UNIVERSITÄT LEIPZIG

FRIEDRICH-LOEFFLER-INSTITUT
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

# Technical and anatomical considerations for reproducible inactivation of large animal carcasses by steam sterilization

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ABSA International
62nd Annual Biological Safety Conference
November 15-20/11/2019

Birmingham | 11/20/2019

# Why large animals?

## The mice crisis

**The "Crisis of Reproducibility" of Animal Studies: Causes and Solutions**

Lisa M Shollenberger, Hannes Hentze and Eric Ibsen

J Immunol May 1, 2019, 202 (1 Supplement) 131.10;

"According to a 2012 study published by Amgen researchers, **90%** of the 50+ in vivo oncology studies were **irreproducible**"

Perspective

**Unstressing intemperate models: how cold stress undermines mouse modeling**

JEM Journal of Experimental Medicine

Christopher L. Karp

DOI: 10.1084/jem.20120988 | Published June 4, 2012 Check for updates

"**...cold stress** to which laboratory mice are ubiquitously subjected profoundly **affects mouse physiology** in ways that **impair the modeling of human homeostasis and disease.** Experimental attention to this key, albeit largely **ignored...**"

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## Genetically modified pigs on the way

Boar 1339 was genetically engineered to have diabetes; its body parts, now in the Munich MIDY-PIG Biobank in Germany, are freely available to researchers.

ANIMAL MODELS

**Inside the first pig biobank**

*Elaborate array of tissue samples provides powerful animal model for studying diabetes.*

**Tissue Sampling Guides for Porcine Biomedical Models**

Toxicologic Pathology
2016, Vol. 44(3) 414-420
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DOI: 10.1177/0192623316631023
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**Barbara Albl[1,2], Serena Haesner[1], Christina Braun-Reichhart[3], Elisabeth Streckel[3], Simone Renner[3], Frank Seeliger[4], Eckhard Wolf[3,5], Rüdiger Wanke[1], and Andreas Blutke[1]**

**Trends in Microbiology**

Volume 20, Issue 1, January 2012, Pages 50-57

CellPress

Review

## The pig: a model for human infectious diseases

François Meurens [1] ✉, Artur Summerfield [2], Hans Nauwynck [3], Linda Saif [4], Volker Gerdts [5]

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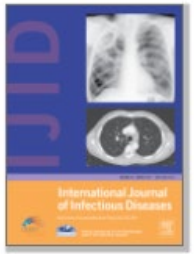
# Why large animals?

## Ruminants in tuberculosis disease modelling

Review

Experimental animal modelling for TB vaccine development

Pere-Joan Cardona [a], Ann Williams [b]

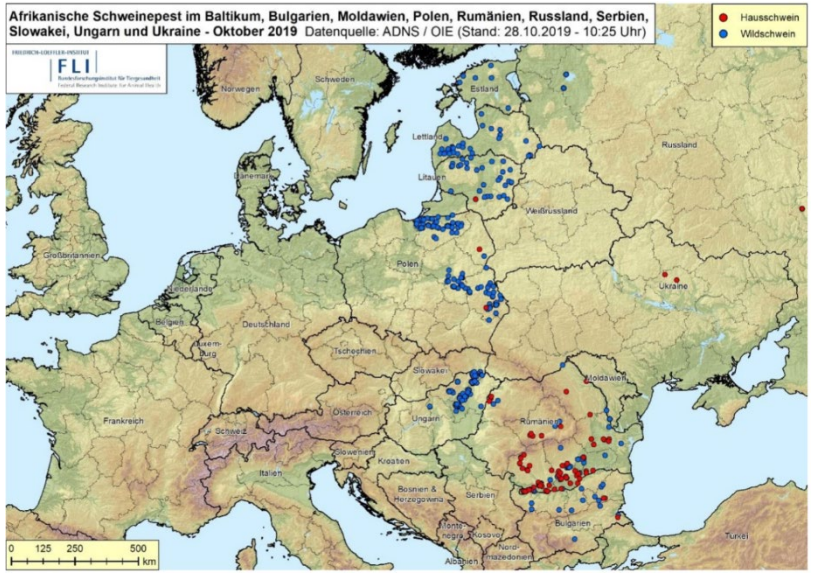



Experimentally induced disease

Experimental Infection of Goats with *Mycobacterium avium* subsp. *hominissuis*: a Model for Comparative Tuberculosis Research

