiotics to control outbreaks and reduce economic loss**
- **Antibiotic misuse contaminates water systems and the environment.**

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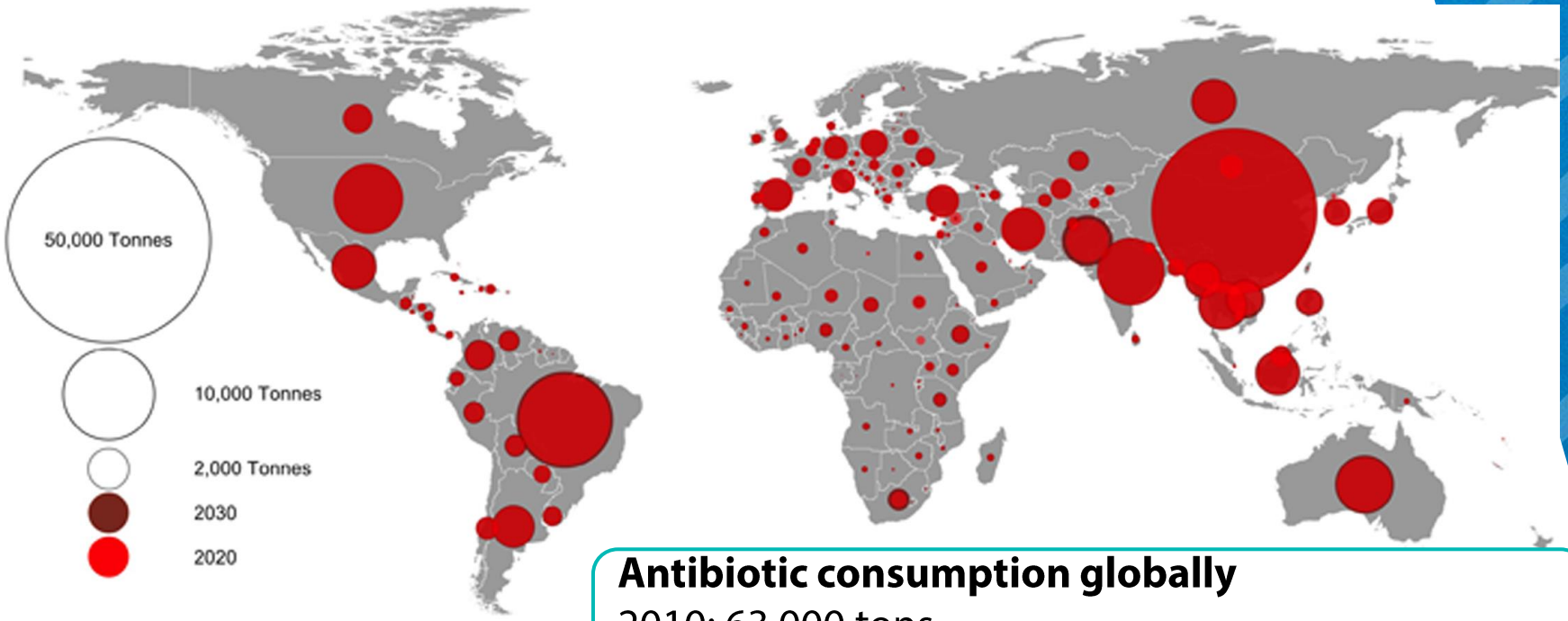
Antibiotic use for livestock and aquaculture in Nigeria



Mulchandani et al., 2023

Antibiotic consumption globally
2010: 63,000 tons
2030: 106,000 tons **Nigeria (163%)**

(Laxminarayan et al., 2015; Van Boeckel et al. 2015

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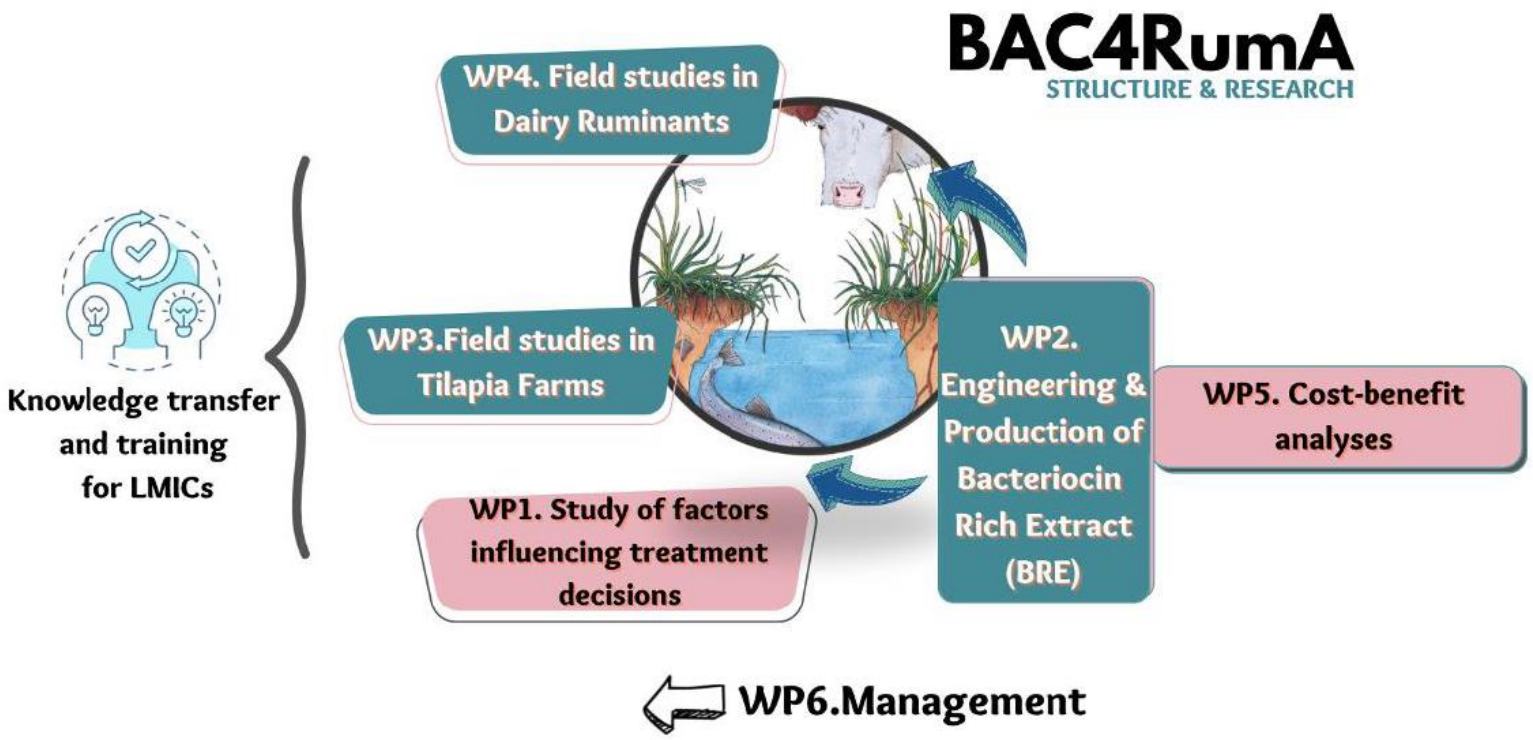
Our project



