Can insects be safely reared on residual streams?

Elise F. Hoek – van den Hil

4 September 2024, EAAP

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SAFE INSECTS

General introduction

- Why insect rearing on residual streams?
- Is it safe?

- Substantiate safety for EU legislation

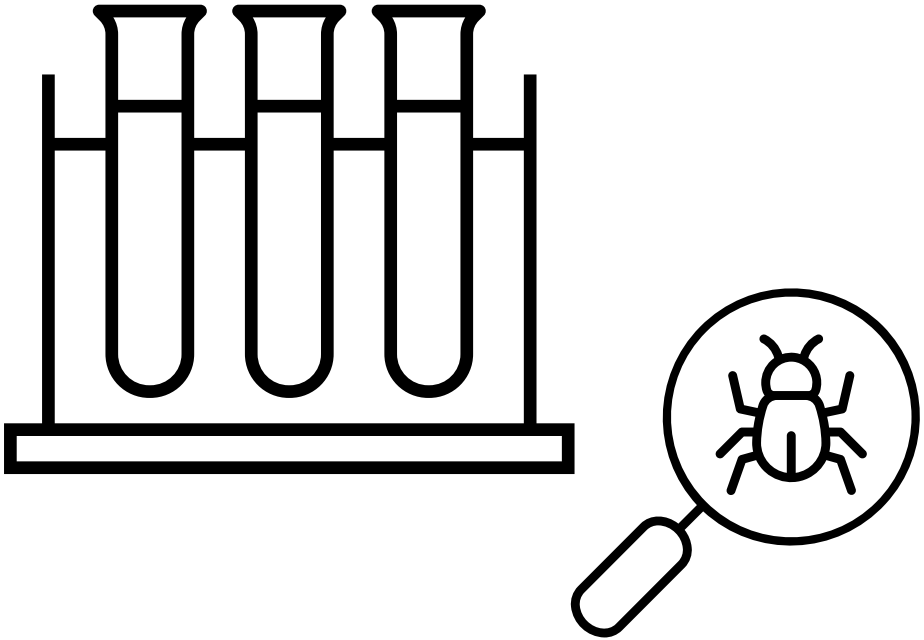
Insects and residual streams

- Black Soldier Fly *(Hermetia illucens)*
- Mealworm *(Tenebrio molitor)*

What about food safety?
Chemical + microbiological analyses of substrate, larvae, frass samples

- Broiler manure (BM)
- Category 2 meat meal
- Household (kitchen) waste
- Supermarket mix

Chemical safety



- Heavy metals
- Pesticides
- Dioxins and PCBs
- Antibiotics and coccidiostats

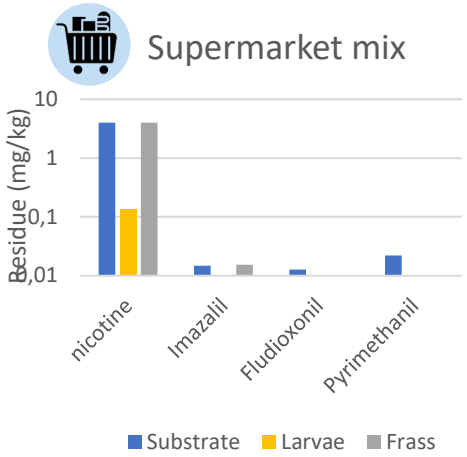
(Heavy) metals

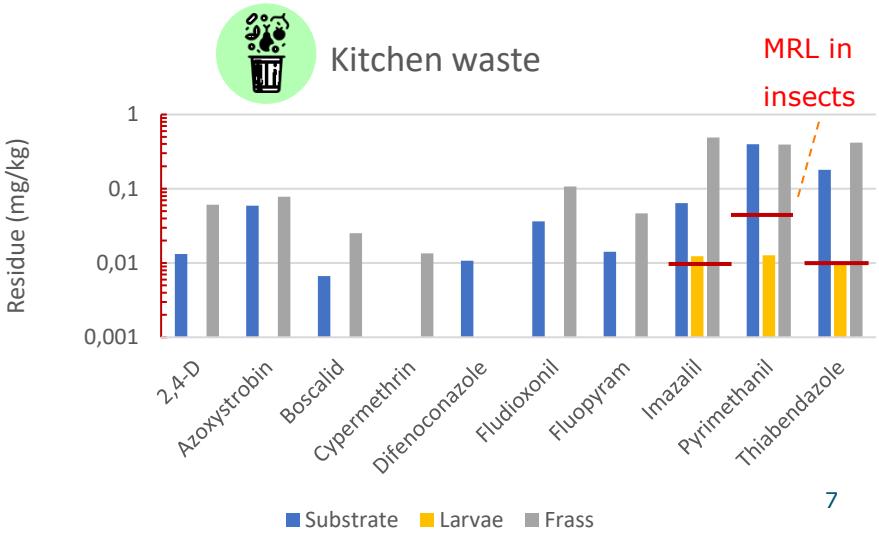
Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Hg	Tl	Pb	U

- Legal limits in feed for Cd, Hg, Pb, As: **not exceeded** in any larval samples
- **Bioaccumulation**: cadmium in BSFL
- Broiler manure samples: overall highest for all elements

Pesticides

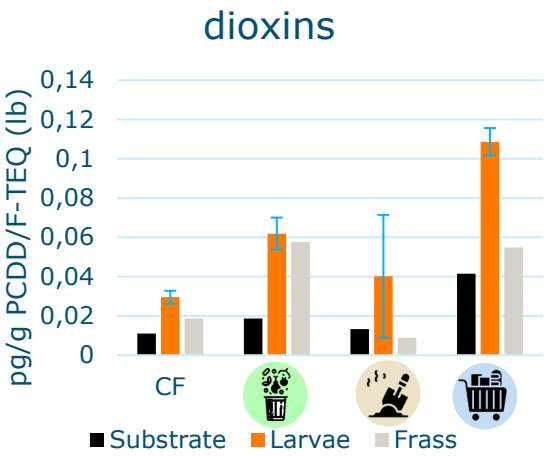
- Pesticide residues detected only in control, kitchen waste and supermarket mix
- Nicotine very high in SW substrate (~2 mg/kg)
  - Tobacco residues?
- Lower yields caused by pesticide residues?