J. Schinköthe *, P. Möbius †, H. Köhler †, E.M. Liebler-Tenorio †

## Livestock infectious diseases (FMDV, ASFV …)



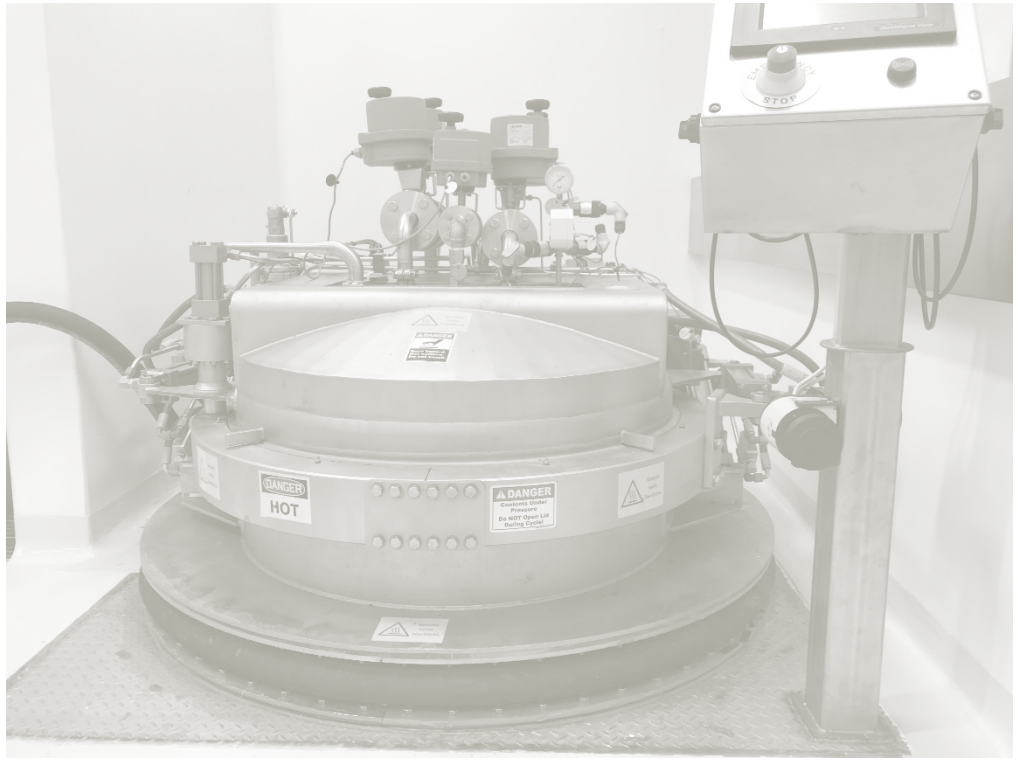

Radar Bulletin-October-2019

# Why steam autoclaving?

## Two main reasons

- **Malfunction:** tissue grinder or alkaline hydrolysis

- **There is no** tissue grinder or alkaline hydrolysis in your institution



# Initial testing

## Specifications:

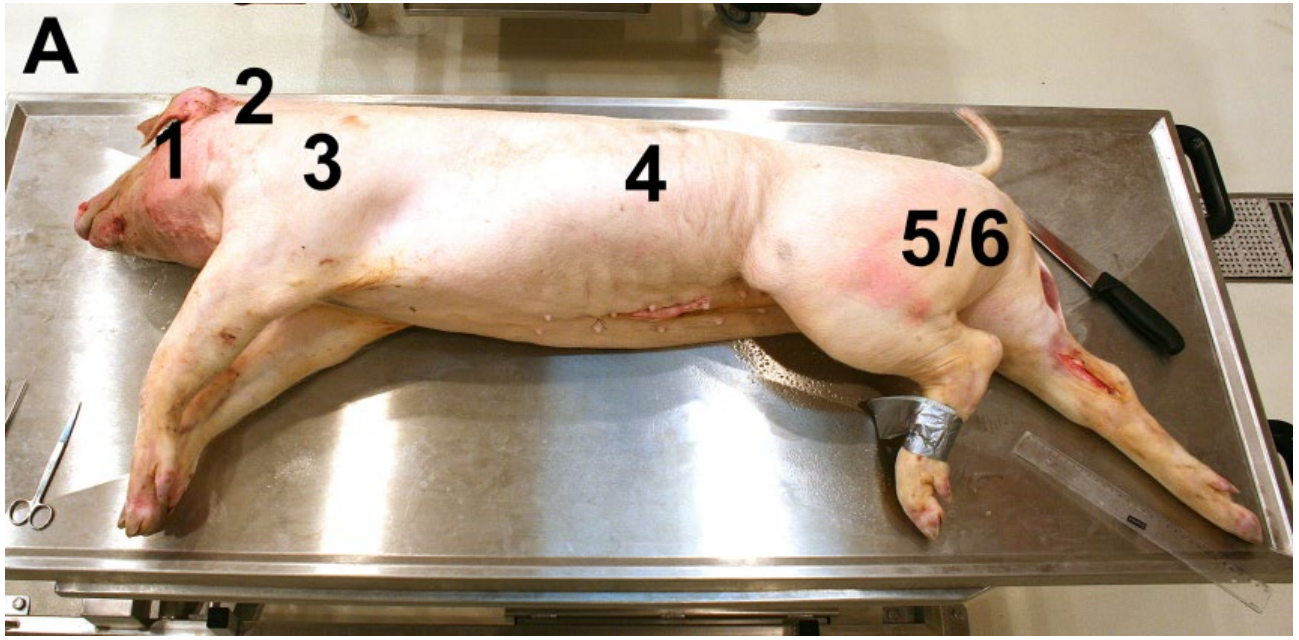
- Pig: 49.0 kg, carcass temperature (~0.5°C)
- Autoclave: chamber volume: 2.63 $m^3$,

  17-9-14 HS2, PST-series, Belimed, Switzerland
- Cycle programm: Liquid cycle, manufacturers protocol

  (134°C, 300 min)

# Initial testing

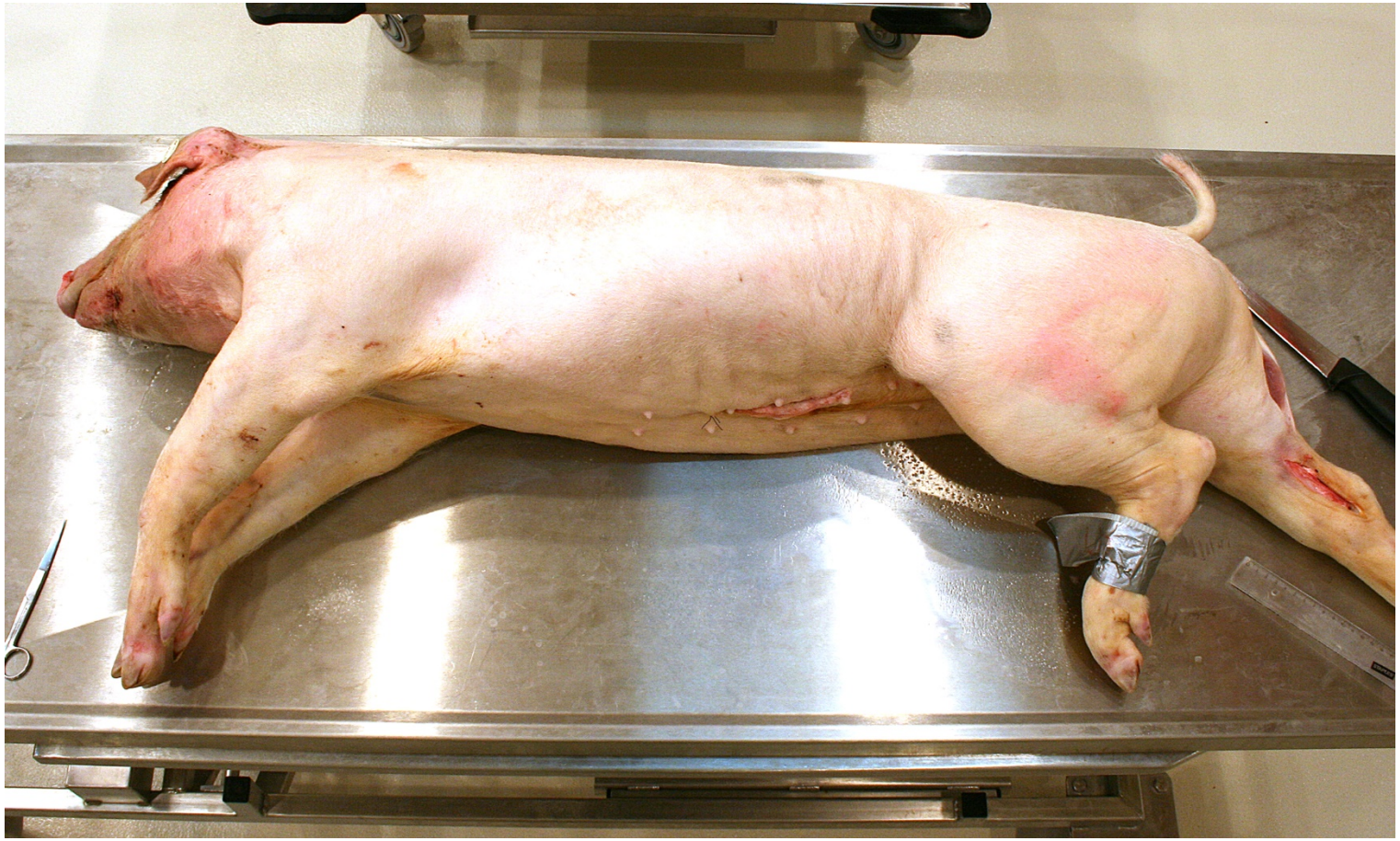
## Carcass preparation:

