



**SUSINCHAIN**  
SUSTAINABLE INSECT CHAIN

WP 6: Safety along the insect value chain

**KU LEUVEN**

Research Group for Insect  
Production & Processing

# HORIZONTAL TRANSFER OF FOOD PATHOGENS FROM SUBSTRATE TO INSECTS DURING REARING

DRIES VANDEWEYER

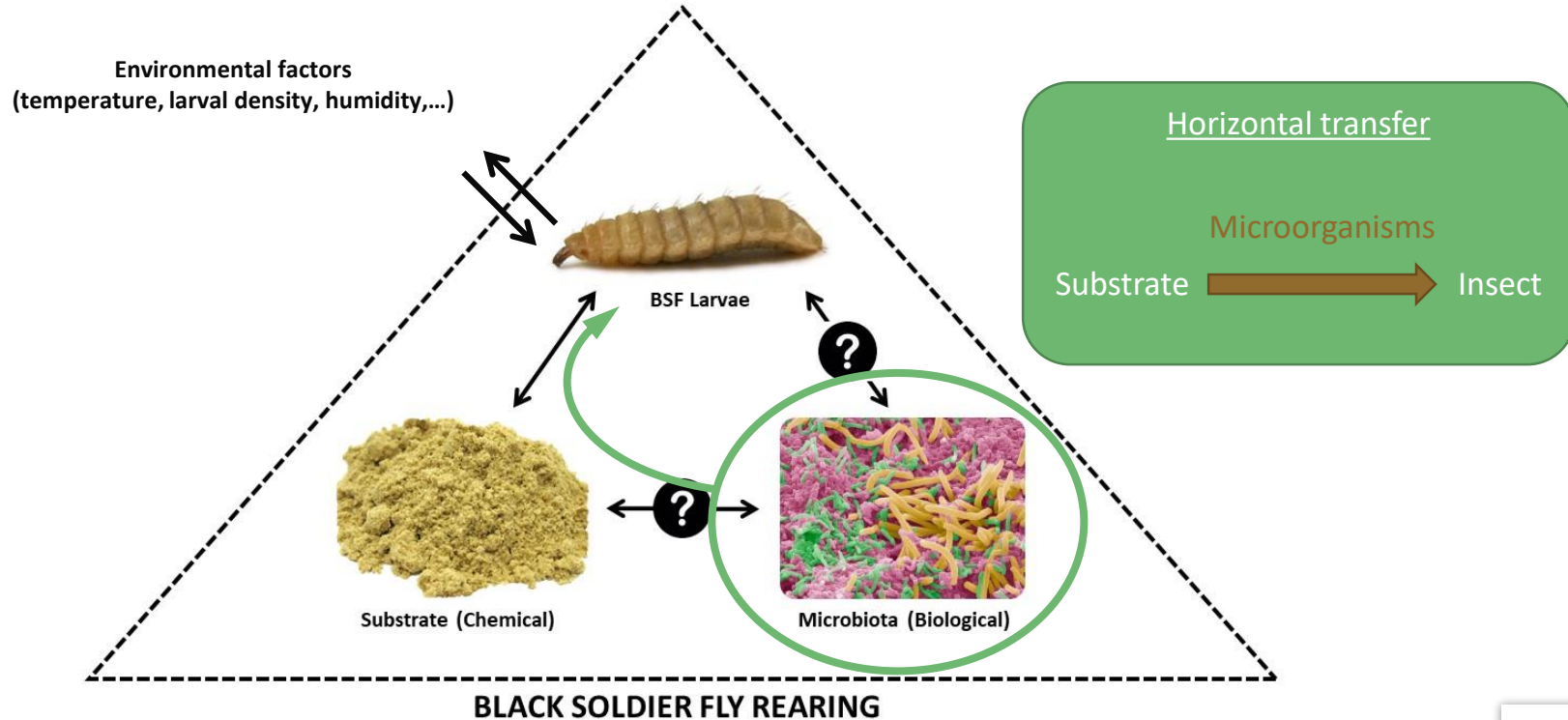
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# Insect-microbe-substrate interactions



# Relevant foodborne pathogens in insect rearing

- Literature study: microbiological safety evaluation
  - Substrate ingredients
  - Insects as food and feed
- Most relevant pathogens:

## Pathogen

*Salmonella* spp.