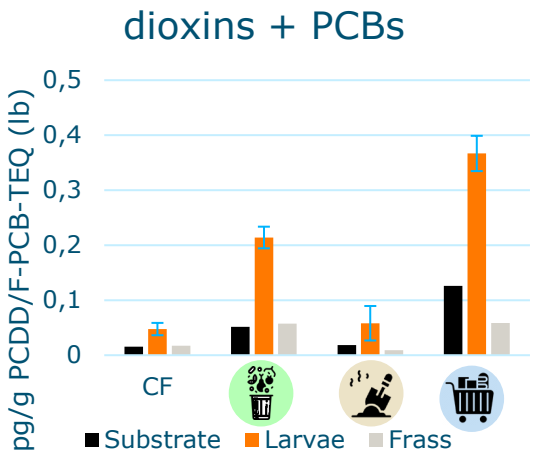


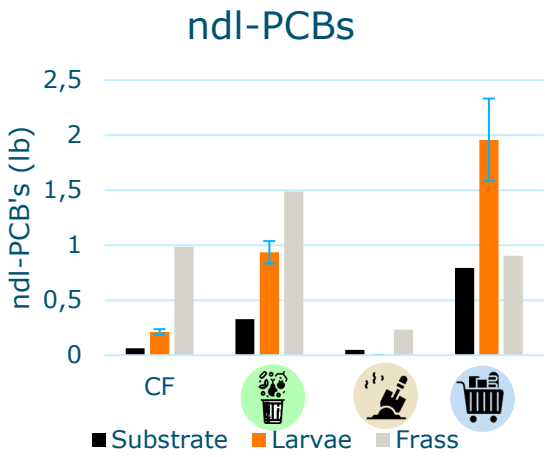


Dioxins

- Some accumulation, all concentrations below legal limits
- Larval concentrations below legal limits (Regulation (EU) 2023/915)







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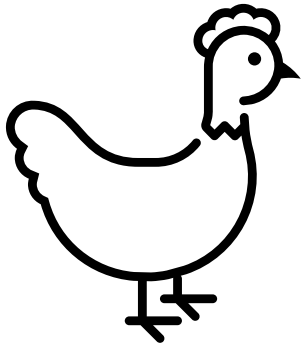
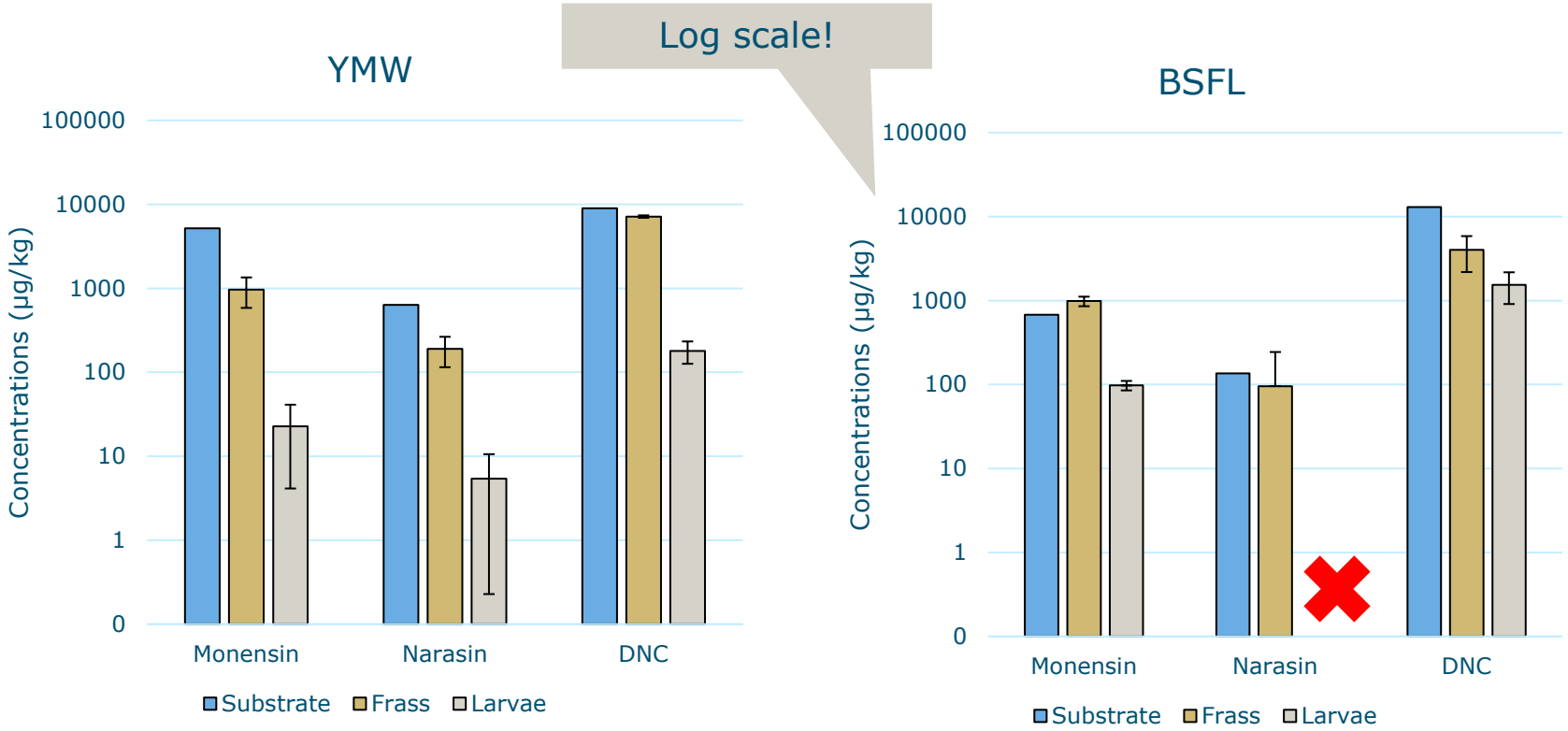
CF = control feed

Antibiotics and coccidiostats in broiler manure

- **Veterinary medicine**: administered to treat disease, requires veterinary prescription
- **Coccidiostats**: feed additive to treat parasitic disease in poultry, preventative: monensin, narasin, DNC **detected**
- **Legal limits** for carry-over of coccidiostats in food and feed: exceeded for all larval samples
    - Except narasin – BSFL (<LOQ)