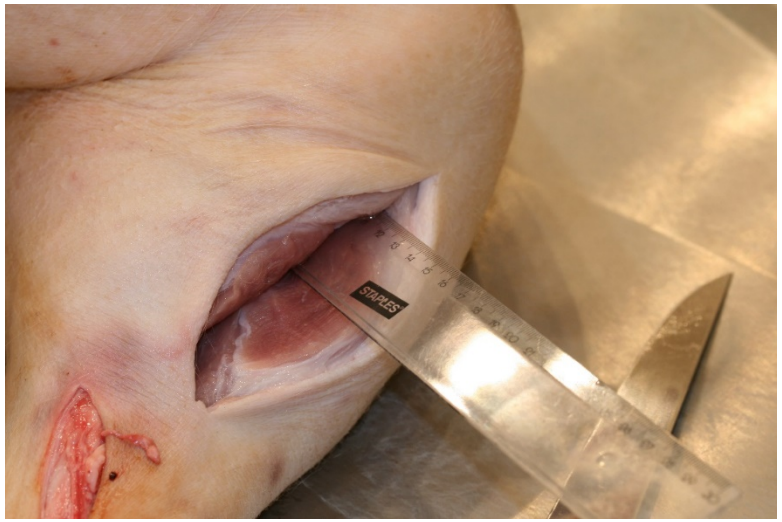


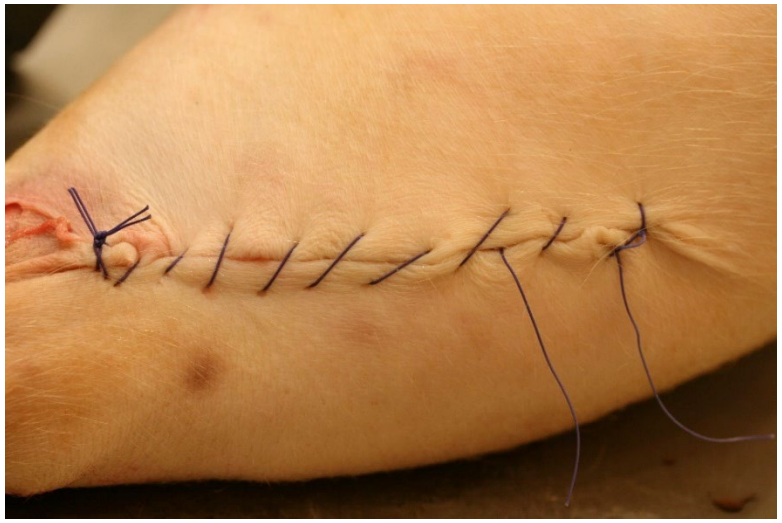
https://www.ebro.com/fileadmin/pics/Kataloge/Data_sheet_gesamt_DE_20.11.2017.pdf

- Magna Amp and ProSpore, MESA LABS, with $10^6$ *Geobacillus stearothermophilus* spores
- Data logger, EBI-12, EBRO