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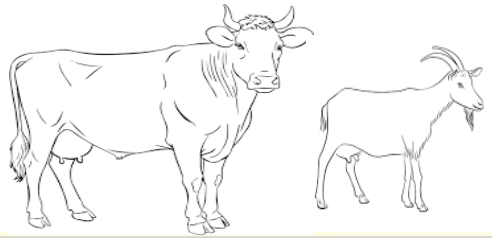
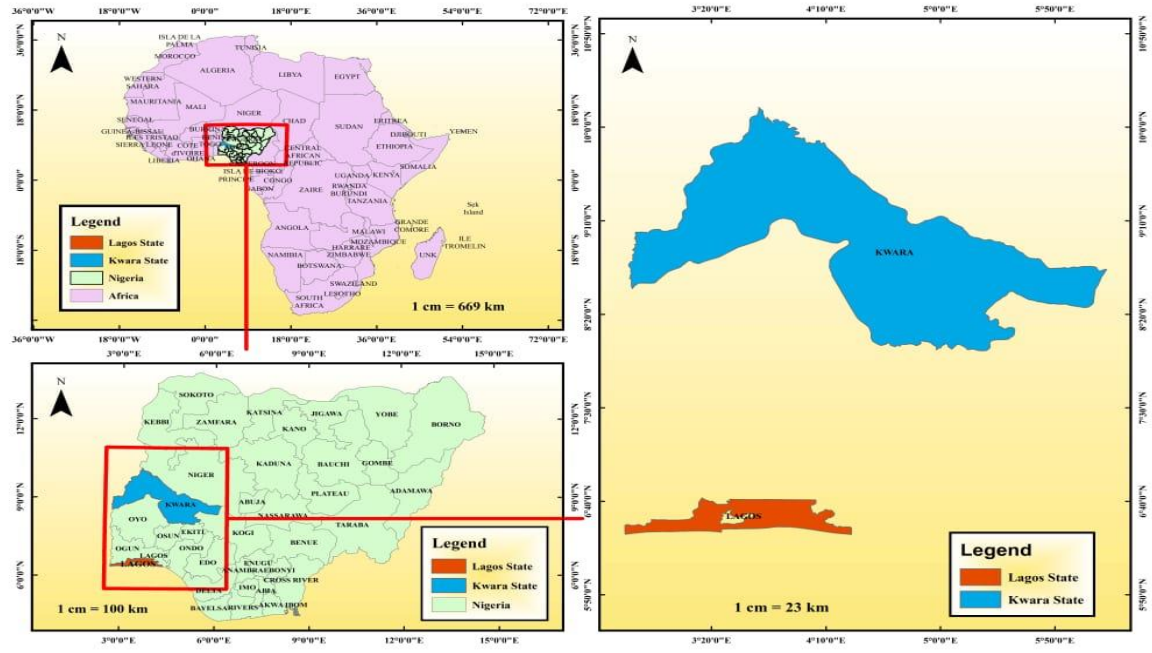
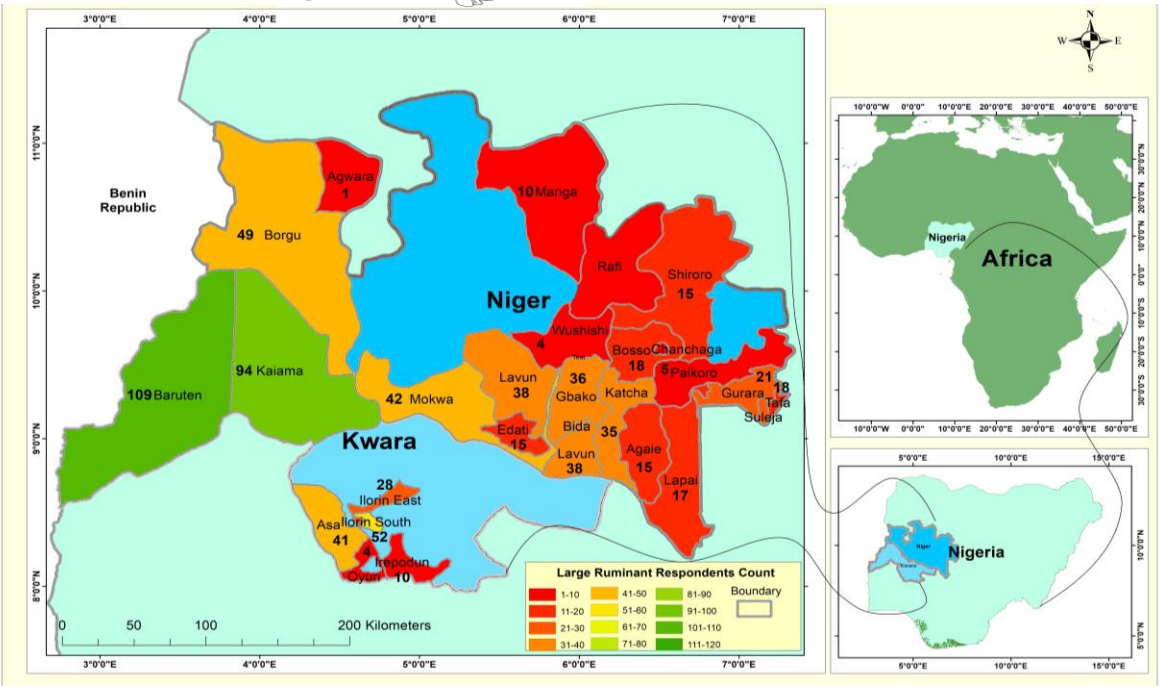
Farm characteristics and treatment decisions among farmers