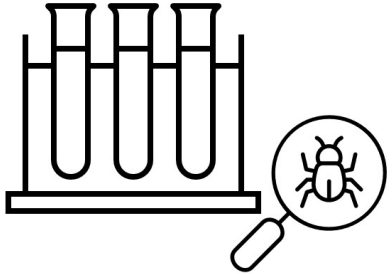


Coccidiostats in broiler manure



DNC: 4,4'-dinitrocarbanilide: Marker for nicarbazin

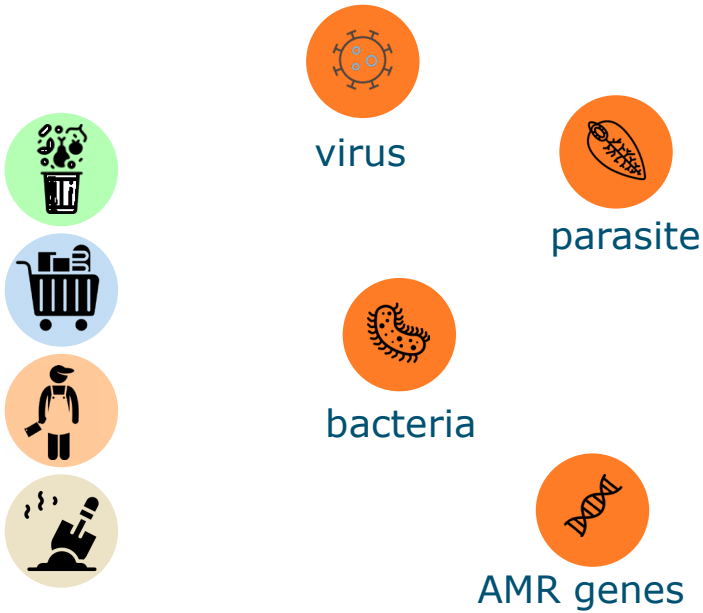
Chemical safety



- **Pesticides** more likely to be in vegetable-based organic residual streams (kitchen waste/supermarket mix)
- Some accumulation **dioxins**, **heavy metals** in the larvae– but concentrations generally below legal limits
- Concentrations **coccidiostats** in broiler manure very high; transfer problematic

Microbiological safety – residual streams

- Transmission of pathogens from residual streams to animals
- Do they remain infectious?



Pathogens in residual streams?

Virus	Perhaps	Perhaps	No	Perhaps
Parasite	Perhaps Yes	Perhaps Yes	No	Yes
Bacteria	Yes	Yes	No	Yes
AMR genes	Yes	Yes	No	Yes

Do they remain infectious in insects?

Spike experiments in Black Solder Fly larvae (BSFL)

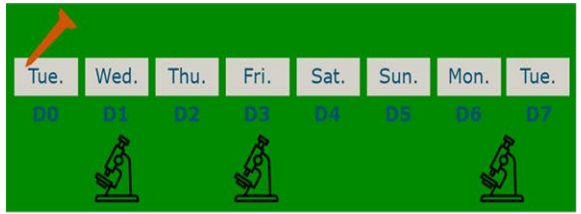
Dry matter content 40%

Viruses
- FMDV
- CSFV
- LPAIV
- SVDV
- HEV

Parasites
Combined:
- *E. acervulina*
- *E. maxima*
- *E. tenella*

Bacteria
- Salmonella enterica subsp enterica Abaetetuba
- Klebsiella pneumoniae
- Clostridium perfringens
- Bacillus cereus

AMR resistance genes
- *ermB (Macrolide (erythromycine)*
- tetW (Tetracyclines)
- aph3 (Aminoglycoside)
- *sul2 (Sulfamethoxazole)*

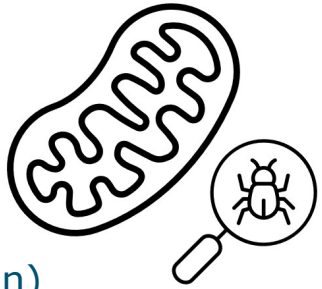


Do tested pathogens remain infections in BSFL?

	Main results
	• rapid inactivation of virus in broiler manure • only SVDV recovered from BSFL on day 1
	• Eimeria: oocysts isolated from BSFL on day 7
	• *K. pneumonia* and *B. cereus*: no decline • *S. enterica* and *C. perfringens*: decline
	• Tested AMR genes were persistently present

SVDV = *Swine vesicular disease virus*

Microbiological safety



- Main concerns
  - Food: food-borne pathogens, spore-forming bacteria
  - Feed: viruses (ban on swill feeding) and prions (TSE regulation)
- Microbiological analysis: Cat2 material most safe; possible issues with spore-formers, *C. perfringens* and *B. cereus* in some other residual streams
- Nuance: different batches may contain different types / concentrations of micro-organisms
- Spike experiments
  - *K. pneumonia* and *B. cereus* found in the larvae
  - Most viruses rapidly inactivated in BM, not detected in BSF except SVDV
- RA: very low levels of BSE exposure of cattle from substrates for insect production

Inactivation of the relevant microorganisms

Vegetative cells and viruses

- *Listeria monocytogenes*
- *Salmonella* (on liquid products)
- *Staphylococcus aureus*
- *Campylobacter* spp.
- *Klebsiella pneumoniae*
- Swine vesicular Disease (SVDV)
- Hepatitis E virus
- Avian Influenza
- Avian Influenza on dried egg white

Spores

- *Bacillus cereus*
- *Clostridium perfringens*

Salmonella (on dry matrix)

example heating: 90°C/2 min

>5 Log reduction	Not inactivated	Not inactivated

Spore outgrowth needs to be prevented low pH, T, Aw

To conclude

- Is insect rearing on residual streams safe?
  - Residual stream
  - Insect species
  - Feed or food
  - Microbiological and chemical hazards

Conclusions

Broiler manure	Cat 2 meat meal	Kitchen waste	Supermarket mix
• Contains (heavy) metals, coccidiostats, possibly other veterinary medicines	• Severe heat-treated resulting in low microbiological contamination	• Assumed to be food grade: but can contain variety of contaminants; control / traceability difficult	

- Processing reduces microbiological risk in most cases
- Very low levels of BSE infectivity to cattle (for each substrate)

Acknowledgements

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Nathan Meijer, Winnie Tao, Kristian Zadelhoff, Yvette Hoffmans, Marko Appel, Adriaan Antonis, Clazien de Vos, Olga Haenen, Marieke Bruins, Anita Dame, Jan Boonstra, Helmi Fijten, Hasmik Hayrapetyan, Marieke Bruins, Teun Veldkamp, Somaya Naser El Deen

Project partners:

Project SAFE INSECTS is financed by TKI AgriFood that is funded by the ministry of LVVN and partners of the project

Microbial hazards

	Residual stream	TBC	Spore counts	Salm.	Camp.	C. perf.	L. monoc.	B. cereus	
3	 **BM**	<1.0E9 2,2E10 1,8E13	2,2 x 10^7 | 2,0 x 10^5 7,1 x 10^5 | 5,4 x 10^4 3,3x10^5 | 1,3x10^3	ND	ND	>3000 >3000 45	<10	<100	**HEV** (Type 1-4), **AIV**: negative AMR detected Coccidia / parasite eggs
2	 **Ct2**	<1.0E9	neg (2x) ; 1x neg, 1x 1.0E4 cfu/g	ND	ND	<10	<10	<100	
1	 **KW**	8.9E7	4.6x10E5 4.2x10E5	ND	ND	<100	<100	1.3E5	**HEV** (Type 1-4) : negative MRSA negative
2	 **SW**	<1.0E9 2.2E8	1,4 x 10^6 | 4,4 x 10^4 4,4x10^4 | 3,3x10^3	D/ND	ND	1.5E3 >1.5E4	<10 <100	<100 6,3E4	**HEV** (Type 1-4) : negative MRSA negative
		5.0E6 cfu/g				1,0E5 cfu/g`	100 `cfu/g	1.0E5 Cfu/g	
		Hygiene criteria				RTE foods (national law)	RTE foods	RTE foods (national law)	

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PO | Could these pathogens remain infectious?