# Initial testing





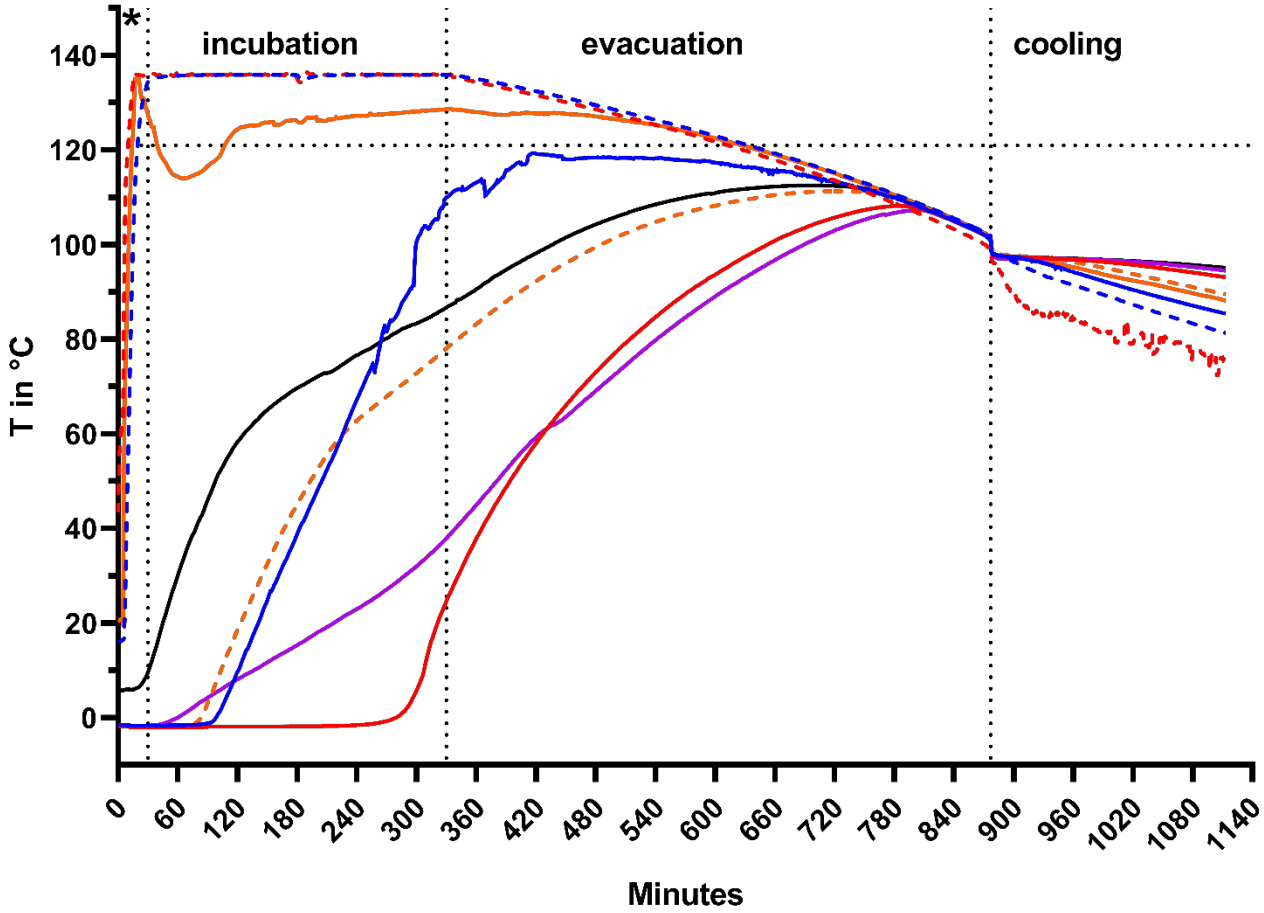



# Initial testing

# Initial testing

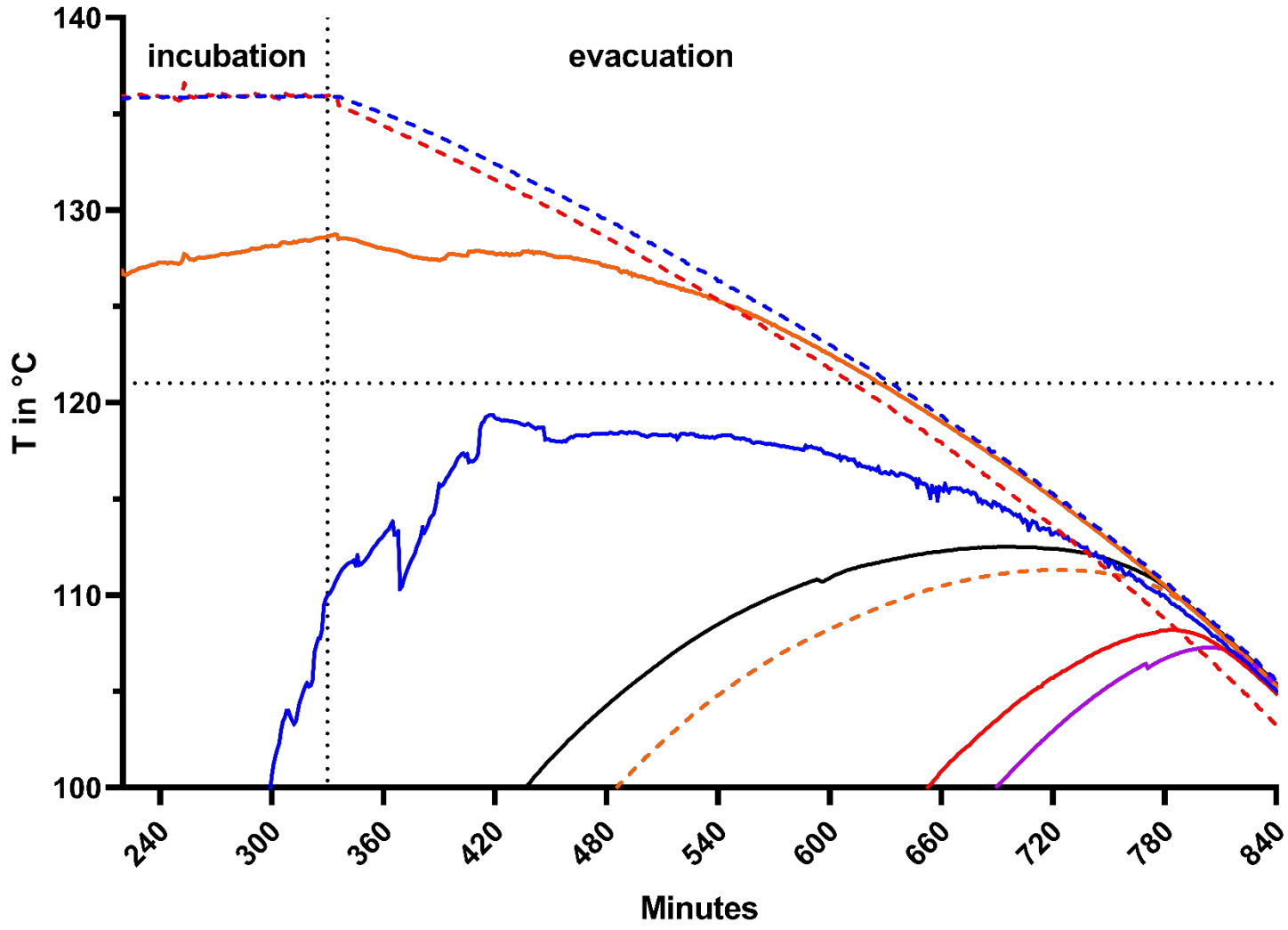
# Initial testing

| Weight in kg | T in °C | P in mbar | Vacuum in min | Heating in min | Incubation in min | Evacuation in min | Cooling in min | Σ min | Σ h |
|---|---|---|---|---|---|---|---|---|---|
| 49 | 134 | 3344 | 5 | 30 | 300 | 547 | 231 | **1113** | **18.5** |




- reference vessel (50 l $H_2O$)
- Autoclave chamber
- Head (1)
- Neck (2)
- M. triceps brachii i.m. (3)
- Abdominal cavity (4)
- M. biceps femoris s.c. (5)
- M. biceps femoris i.m. (6)

# Initial testing

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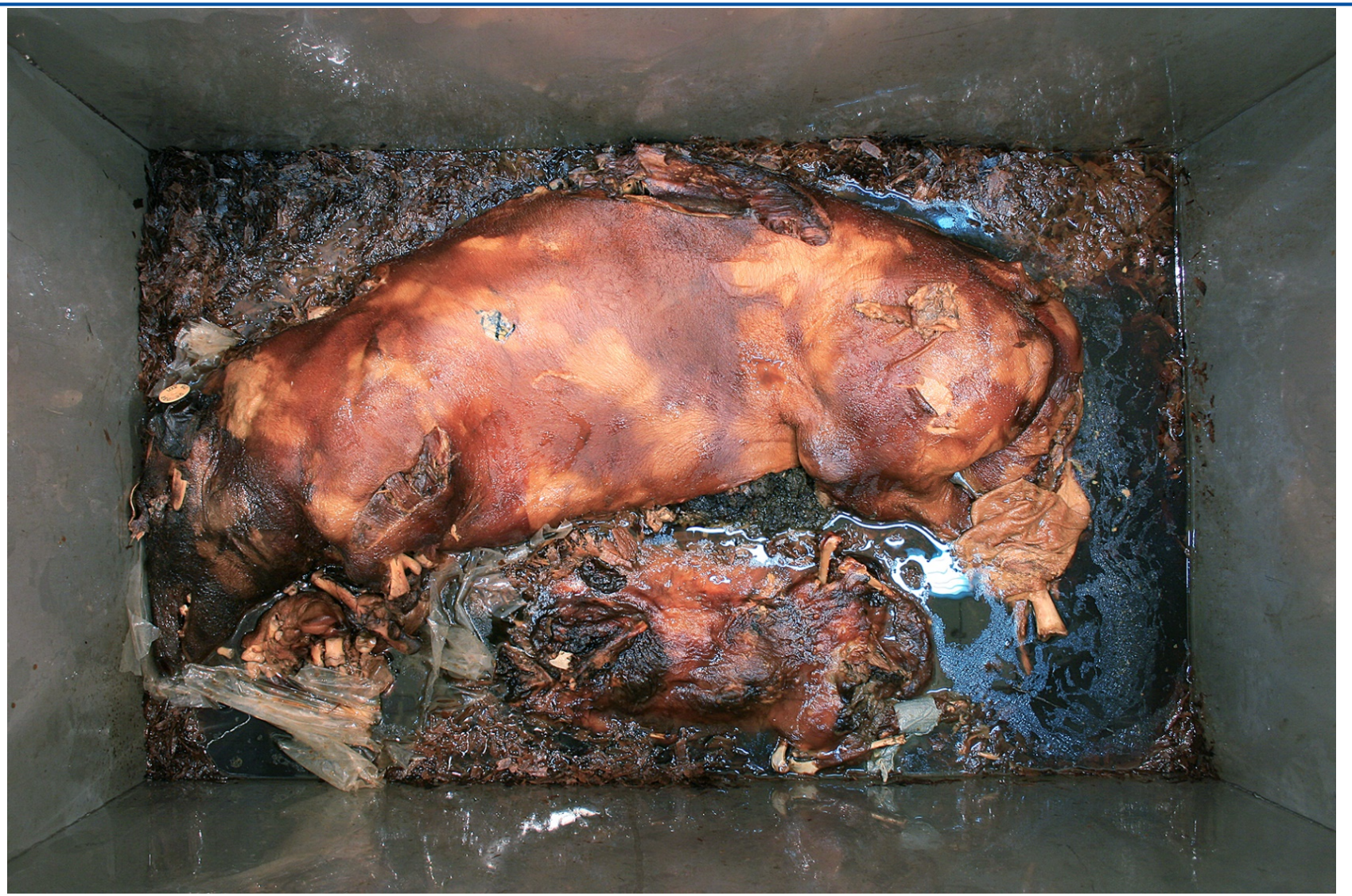
- M. biceps femoris i.m. (6)

- **T (Min):** 107.28°C, **after ~13.5 h**, abdominal **cavity**
- **T (Max):** 135.07°C, after 18 min, *M. biceps femoris s.c.*

# Initial testing



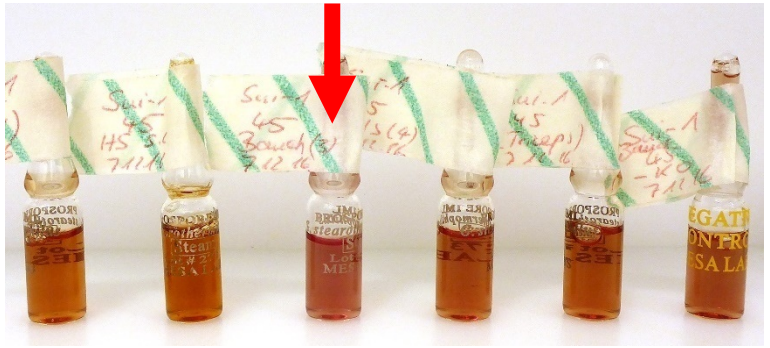
# Initial testing

# Initial testing

## Results

- Considerable amounts of condensate and meat fluid

- < 121°C in almost all probed tissues



→ 1 BI positive = no 6 $log_{10}$reduction

# Initial testing

| Conclusions: | | Adjustments: |
|---|---|---|
| 1. Avoid low starting temperatures[1] | → | Start at room temperature (20°C) |
| 2. No wrapping of carcass | → | Remove embedding material/bags |
| 3. Condensate/meat fluid | → | Lift carcass, grid above 10 cm |
| 4. Worst place = abdominal cavity | → | Put there the reference probe |
| 5. Energy and time consumption | → | 121°C, 120 min |
| 6. Reduction of tissue volume | → | Partial dissection |