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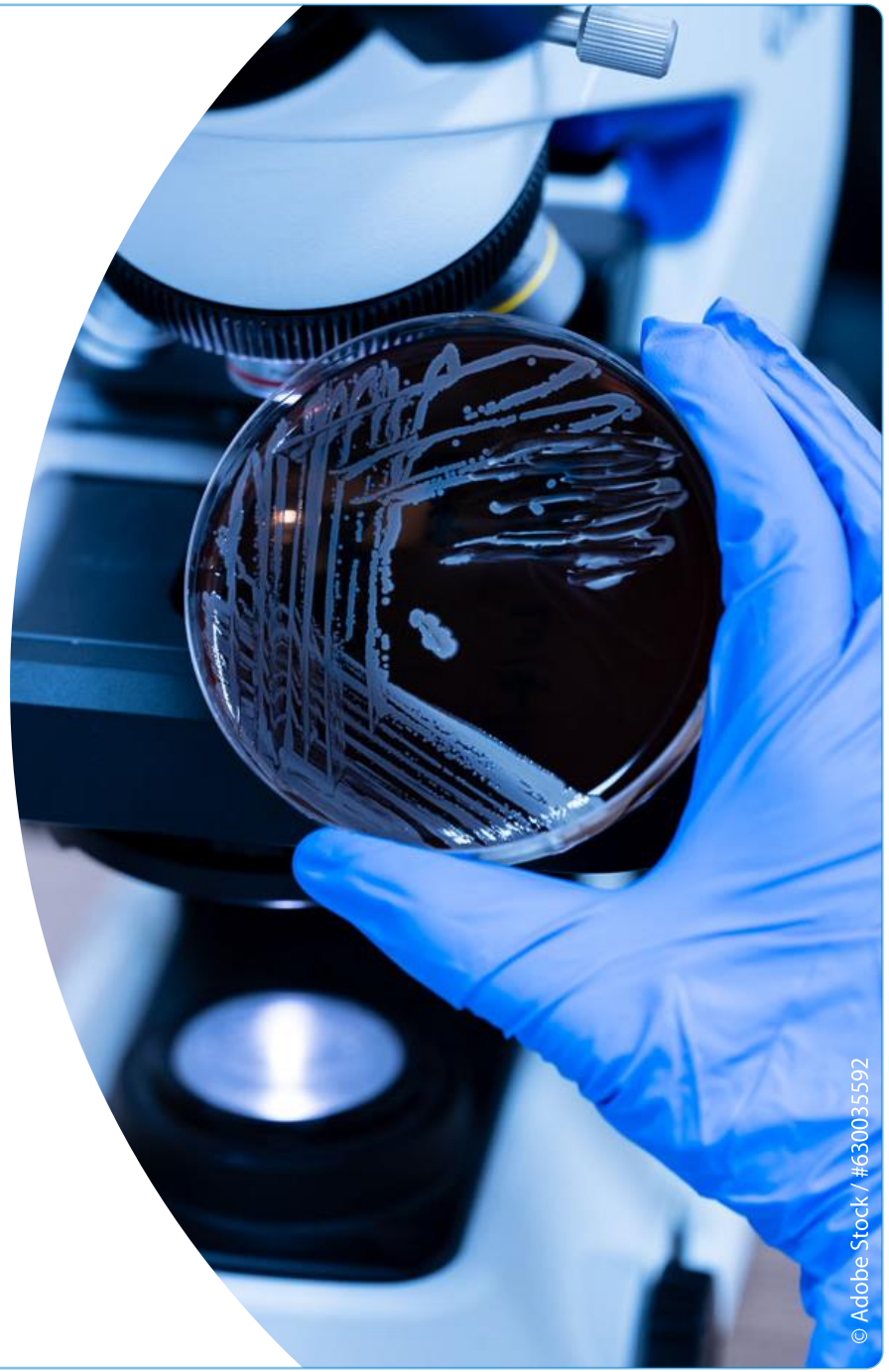
Tilapia farm survey: Key findings

- **More than 30% of farmers reported having a disease problem in the last production cycle.**
- **Disease affects all farmers indiscriminately, regardless of demographics.**
- **Farmers had a wide range of years of experience in aquaculture.**
- **Tilapia farmers still use antibiotics in fish feed.**



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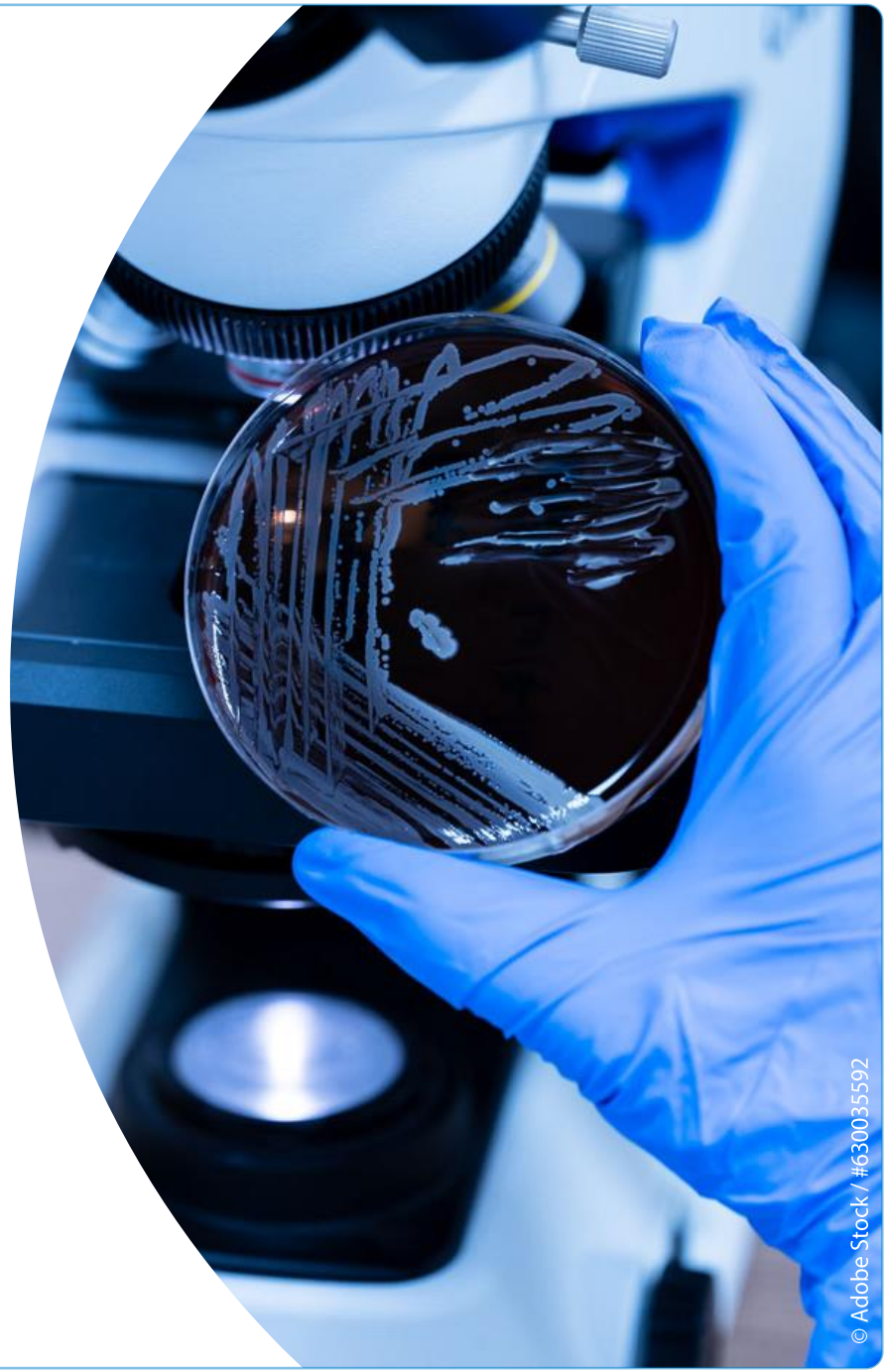
Tilapia farm survey: Key findings

- **There is limited knowledge of AMR amongst tilapia farmers.**
- **Few farmers highlighted the harmful effects of antibiotic misuse, while others listed the antibiotics commonly used in tilapia farming.**
- **More farmers receive training from government organisations than from other sources, except for feed management, where more farmers receive training from feed companies.**
- **Training does not affect mortality rates or disease outbreaks, regardless of who delivers the training.**



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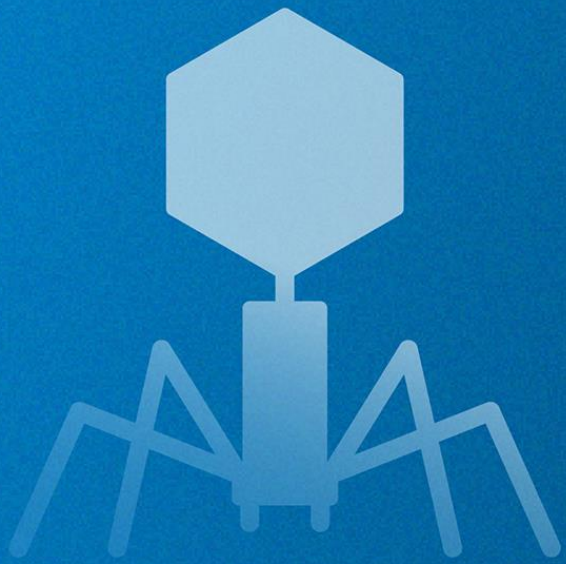
Ruminant farm survey: Key finding



- >30% of farmers reported having mastitis in their cattle in the last 12 month.
- The average number of cases was 2 per farm over the last 12 month.
- >25% of cases were clinical mastitis.
- >46% of farmers started treatment of mastitis without consulting vets.
- >44% of farmers are using ABs without prescription.
- >56% cull animals due to persistent mastitis.