				
	Yes	Yes	NA	Yes, but...
	Yes	Yes	NA	Yes
	Yes	Yes	NA	Yes, but...
	Yes	Yes	NA	Yes

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Low pH might affect the infectivity

PIPO ➔ do they remain infectious in insects?

Spike experiments in Black Solder Fly larvae (BSFL)

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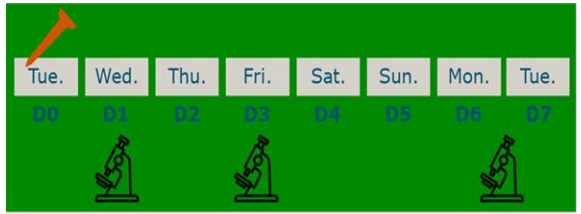


Spiked pathogen (infectious dose/ml)

DPI	0	1		3		7	
Control feed CF							
BM Negative control							
BM Spiked							
BSFL							

Foot & mouth disease virus *(10^6 $TCID_{50}$/ml)*

DPI	0	1		3		7	
Control feed CF	Neg.	Neg.	Neg.	Neg.	Neg	Neg.	Neg.
BM Negative control	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
BM Spiked	$10^{4,1}$	Neg.	$10^{2,4}$	Neg.	Neg.	Neg.	Neg.
BSFL	Neg.						

Avian influenza virus (*10^6 $TCID_{50}$/ml*)

DPI	0	1		3		7	
Control feed CF	Neg.	Neg.	Neg.	Neg.	Neg	Neg.	Neg.
BM Negative control	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
BM Spiked	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
BSFL	Neg.	No infectious virus, but PCR positive!					

Swine vesicular disease virus (*10^6 $TCID_{50}$/ml*)

DPI	0	1		3		7	
Control feed CF	Neg.	Neg.	Neg.	Neg.	Neg	Neg.	Neg.
BM Negative control	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
BM Spiked	$10^{6,4}$	$10^{4,86}$	$10^{5,7}$	Neg.	Neg.	Neg.	Neg.
BSFL	Neg.						

Klebsiella pneumoniae *(3,6x10^9)*

DPI	0	1		3		7	
Control feed CF	Neg.	 3.1 E5	 Neg.	 3.2 E5	 E5*	 E4*	 E4*
BM Negative control	Neg.	E4*	E4*	E4*	E1*	E4*	E1*
BM Spiked	2.6 E9	3.5 E7	1.8 E7	E7/E8*	E5	E8*	E7*
BSFL	2.6 E5						

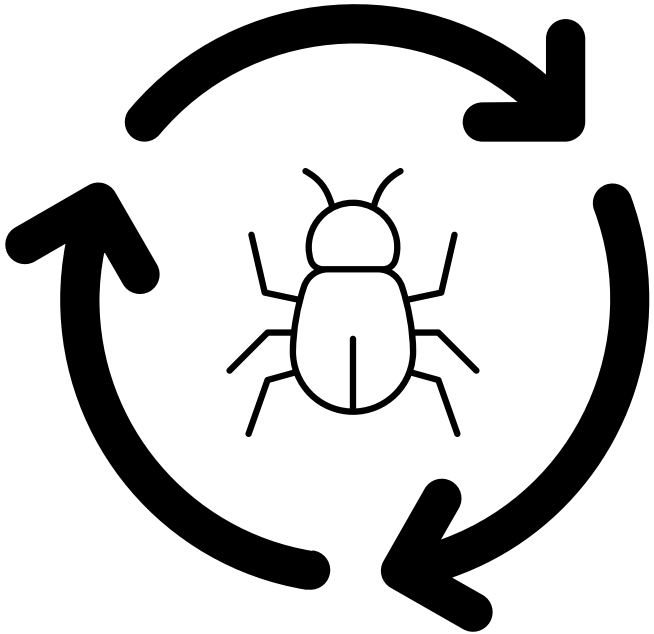
Salmonella enterica *subsp enterica abaetetuba* *(9,0x10^9)*

DPI	0	1		3		7	
Control feed CF	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
BRM Negative control	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
BRM Spiked	4.0 E9	3k -7 10k -5	3.0 E9	E6*	E6*	1.0 E2	2.0 E2
BSFL	Neg.						

Eimeria (maxima, tenella & acervulina)| OPG

DPI	0	1		3		7	
Control feed CF	0 21A	0/0 21C	0 21B	0/0 21E	0 21D	0/0 21G	0 21F
BM Negative control	0 22A	0/0 22C	0 22B	0/0 22E	0 22D	0/0 22G	0 22F
BM Spiked	113867 23A	0/0 23C	4247 23B	0/0 23E	8820 23D	33/167 23G	21920 23F
BSFL	0/0 24A						

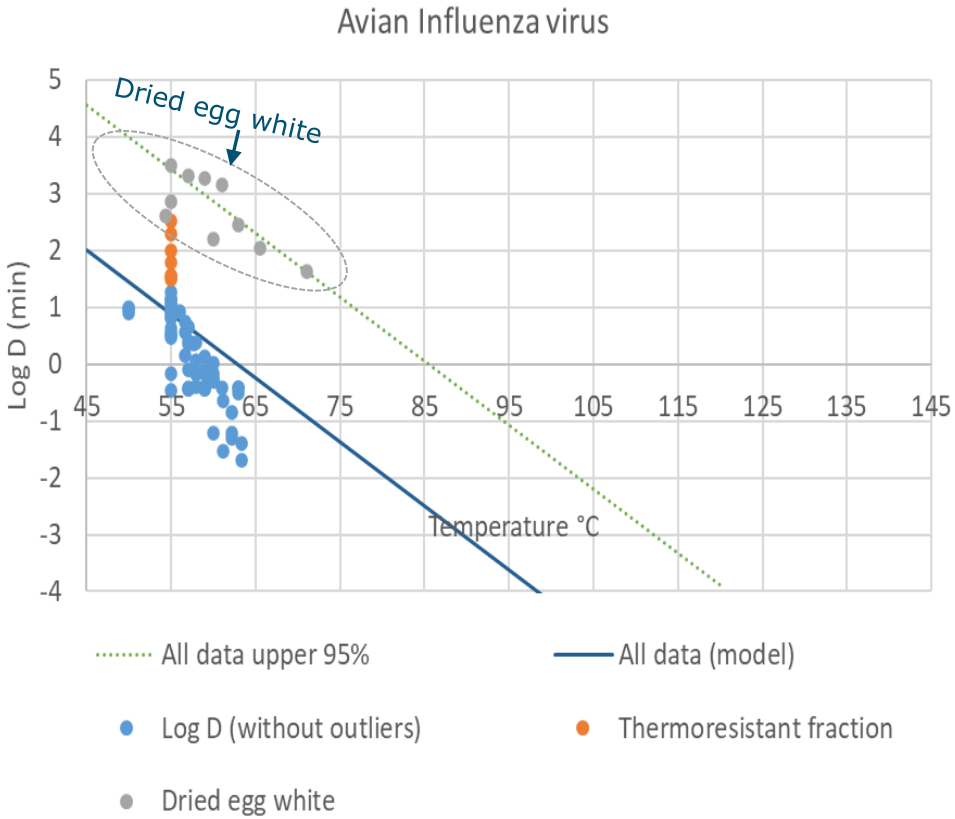
Processing (Hasmik Hayrapetyan)