[1]Bearss JJ, et al. *Applied Biosafety*. 2017;22(1):33-37

# Pigs

## Partial dissection

## Grid

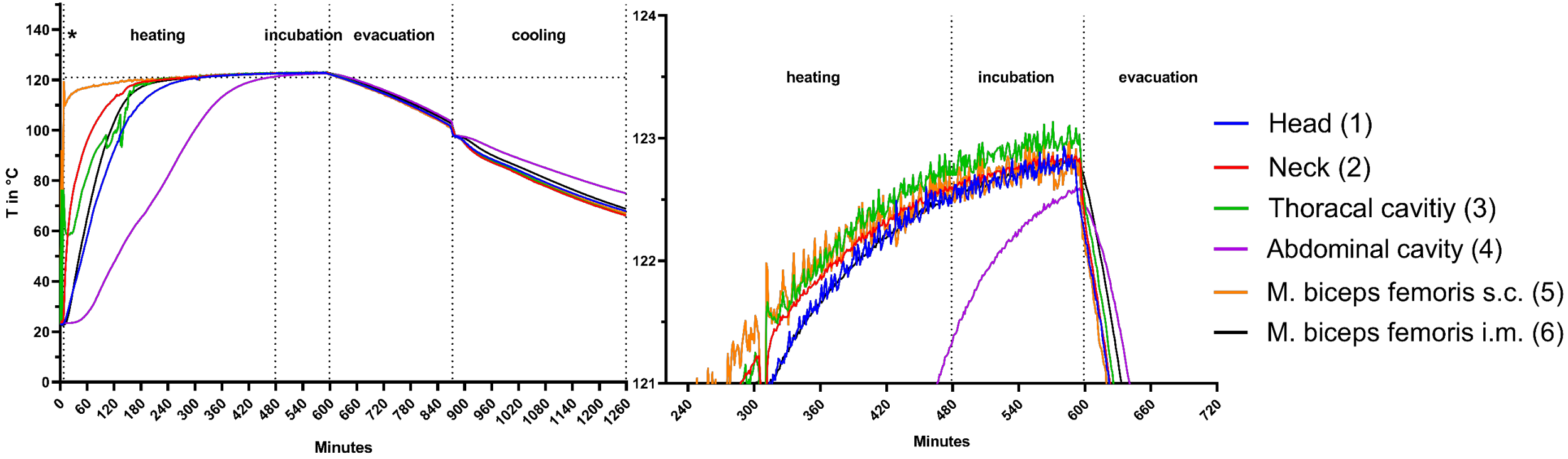
# Pigs

## Grid

## Final pig load (2x)

# Pigs

| Run | Weight in kg | T in °C | P in mbar | Vacuum in min | Heating in min | Incubation in min | Evacuation in min | Cooling in min | Σ min | Σ~ h |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 42/37 | 121 | 2269 | 8 | 471 | 120 | 274 | 386 | **1259** | **21.0** |
| **2** | 39.5/34.5 | 121 | 2228 | 7 | 382 | 120 | 264 | 337 | **1110** | **18.5** |
| **3** | 39.5/25.5 | 121 | 2236 | 8 | 351 | 120 | 263 | 302 | **1044** | **17.5** |



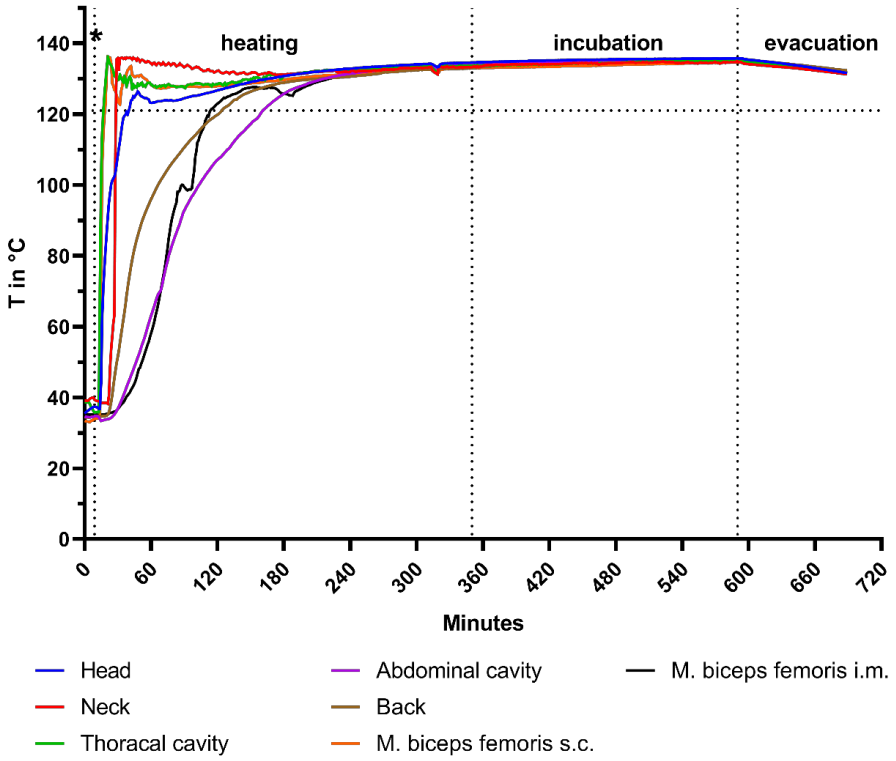
# Pigs

# Calves

# Calves

| Run | Weight in kg | T in °C | P in mbar | Vacuum in min | Heating in min | Incubation in min | Evacuation in min | Cooling in min | Σ min | Σ~ h |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 135 | 134 | 3318 | 9 | 341 | 240 | 545 | 224 | **1359** | **22.5** |
| 2 | 123 | 121 | 2245 | 9 | 401 | 120 | 270 | 352 | **1152** | **19.0** |
| 3 | 101 | 121 | 2259 | 9 | 688 | 120 | 270 | 381 | **1468** | **24.5** |

A

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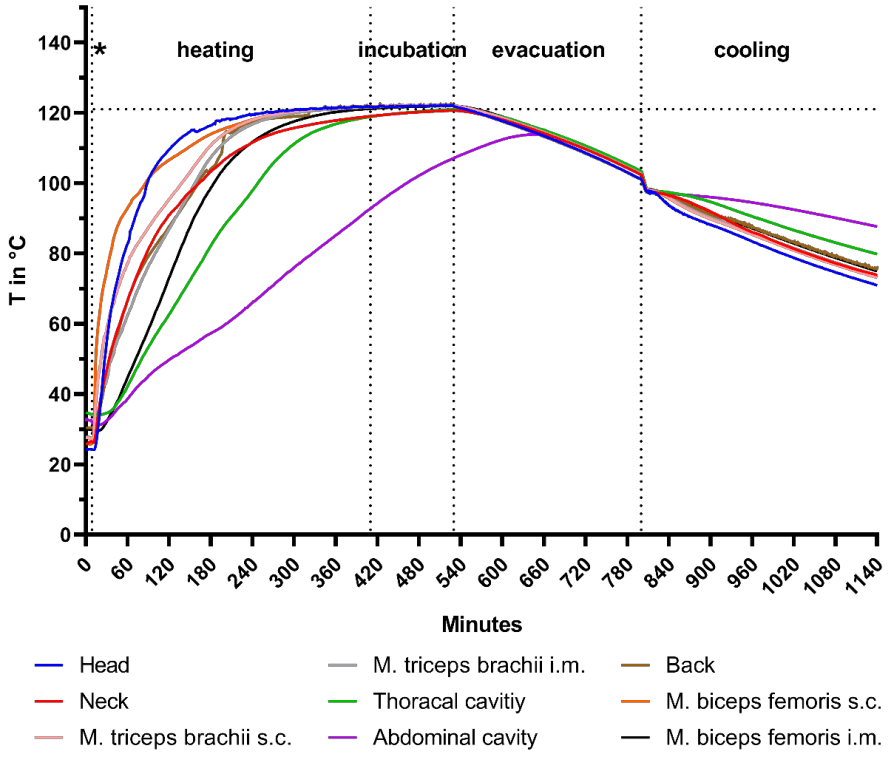