*Staphylococcus aureus*

*Bacillus cereus* s.l.

*Clostridium perfringens*



- Most relevant insect species:

## Insect

Mealworm

Black soldier fly larva (BSFL)

House cricket/Grasshopper

*Journal of Insects as Food and Feed*, 2021; 7(5): 807-822

**SPECIAL ISSUE:** Advancement of insects as food and feed in a circular economy



## Biological contaminants in insects as food and feed

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# Pathogen x insect matrix

Pathogen \ Insect	Mealworm	Black soldier fly larva	House cricket Grasshopper
<i>Salmonella</i> spp.	Experiments “Horizontal transfer of food pathogens from substrate to insects during rearing”		
<i>Staphylococcus aureus</i>			
<i>Bacillus cereus</i> s.l.			
<i>Clostridium perfringens</i>			

Food Control 100 (2019) 227–234



Risks related to the presence of *Salmonella* sp. during rearing of mealworms (*Tenebrio molitor*) for food or feed: Survival in the substrate and transmission to the larvae

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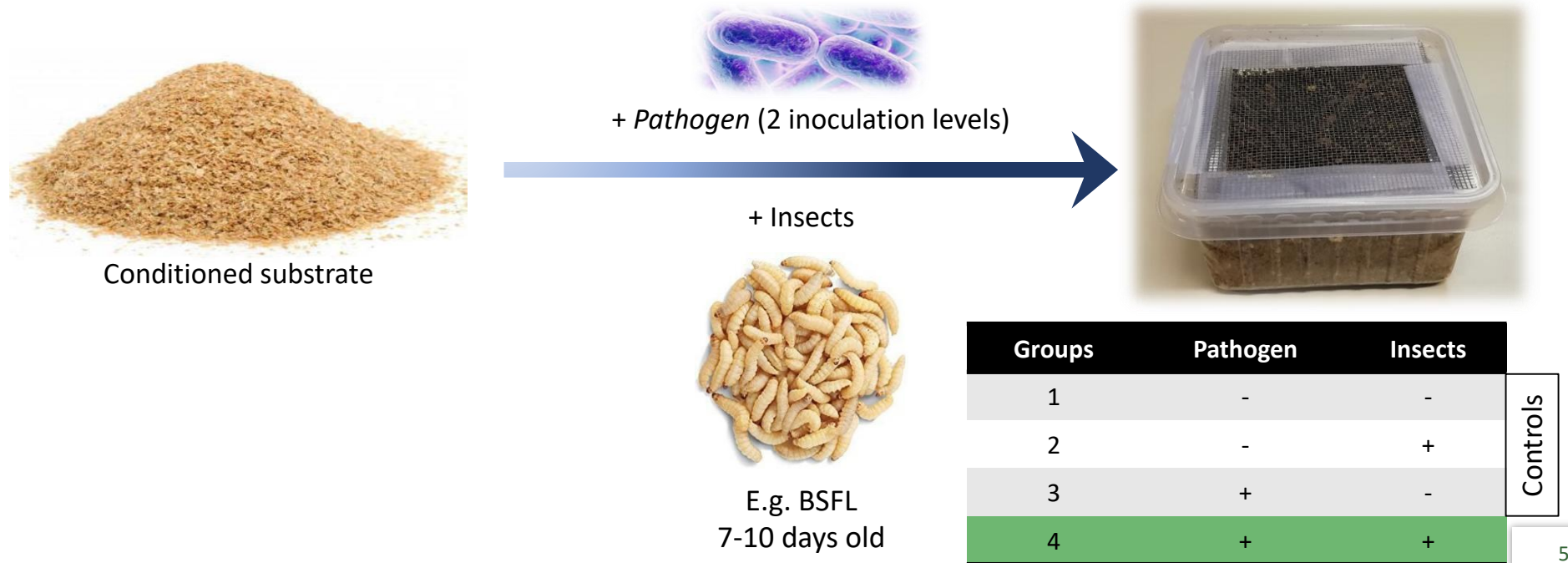
**Dynamics of *Salmonella* inoculated during rearing of black soldier fly larvae (*Hermetia illucens*) on chicken feed**

J. De Smet, D. Vandeweyer, L. Van Moll, D. Lachi, L. Van Campenhout

doi: <https://doi.org/10.1101/2021.04.13.439665>

This article is a preprint and has not been certified by peer review [what does this mean?].