Heating types for processing of insects

- Heating (conventional)
- Tyndallisation as a heating method for spores
- Microwave heating
- Extrusion

Heat resistance of Avian influenza virus on dry and wet matrixes



	$D_{(70C)}$ (min)	Log Reduction at 90°C/2 min
mean	0.15	>2000
upper 95% (dried egg white)	56.27	6.3

- D value on dry matrix is 100 fold higher!

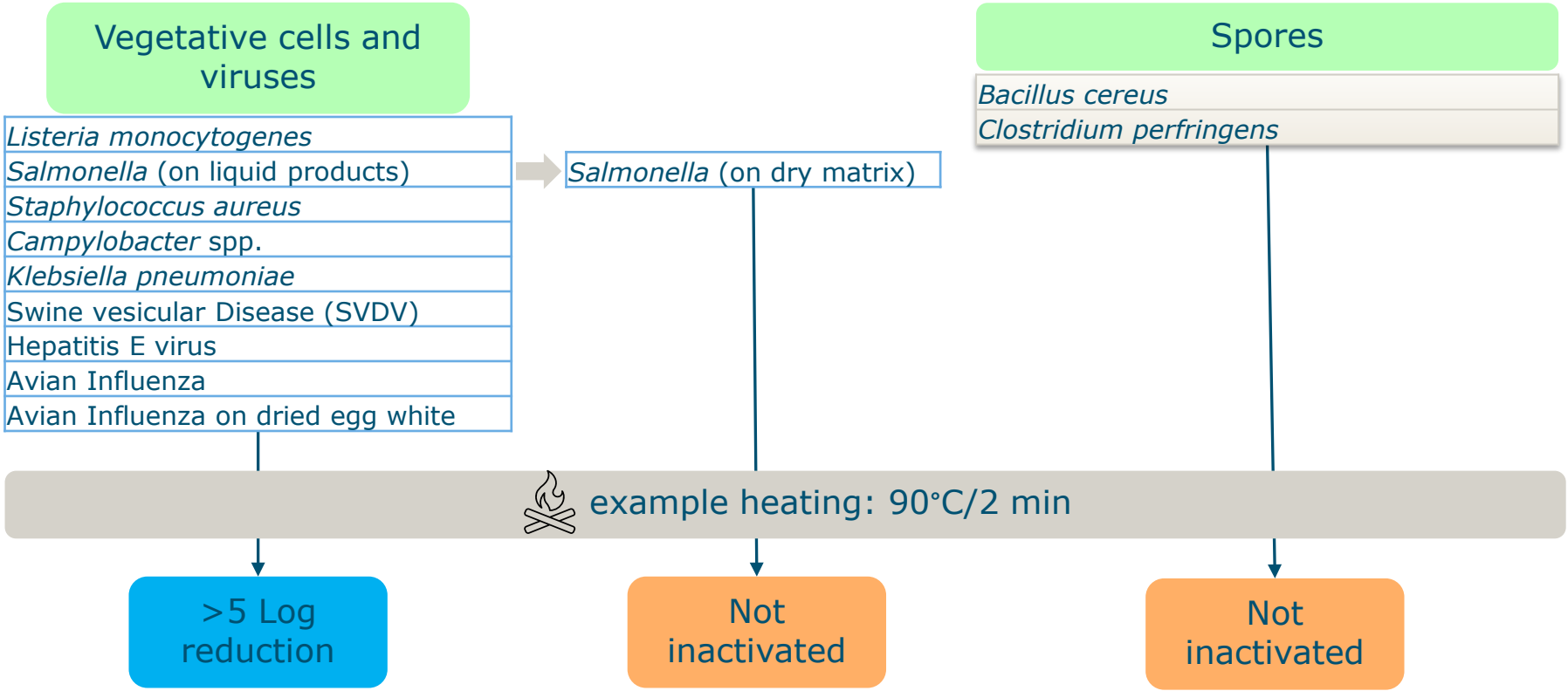
>5 Log reduction

✓

Similar matrix effect for *Salmonella*!

REFRESH project report
Hayrapetyan et al., 2019

Inactivation of the relevant microorganisms



Spore outgrowth needs to be prevented.

Spores as insect contaminants

- gut microbiota of insects
- substrate contamination

Blanching mealworm larvae

Inactivated vegetative cells ✓	10 sec No effect on spores	20 sec Spore counts increased!	40 sec 0.8 log spore decrease

Blanching followed by microwave drying (13min) resulted in 1 Log spore reduction.

Osimani 2021

Vandeweyer 2017[36]

Tyndallisation as a spore inactivation method

Tyndallization:

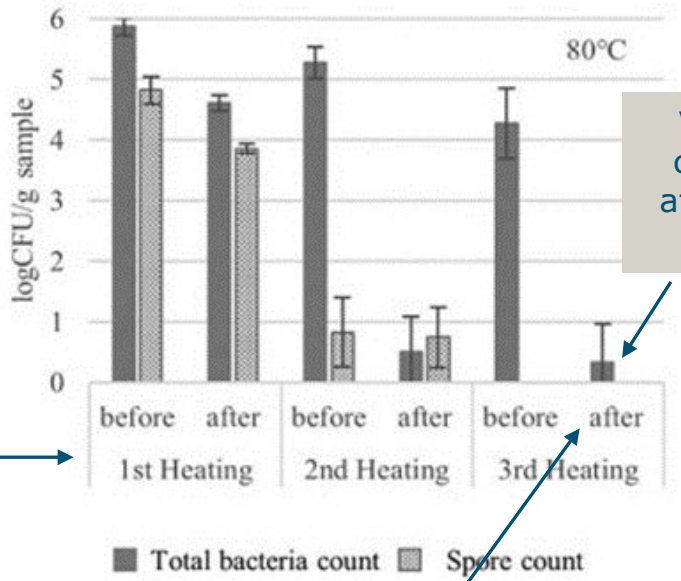
-alternative sterilization method

-heating the substance for multiple times

-with a resting period between cycles

(Tyndall, 1877).