# Calves

# Calves

**Hypothesis:** ruminant gastro-intestinal system → high fluid and ingesta content increasing evaporation of fluids → displacement of abdominal organs → moving the reference probe

# Final conclusions

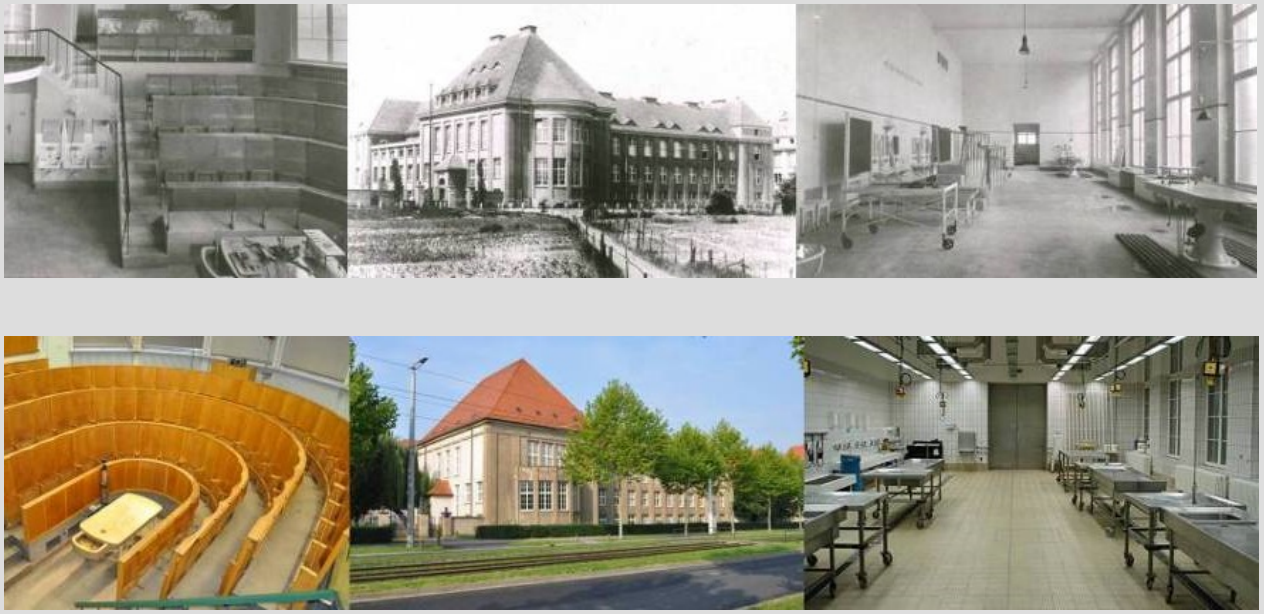
1. Minimal dissection, no wrapping
2. Reference probe at worst location (abdominal cavity)
3. Energy and time consumption (up to 24 h)
4. Highly reproducible for pigs (up to 42 kg)
5. Variable results for calves (multi-gastric species, 134°C)

→ tissue grinder or alkaline hydrolysis method of choice

# Acknowledgement


**Institute of Veterinary Pathology**
Reiner Ulrich

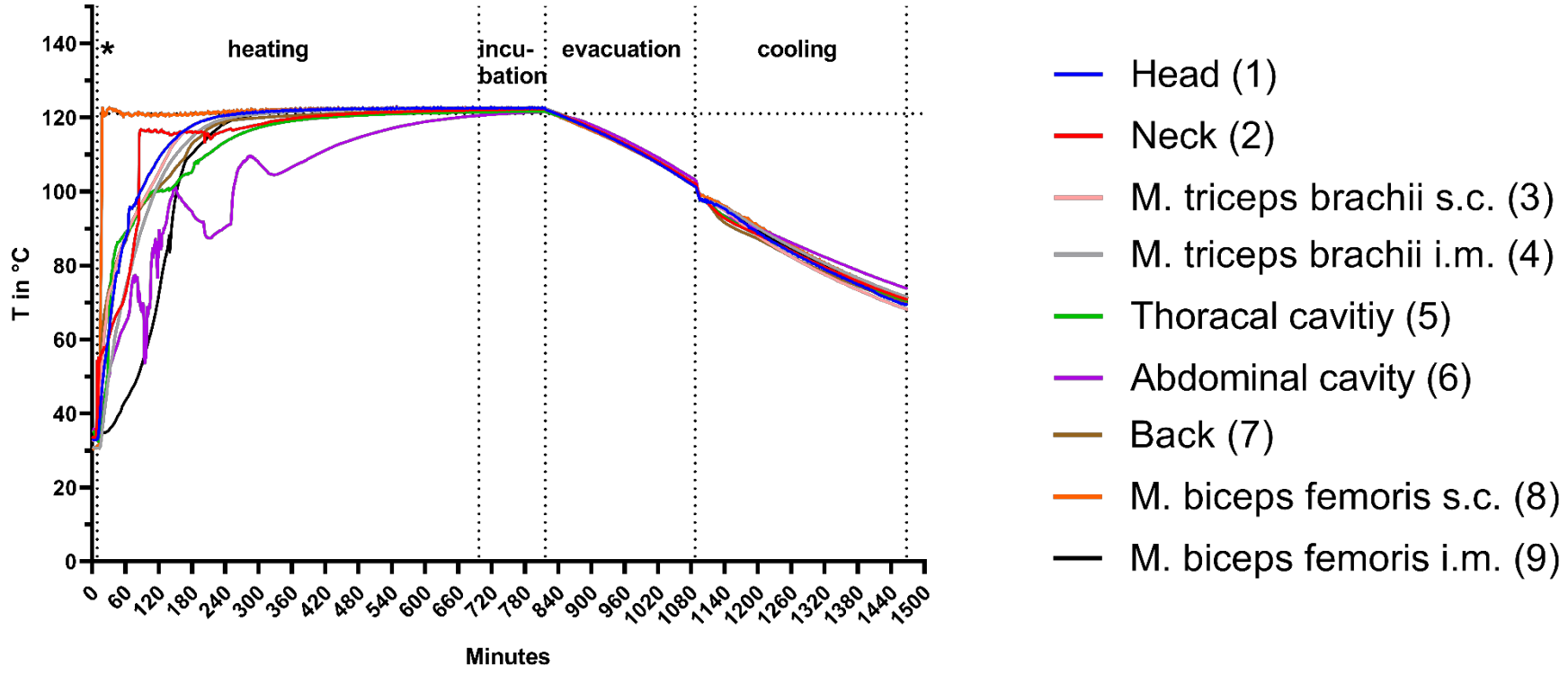
**FLI**
Thomas C. Mettenleiter
Bärbel Hammerschmidt, Charlotte Schröder, Matthias Jahn, Frank Klipp, Markus Ball and Holger Blank