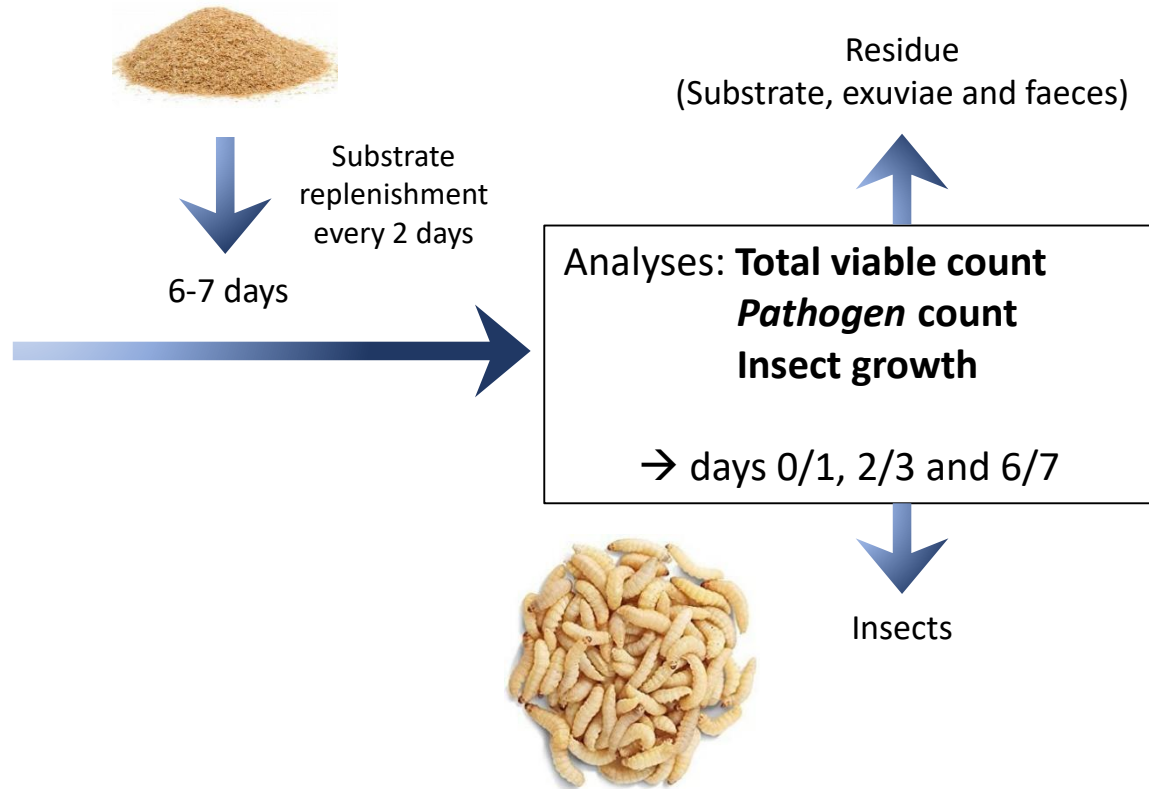
# Experimental set-up



## Experimental set-up (2)

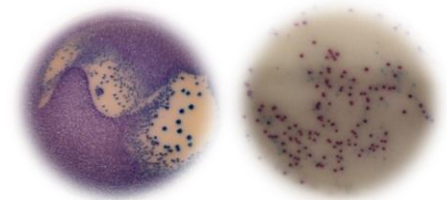


Suitable rearing conditions  
(E.g. BSFL: 28 °C, 65% RH)



# Challenges and insights through experiments

- Background microbiota
  - Selective agar plates are not always selective enough
  - Use of antibiotic-resistant pathogen strain
    - *Salmonella* Typhimurium KAN<sup>R</sup>, *Salmonella* Infantis KAN<sup>R</sup>
    - *Staphylococcus aureus* KAN<sup>R</sup>



**Figure:** RAPID<sup>®</sup>Salmonella agar without (left) and with (right) kanamycin. High numbers of background microbiota (blue colonies) impede correct *Salmonella* (purple colonies) counting. By adding an antibiotic, inoculated antibiotic-resistant *Salmonella* cells can easily be counted.