Bacillus subtilis spores on cured surimi.



Vegetative cells grown after the last cycle

3x heating and incubation cycles

Beware of superdormant spores: not 100% might germinate or might germinate later.

Control measures are needed to prevent the outgrowth!

Keratimanoch et al, 2022

Control factors preventing spore outgrowth

- ✓ pH
- ✓ Water activity (a_w)
- ✓ Temperature
- ✓ …

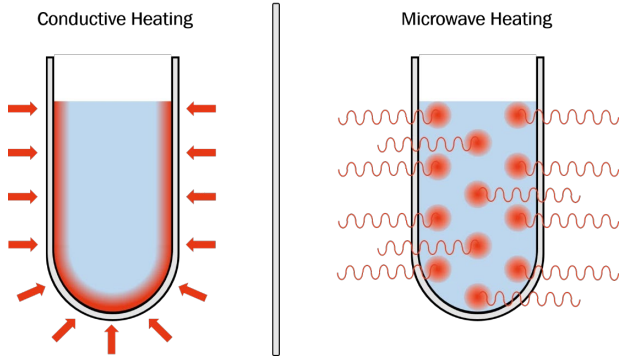
Pathogen	Conditions to prevent the outgrowth.
B. cereus	a_w<0.92 T<4°C pH<4.3-4.6
C. perfringens	a_w <0.93 T<10°C pH<5.0

Spores cannot grow on a dry matrix such as insect powder.
However when the insect powder is used to make a wet product, the other controls need to be in place.

Example of microwave heating for insects

- 100°C for 1h
- Vegetative pathogens and viruses inactivated
- *C. botulinum* (proteolytic) inactivated by 2.3 Log.
  - ➢ After 2h: by 4.6 Log.

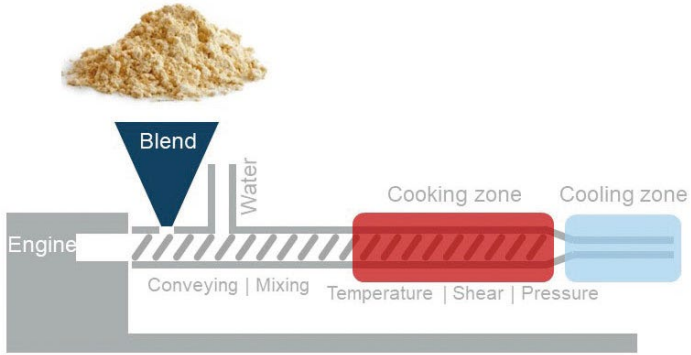
Beware of cold spots (apply mixing)



https://cem.com/microwave-heating-mechanism-and-theory?___store=en&___from_store=de
Bozkurt-Cekmer 2017

Extrusion as heat processing

High moisture extrusion of insects: 160-170°C (40-45% water)



Prevent re-contamination!

The effect can be calculated based on the residence time at temperatures higher than 95°C (Likimani 1990).

Extrusion at 115.6°C resulted in 4–5 log reduction of *Clostridium sporogenes* spores (Hsieh 1993).

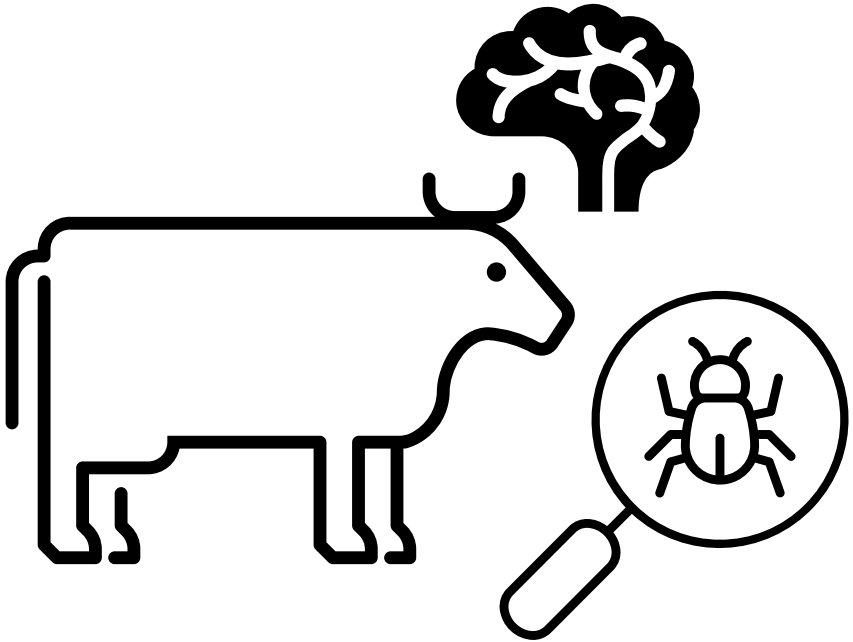
https://www.dlg.org/en/food/topics/dlg-expert-reports/food-technology/extrusion

Conclusions: heating

- Easier to inactivate on a liquid matrix than dried
- Spores are harder to inactivate: growth needs to be prevented by low pH, T, a_w

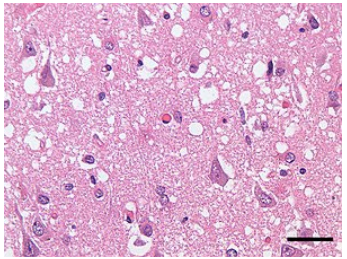
- Spore inactivation is a good future research topic
- Also other inactivation methods can be considered: e.g. irradiation or E-beam

Risk assessment prions (Clazien de Vos)



BSE

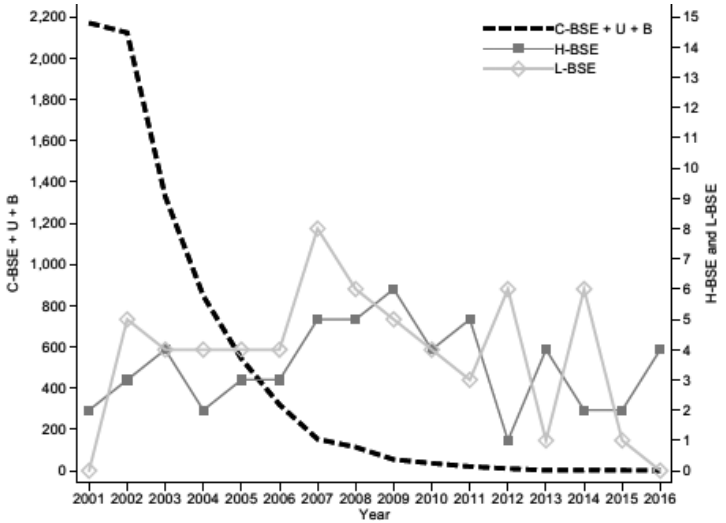
- BSE = prion disease of cattle
  - First detected in UK in 1980s
  - Other prion diseases: scrapie (sheep), CWD (deer), CJD (human)
- Pathogen = misfolded prion protein
  - Extremely heat resistant
  - Classical vs atypical BSE strains
- Fatal neurological disease
  - Long incubation period
  - Clinical signs: behavioural changes, mobility disorders