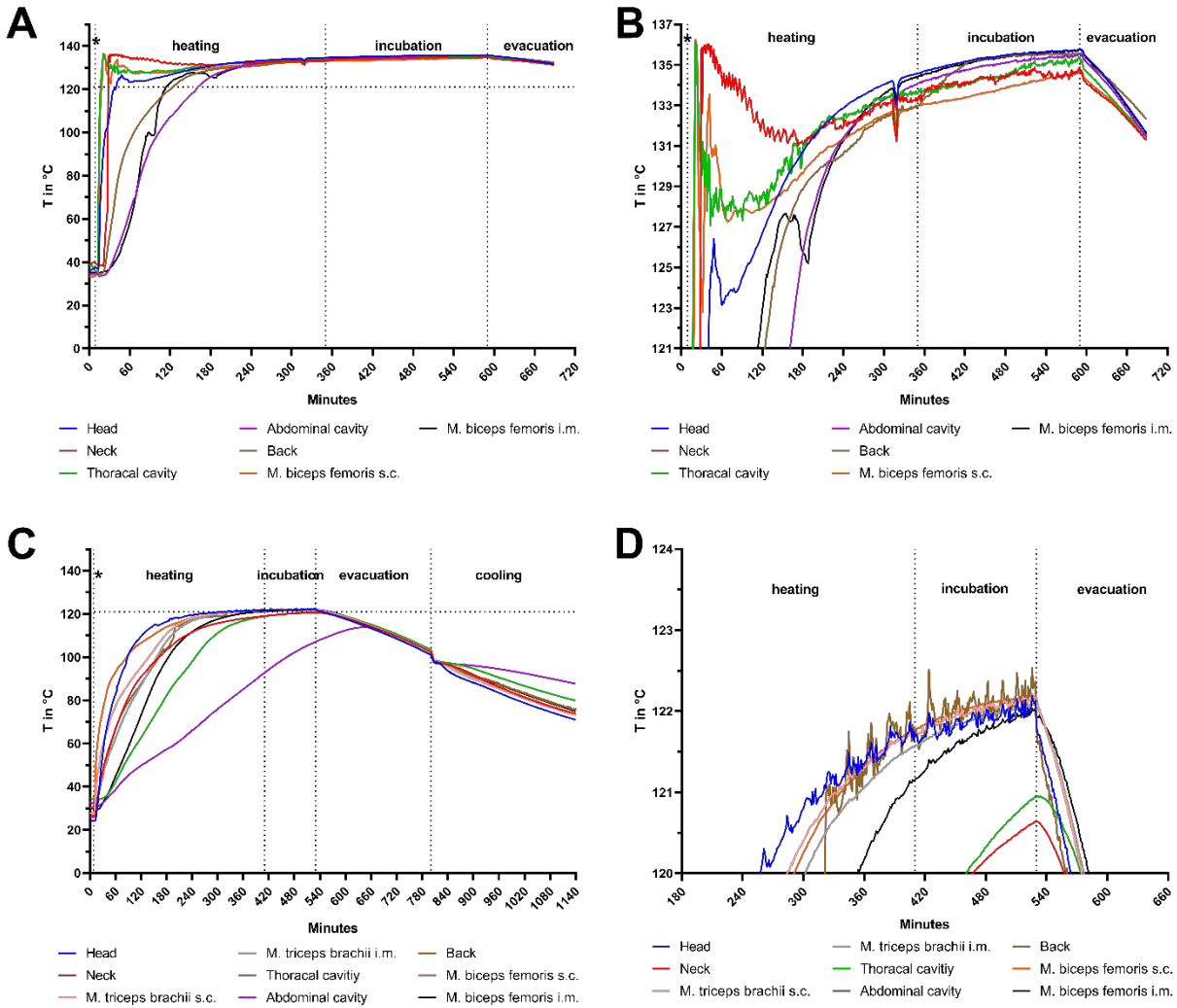
**Thank you for your attention!**

# Calves

| Run | Weight in kg | T in °C | P in mbar | Vacuum in min | Heating in min | Incubation in min | Evacuation in min | Cooling in min | Σ min | Σ~ h |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 135 | 134 | 3318 | 9 | 341 | 240 | 545 | 224 | **1359** | **22.5** |
| 2 | 123 | 121 | 2245 | 9 | 401 | 120 | 270 | 352 | **1152** | **19.0** |
| 3 | 101 | 121 | 2259 | 9 | 688 | 120 | 270 | 381 | **1468** | **24.5** |