- Contamination of controls can occur ← Airborne pathogen
  - Separated incubation per test group
  - Proper disinfection!



4 x



# Results: *Salmonella* x mealworm

Experiment details	
Substrate	Wheat bran
Pathogen strains	S. Infantis + S. Typhimurium + S. Enteritidis
Incubation	Not separated, 7 days, 28 °C, 65% RH

Number of samples testing positive for *Salmonella* sp.

Sample	Target <i>Salmonella</i> sp. contamination level in substrate (log cfu/g)	Number of <i>Salmonella</i> sp. positive samples per batch (6 replicates each)					
		Day 1			Day 7		
		Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
Substrate without larvae	Control (0)						
	2						
	4						
Substrate with larvae	Control (0)						
	2						
	4						
Larvae, not disinfected	Control (0)						
	2						
	4						

- *Salmonella* sp. appears to be reduced in the presence of mealworms
- Lowest contamination level → larvae negative for *Salmonella* sp. after 7 days

BUT: only for the circumstances investigated!

## Results: *Salmonella* x BSFL

Experiment details	
Substrate	Chicken feed
Pathogen strains	<i>S. Infantis</i> KAN <sup>R</sup> + <i>S. Typhimurium</i> KAN <sup>R</sup>
Incubation	Separated, 6 days, 28 °C, 65% RH

Sample	Target <i>Salmonella</i> sp. contamination level in substrate (log cfu/g)	Number of <i>Salmonella</i> sp. positive samples per batch (2 replicates each)					
		Day 0			Day 6		
		Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	batch 3
Substrate without larvae	Control (0) 3						
Substrate with larvae	Control (0) 3						
Larvae, disinfected	Control (0) 3						

- ➔ No reducing effect on *Salmonella* sp. is observed after 6 days
- ➔ Similar observations for higher contamination levels (data not shown)

BUT: only for the circumstances investigated!

# Results: *S. aureus* x BSFL

Experiment details	
Substrate	Chicken feed
Pathogen strains	<i>S. aureus</i> KAN <sup>R</sup>
Incubation	Separated, 6 days, 27 °C, 60% RH

Sample	Target <i>S. aureus</i> contamination level in substrate (log cfu/g)	Number of <i>S. aureus</i> positive samples (6 replicates each)	
		Day 0	Day 6
Substrate without larvae	Control (0)		
	3		
	7		
Substrate with larvae	Control (0)		
	3		
	7		
Larvae, disinfected	Control (0)		
	3		
	7		

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But:

- Higher suppression at day 2 (data not shown)
- Lower detection limit!

→ High suppression of *S. aureus* in presence of BSFL

→ No *S. aureus* detected in the larvae after 6 days

BUT: only for the circumstances investigated!

# Conclusions

- *Salmonella* x mealworm
  - Limited horizontal transfer to mealworms
  - Small reducing effect of mealworms on *Salmonella* presence in substrate
- *Salmonella* x BSFL
  - Horizontal transfer to BSFL
  - No effect of BSFL on *Salmonella* presence in substrate
- *S. aureus* x BSFL
  - No horizontal transfer to BSFL
  - High reducing effect of BSFL on *S. aureus* presence in substrate

Horizontal transfer of food pathogens from substrate to insects during rearing highly depends on circumstances:  
Pathogen (species and contamination level) – insect species – rearing conditions – ...



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<http://www.susinchain.eu>



<https://doi.org/10.3920/JIFF2020.0060> (Biological contaminants in insects)  
<https://doi.org/10.1016/j.foodcont.2019.01.026> (*Salmonella* x mealworm)  
<https://doi.org/10.1101/2021.04.13.439665> (*Salmonella* x BSFL)