Spongiform degeneration of CNS tissue (vacuoles)

Epidemiology of BSE

- Main transmission route: ingestion of prions via feed
  - ➢ Total feed ban for PAP in 2001 to stop BSE transmission



Source: EFSA 2018

Source: EFSA

NOS Nieuws Sport Live Programma's

Voor het eerst in tien jaar weer BSE in Nederland

Redactie
Jan Braakman
parlementair verslaggever

Voor het eerst sinds 2011 is in Nederland koegekte (Boviene Spongiforme Encefalopathie, BSE) vastgesteld. Het gaat om een Zuid-Hollandse koe die op het bedrijf is overleden en bij de destructie is terechtgekomen. Het dier is bij Rendac bemonsterd op de ziekte. De koe was circa negen jaar.

Het gaat om een spontane vorm van BSE die sporadisch voorkomt bij koeien, en die afwijkt van de klassieke BSE die is veroorzaakt door besmet voer. Wageningen Bioveterinary Research heeft vastgesteld dat het om de atypische variant gaat. Het is de vijfde keer dat een atypische variant van BSE in Nederland wordt vastgesteld.

NOS Nieuws • Woensdag 1 februari 2023, 13:36 •
Aangepast woensdag 1 februari 2023, 19:02

Gekkekoeienziekte op boerderij in Zuid-Holland, geen gevaar voor mensen

Op een boerderij in Zuid-Holland is de gekkekoeienziekte aangetroffen. Dat heeft minister van Landbouw Adema bekendgemaakt. Waar het bedrijf zich precies bevindt, is niet bekendgemaakt. Wageningen BioVeterinary Research heeft het kadaver van het dier onderzocht
niet via veevo

Opgedoken geval gekkekoeienziekte ongevaarlijk voor mensen

1 februari 2023 17:24 • Aangepast 2 februari 2023 06:15

NIEUWE OOGST NIEUWS VEEHOUDERIJ AKKER- & TUINBOUW REGIO VIDEO PODCA

Besmetting koe met BSE komt niet door voer

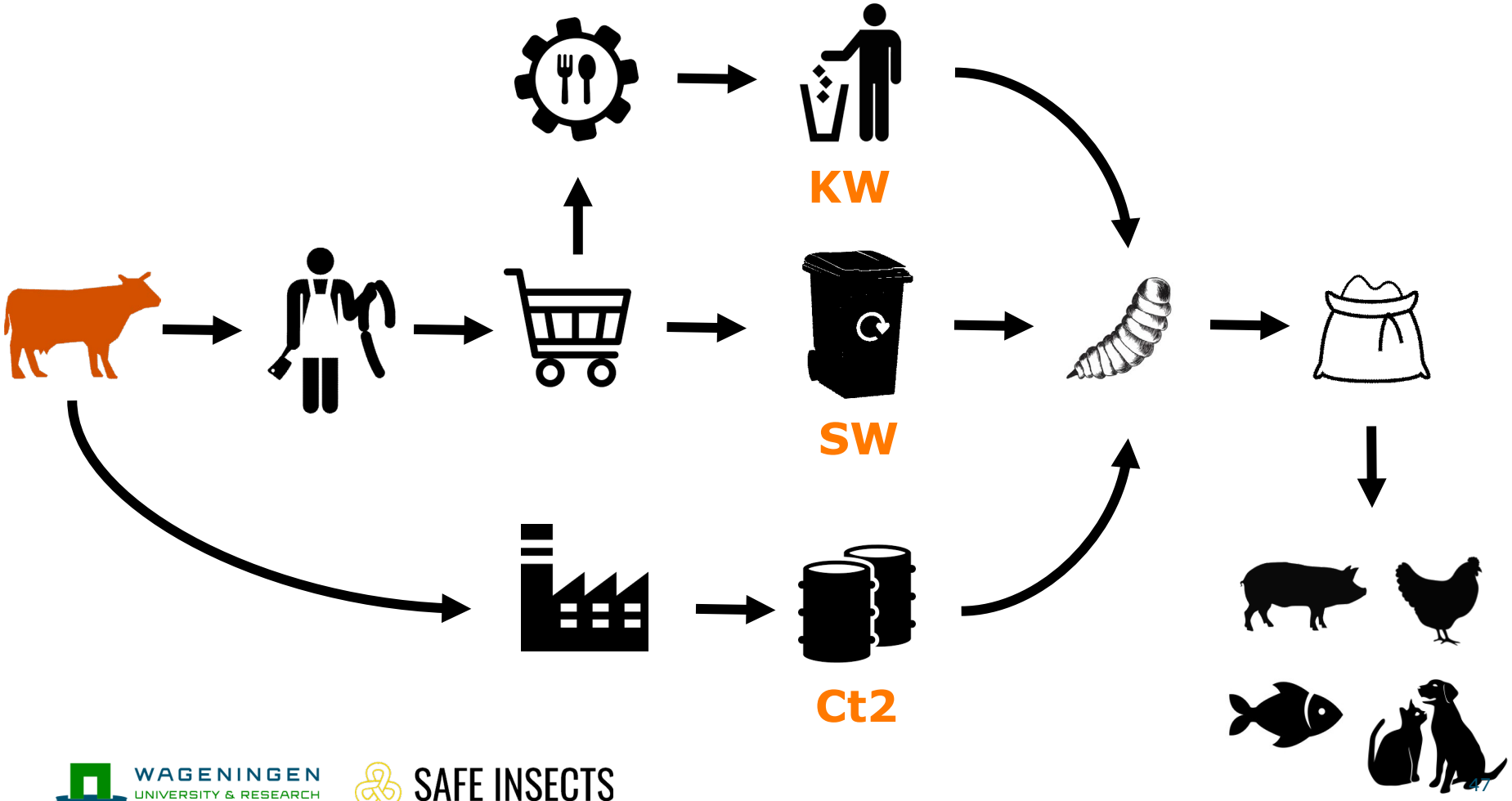
NIEUWS MELKVEE MARTIJN VAN ROSSUM 01 FEB 2023 OM 17:55UUR

De koe op een bedrijf in Zuid-Holland die besmet was met gekkekoeienziekte (BSE), is niet besmet geraakt via het voer. Dat blijkt uit onderzoek van Wageningen Bioveterinary Research (WBVR). Het gaat om de zogenoemde 'atypische variant' die als ouderdomsziekte sporadisch voorkomt bij koeien.