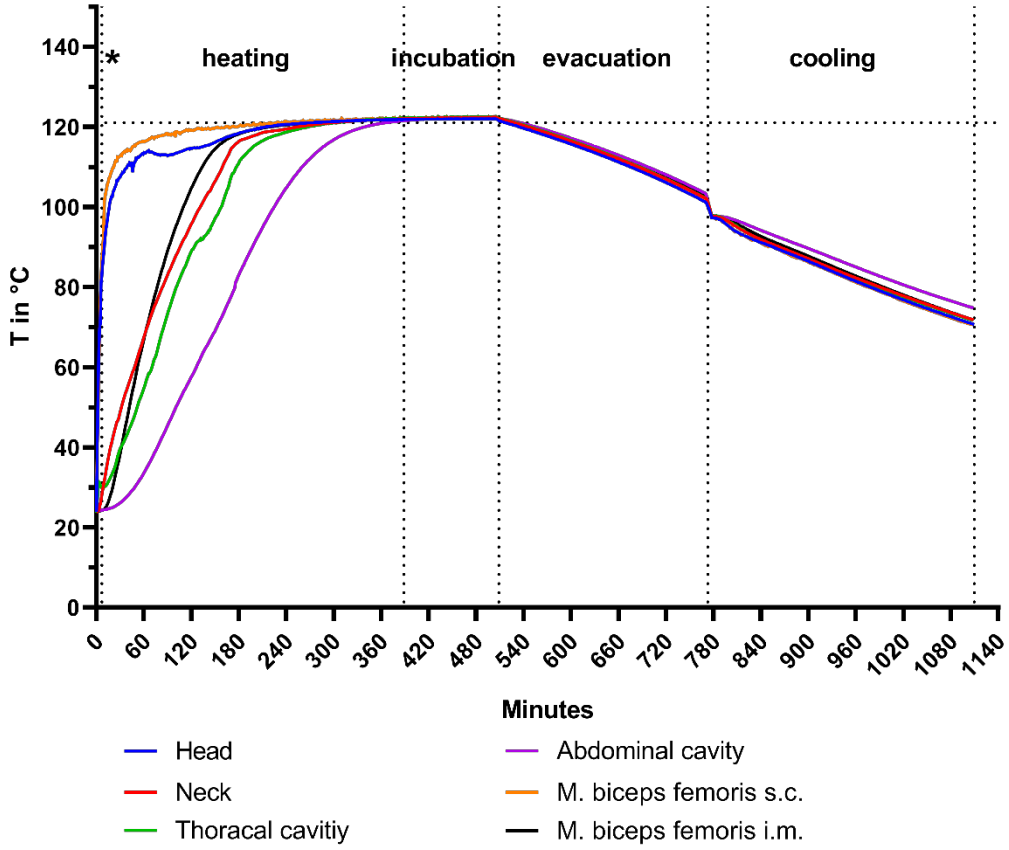


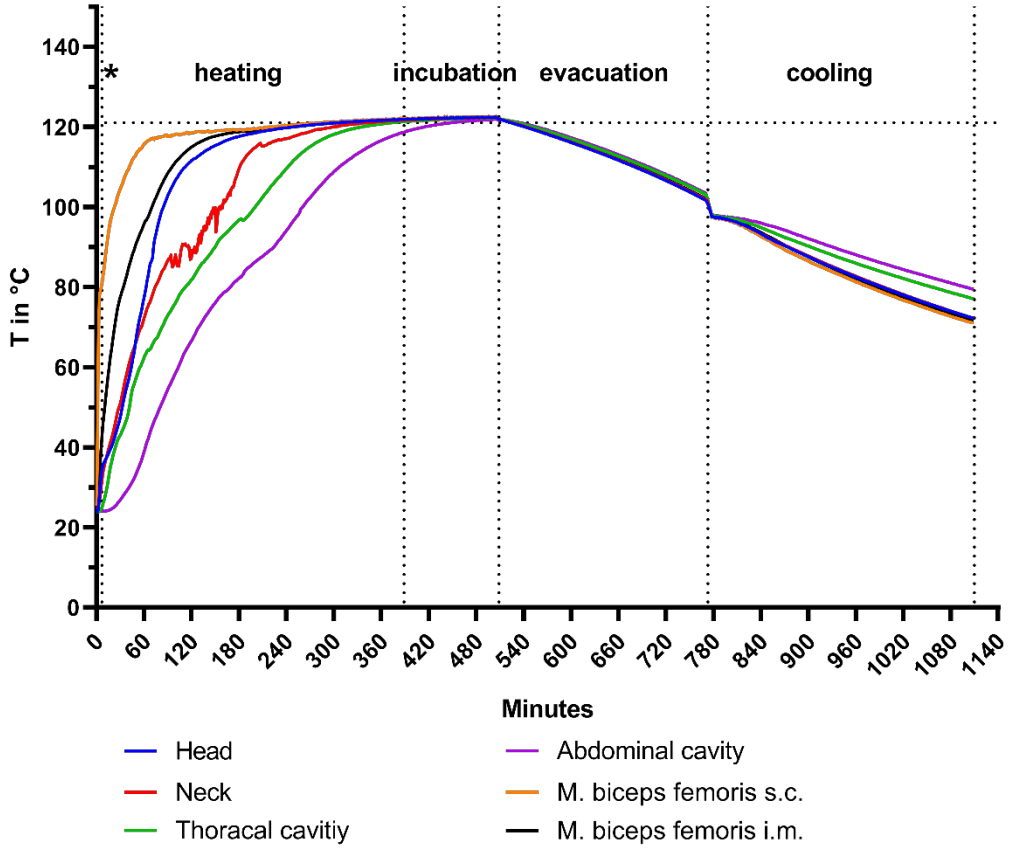
# Extra files


A
B
C
D
heating
incubation
evacuation
cooling
T in °C
Minutes
Head
Neck
Thoracal cavity
Abdominal cavity
Back
M. biceps femoris s.c.
M. biceps femoris i.m.
M. triceps brachii i.m.
Thoracal cavitiy
M. triceps brachii s.c.

# Extra files

## Pig run 2



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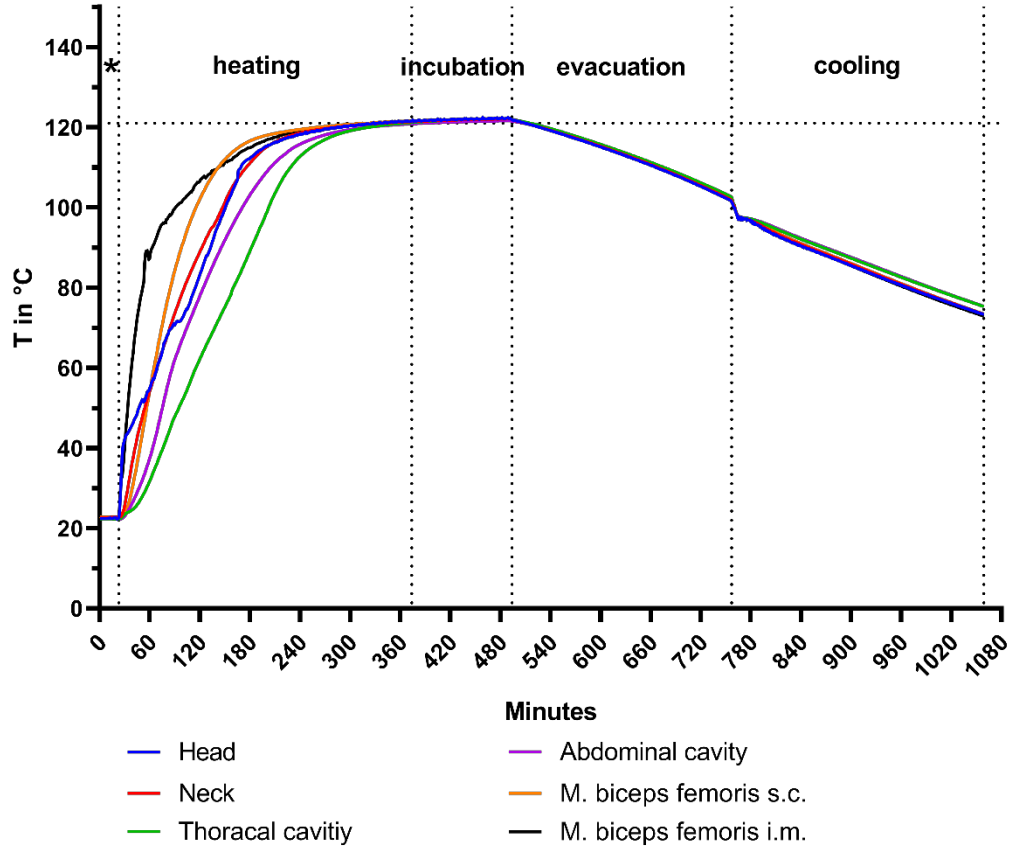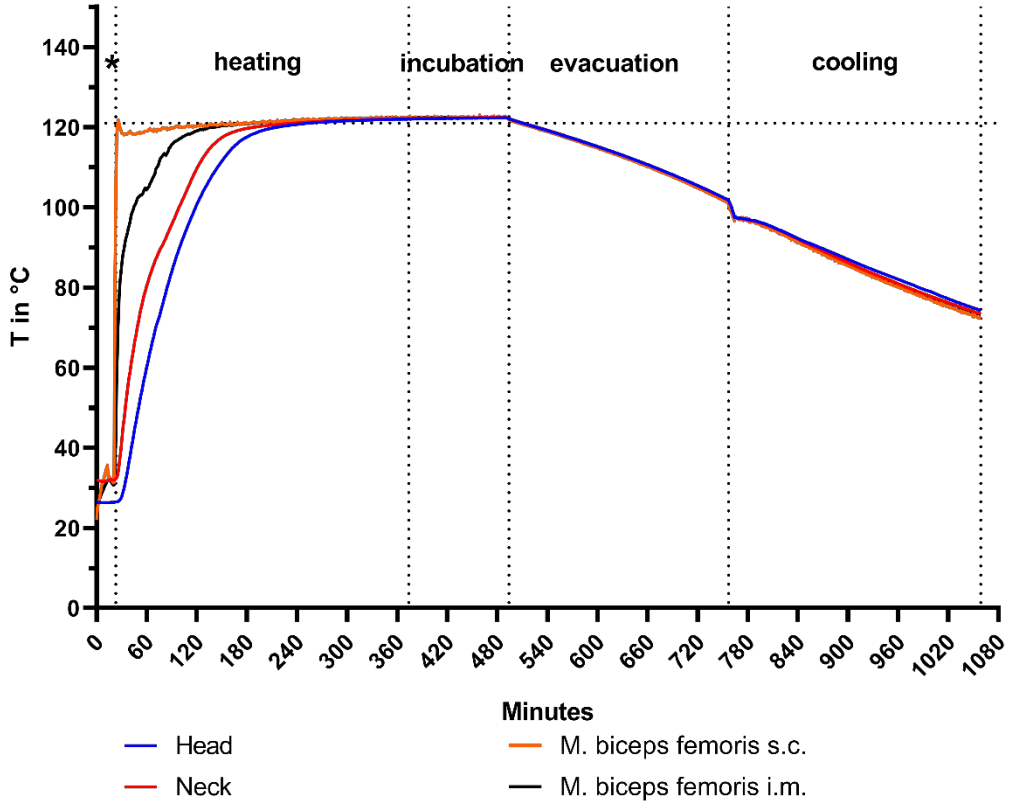
## Pig run 3