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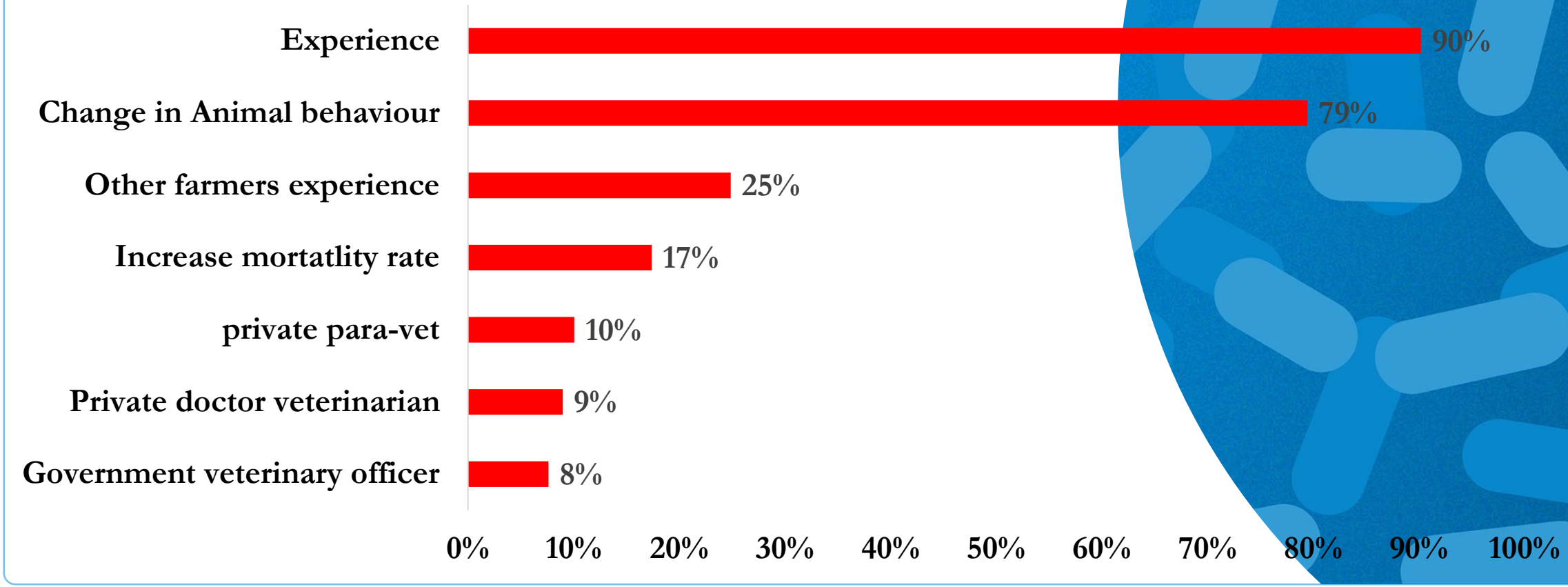
Ruminant survey

How do you know when there is a problem in your farm?



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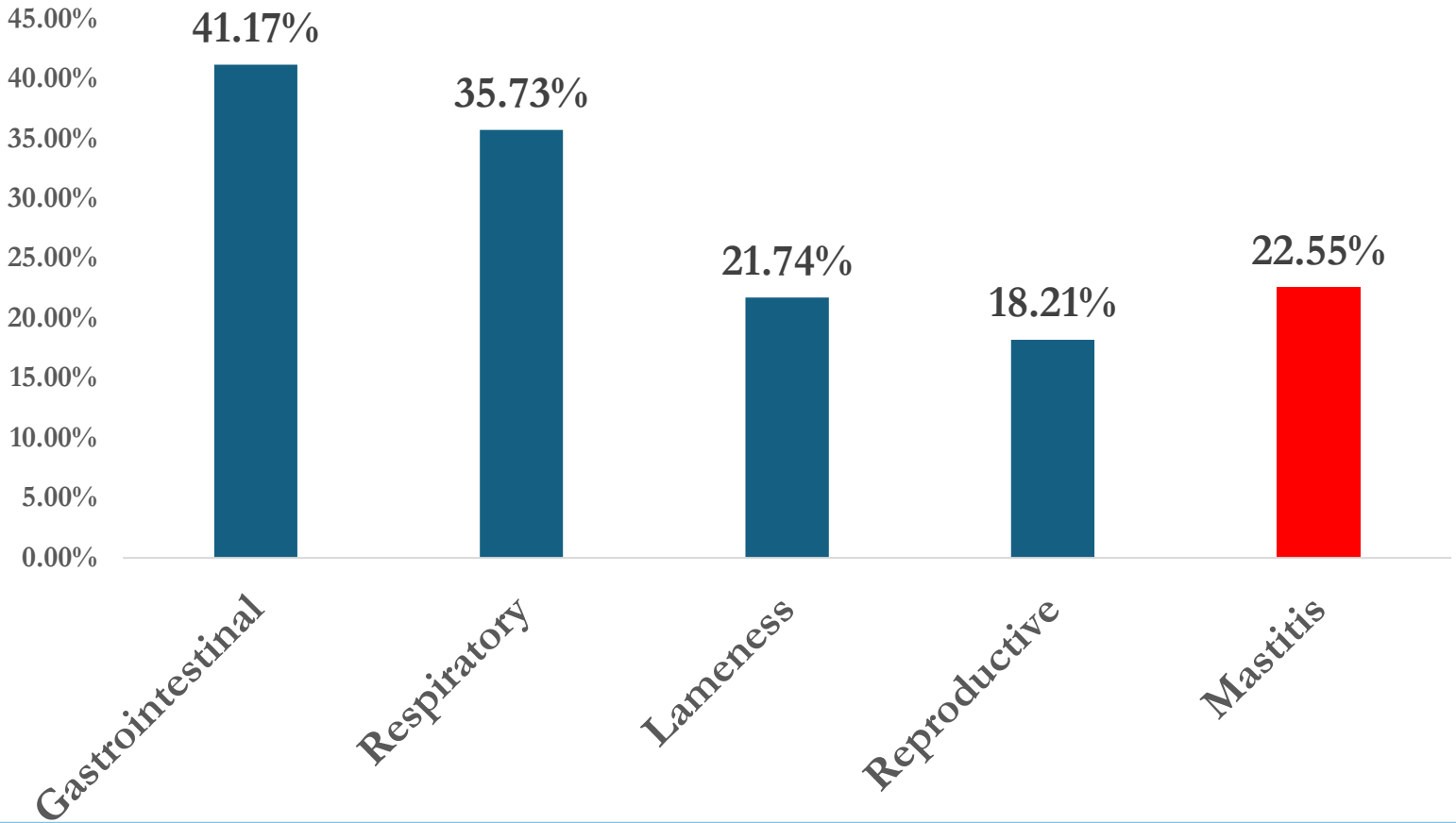
Ruminant survey

What are the main challenges and diseases that affect your herd?