Type here to search

Risk assessment BSE

- Residual streams
  - Broiler manure (BM)
  - Cat 2 meat meal from animal rendering (Ct2)
  - Organic waste from household kitchens (KW)
  - Supermarket waste (SW)
- Main output of risk model
  - CoID50 ending up with cattle if 1 BSE cow is processed



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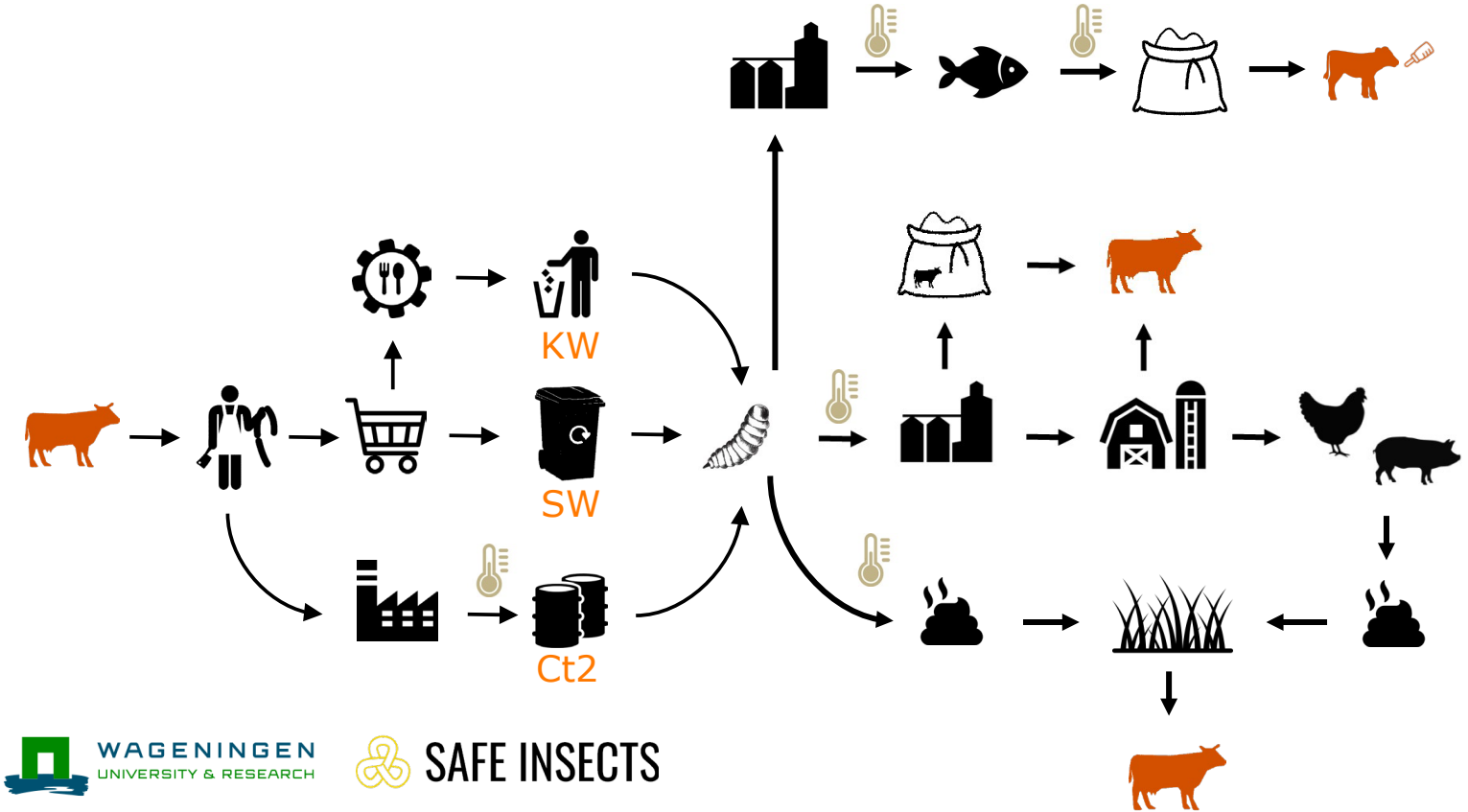
SAFE INSECTS

Exposure routes



Infectivity in substrate

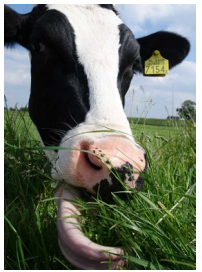
- KW and SW
  - Single BSE infected cow slaughtered at clinical end stage
  - SRM removal, infectivity in other tissues ≤ detection level
  - Probability of contamination at slaughter
  - Meat discarded at supermarket (2.3%) and household level (3.5% in GFE)
- Ct2 meat meal
  - Single BSE infected cow rejected for human consumption at slaughter
  - Negative test result for BSE (?)
  - SRM removal, infectivity in other tissues ≤ detection level
  - Probability of contamination at slaughter
  - Partial inactivation by rendering process (average of 2.3 $\log_{10}$)

Infectivity in insect meal

- Insects lack PrP cells
    - No natural prion disease in insects
    - No biological vector for prions (amplification)
- Worst-case: all infectivity retained in insects and frass
- Division of infectivity across insects and frass
    - Relative weights of insects and frass at harvest
    - Alternatively: weight of gut contents only (most infectivity in frass)
- No inactivation by production of insect meal

Exposure routes

- Contaminated feed
  - Mixed feed mill: cross-contamination / mislabelling
  - Mixed farm: cross-contamination / wrong feed / accidental access



- Grazing
  - Frass used to fertilize grassland
  - Manure of pigs/poultry fed with insect meal to fertilize grassland
  - No inactivation of prions in gastro-intestinal tract of pigs/poultry

- Calf milk replacer
  - Slight inactivation by production of aquafeed (extrusion): 0.2 $\log_{10}$
  - Infectivity retained in small percentage of fish for limited time
  - No inactivation by production of fishmeal

Preliminary results

$R_0 < 1$

- Very low levels of infectivity to cattle for each substrate
- Main data gap
  - Fate of prions in insects
    - → Worst-case approach
- Assumptions
  - Risk of single BSE-infected cow
    - ➢ Small number of BSE cattle slaughtered and processed
  - Equal risk for classical BSE and atypical BSE
  - Homogenous distribution of infectivity in tissues/ products