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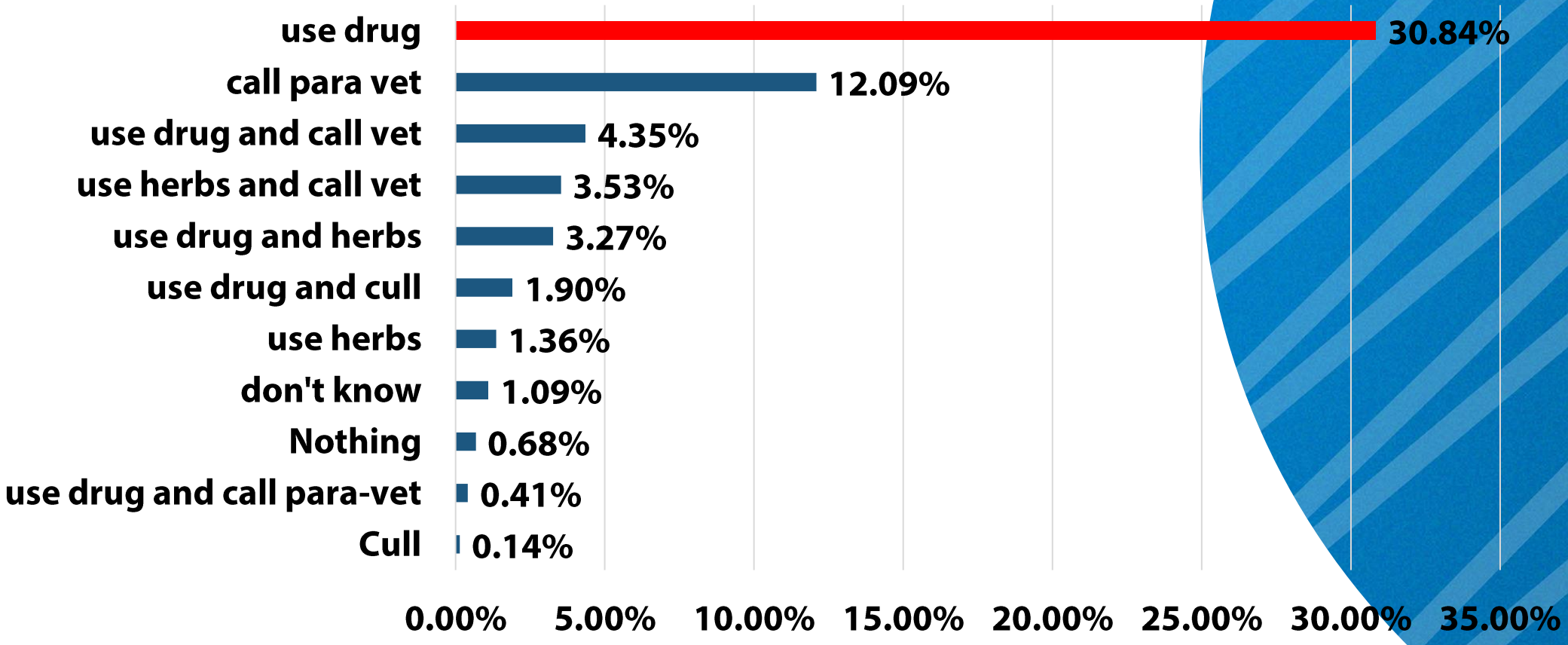
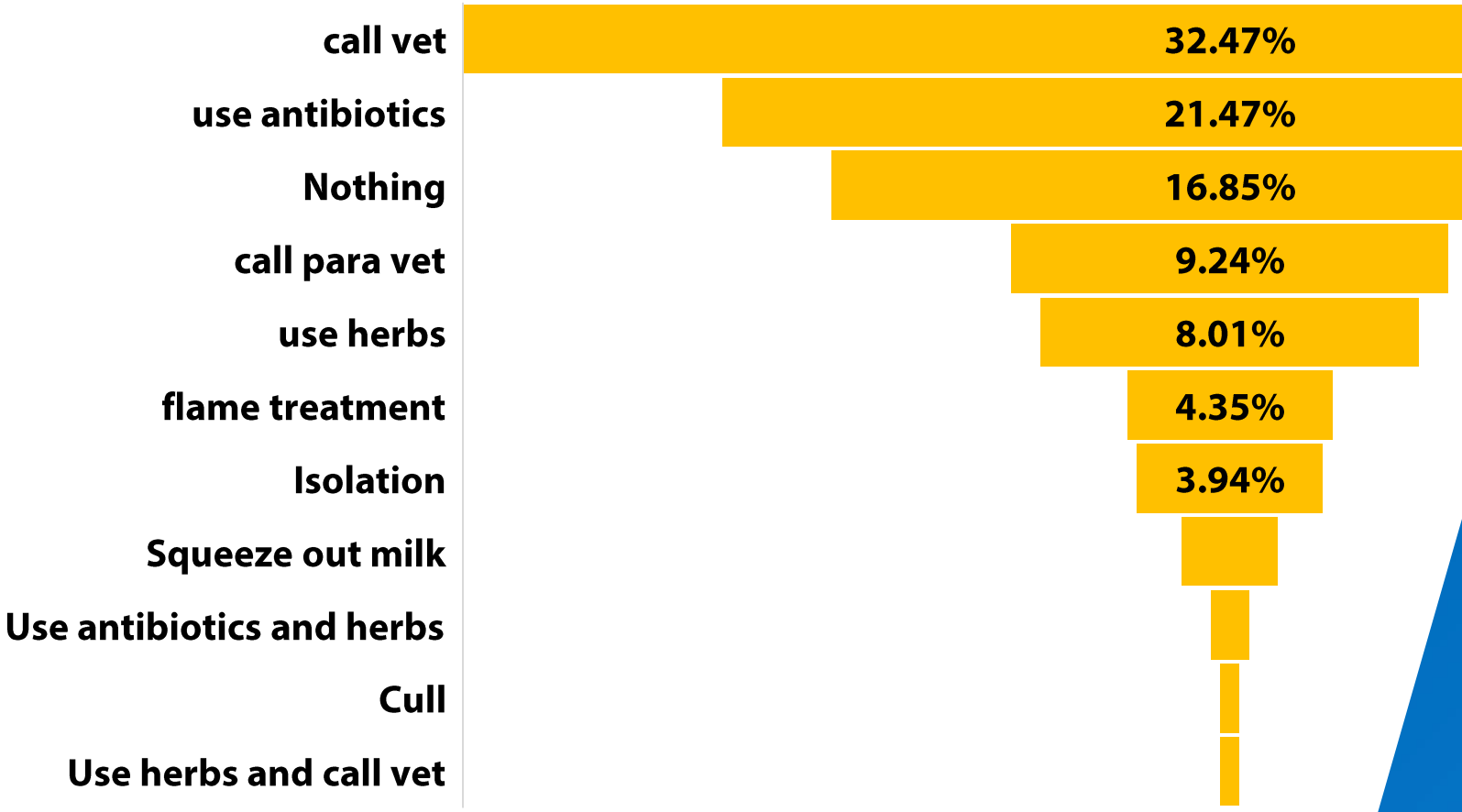
Ruminant survey

What do farmers around you do when they have these diseases?

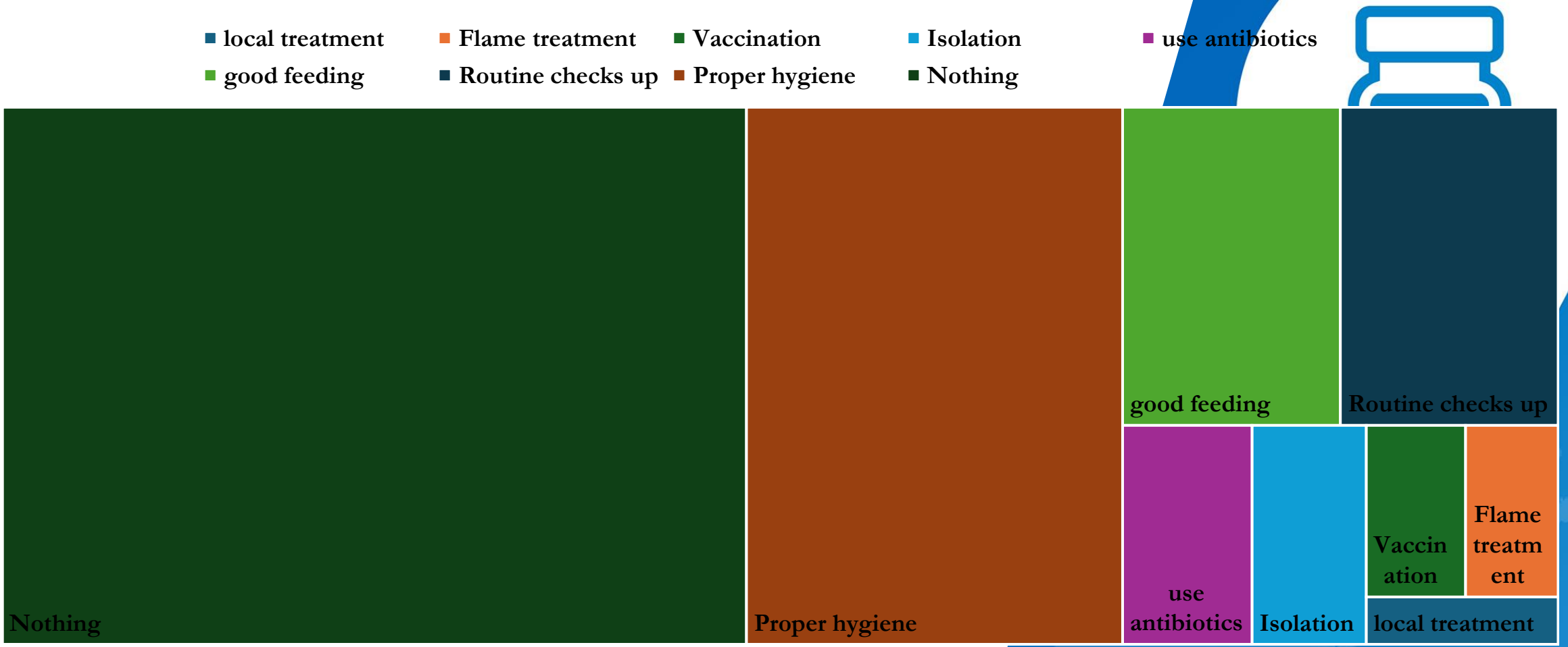


Ruminant survey

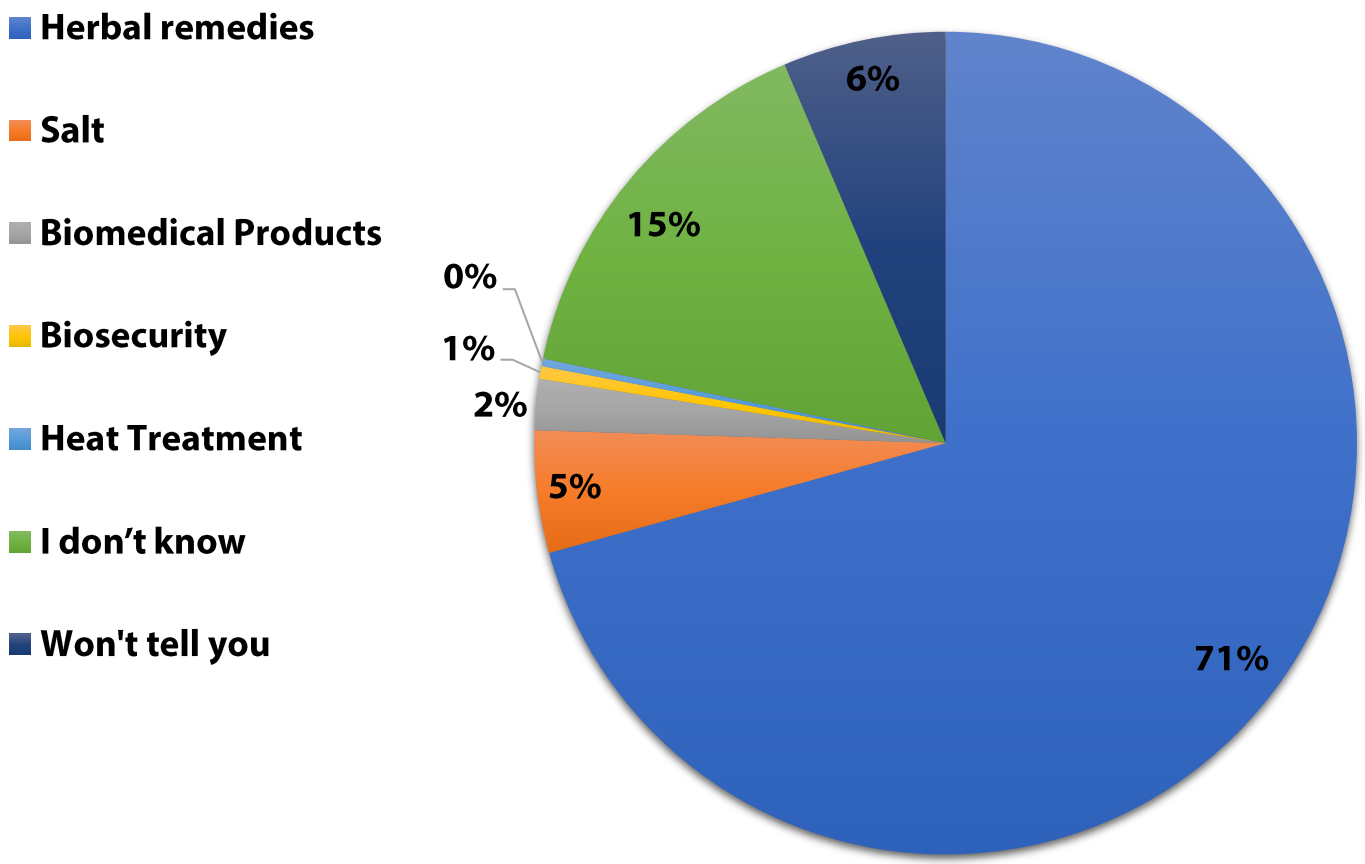
What do you do when you suspect a case of mastitis?

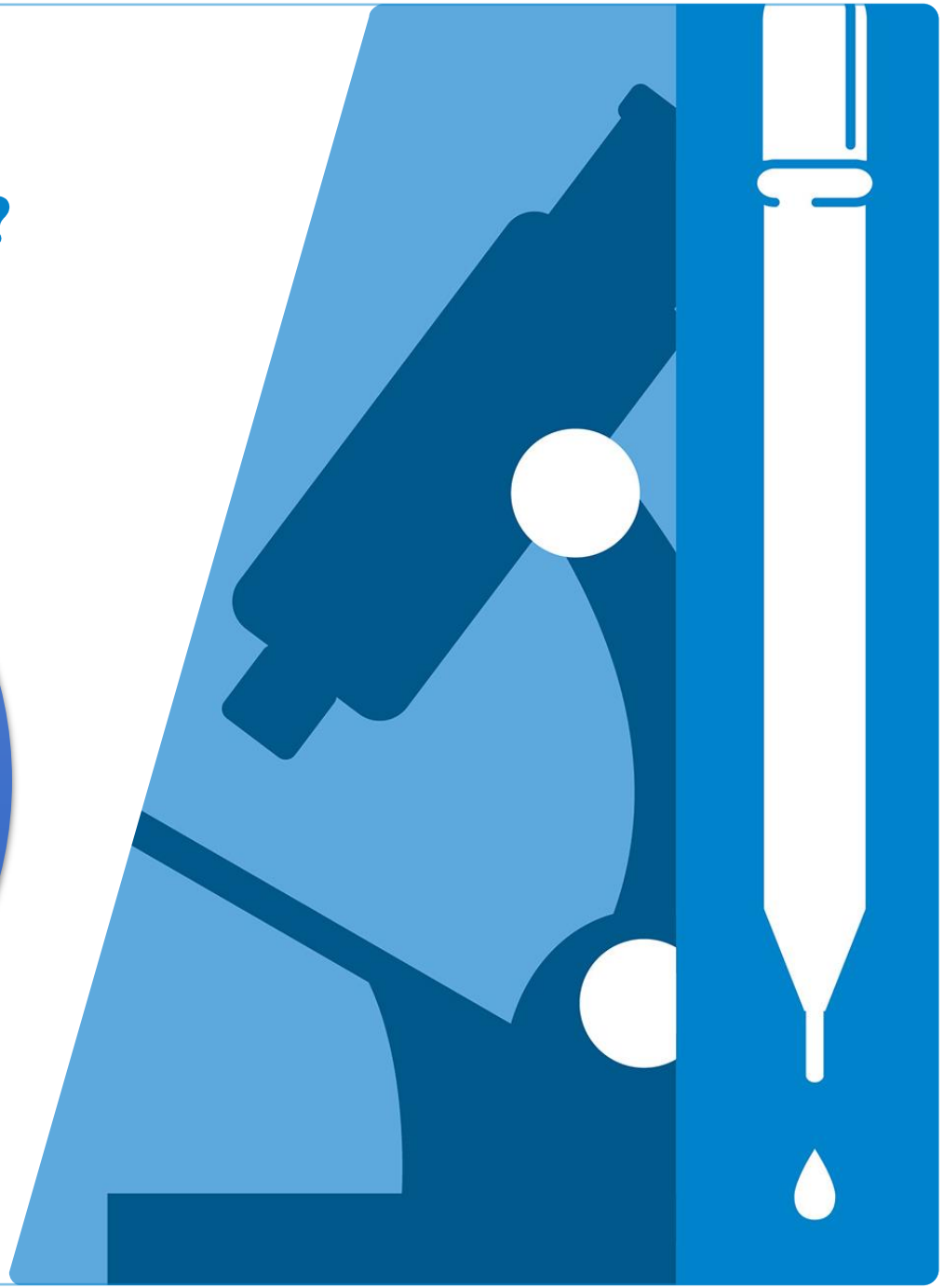


What do you use to prevent mastitis?



What alternatives to antibiotics do you use?

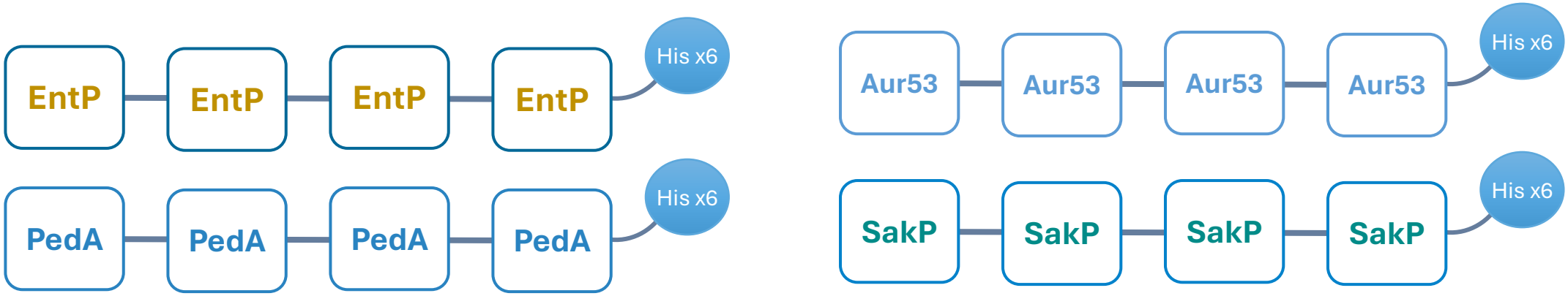




Engineering bacteriocins

Engineering the bacteriocins

Tetrameric concatenation of bacteriocins



x4 monomeric bacteriocins interconnected by **flexible linkers** to allow for combined activity

Roca-Pinilla, R., López-Cano, A., Saubi, C. *et al.* A new generation of recombinant polypeptides combines multiple protein domains for effective antimicrobial activity. *Microb Cell Fact* **19**, 122 (2020). https://doi.org/10.1186/s12934-020-01380-7

Recombinant production hosts

***Lactococcus lactis* NZ9000**

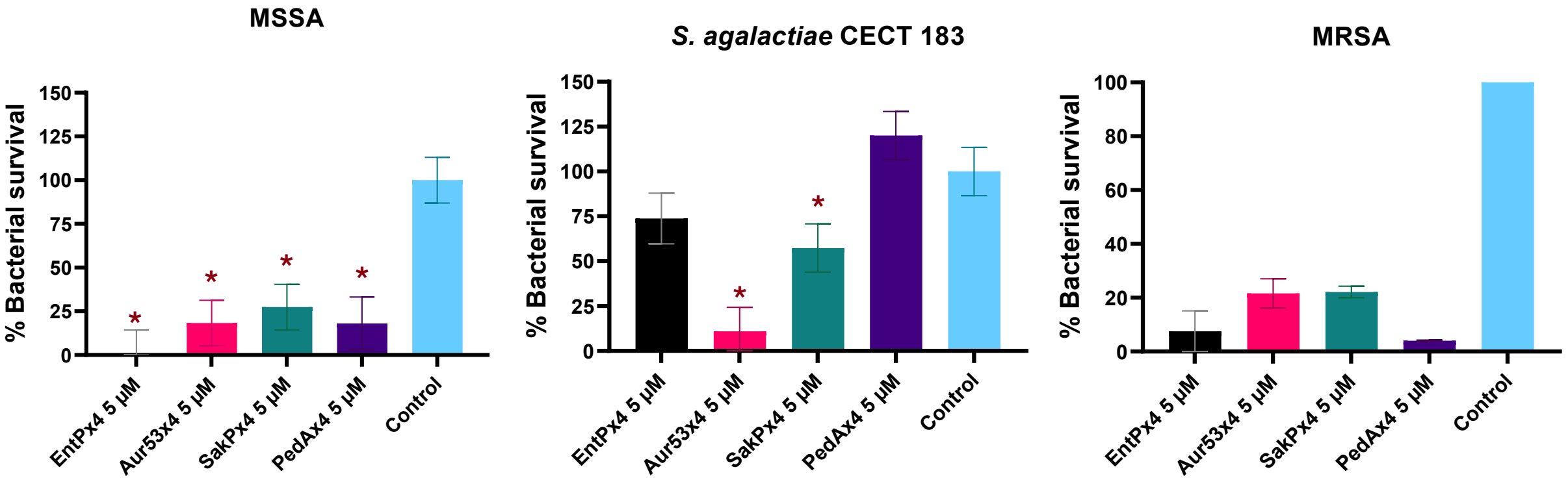
***Escherichia coli* BL21**

Antimicrobial activity assessment *in vitro*

IRTA Institute of Agrifood Research and Technology

Bactericidal activity

BacTiter Glo assay, cell concentration starting at 10^7 cfu/mL for MSSA and 10^8 cfu/mL for both *S. agalactiae* and MSSA



E. coli BL21-produced and intracelluraly purified bacteriocins
Control: positive control for growth - untreated cells
*Data statistically validated with Tukey-HSD

Antimicrobial activity assessment *in vitro*

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Bacteriostatic activity

MIC determination, cell concentration starting at $5 \bullet 10^5$ cfu/mL

	Challenged pathogen, MIC in µM		
Engineered bacteriocin	**MSSA**	**MRSA**	***S. agalactiae* CECT 183**
EntPx4	2.50	2.5	1.25
Aur53x4	1.25	0.6	1.25
SakPx4	2.50	2.5	2.50
PedAx4	2.50	2.5	2.50

E. coli BL21-produced and intracelluraly purified bacteriocins

*MSSA = Methicillin-sensitive *S. aureus* *MRSA = Methicillin-resistant *S. aureus*

EntPx4 and Aur53x4 show to be more effective in inhibiting the growth of these pathogens, showing lower MICs than SakPx4 and PedAx4 in every case

Bioprocess upscalling for *in vivo* experiments

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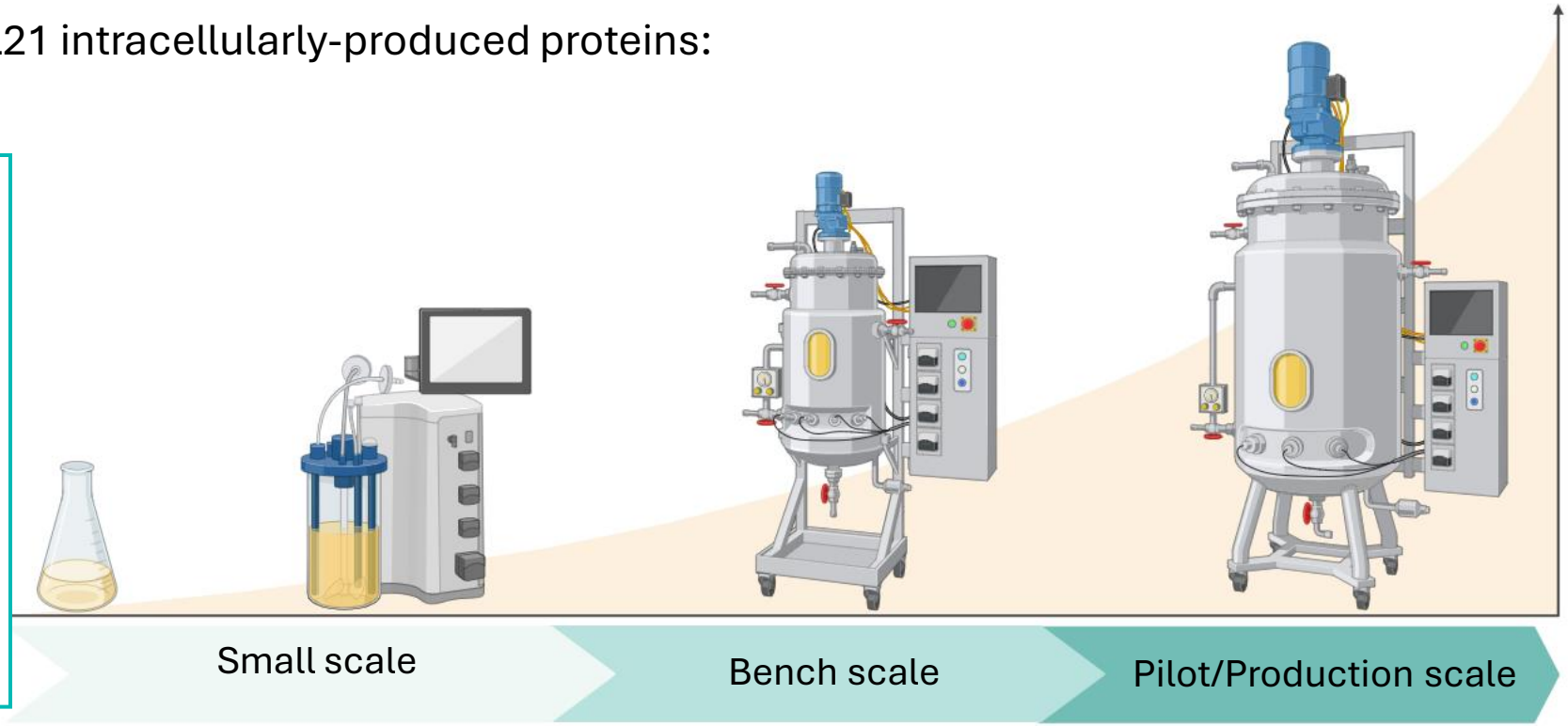
For the *E. coli* BL21 intracellularly-produced proteins:

- **Integrates easily with local dairy and fish-feed systems.**
- **No refrigeration**
- **Residue-free.**
- **Low-cost production**
- **Affordable to smallholders**



	Production rate : mg/L culture	
Bacteriocin	**Small scale - Flasks**	**Small scale - Bioreactor**
EntPx4	0.19	4.5
Aur53x4	0.50	10

→ **x20 increase in bacteriocin bioprocess productivity**

Policy and regulatory pathway

- **Simplified approval frameworks for biologically derived antimicrobials. E.g. NAFDAC.**
- **Public–private partnerships for scale-up.**
- **Policy endorsement will enable commercialization and uptake.**
- **We hope that WOAH's manuals would include bacteriocins and other non-vaccine alternatives.**

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Thank you for listening!