UGhent Center for
Strategic Prophylaxis and
Vaccine Development

PROVAXS

The immune response against *Chlamydia suis* genital tract infection partially protects against re-infection

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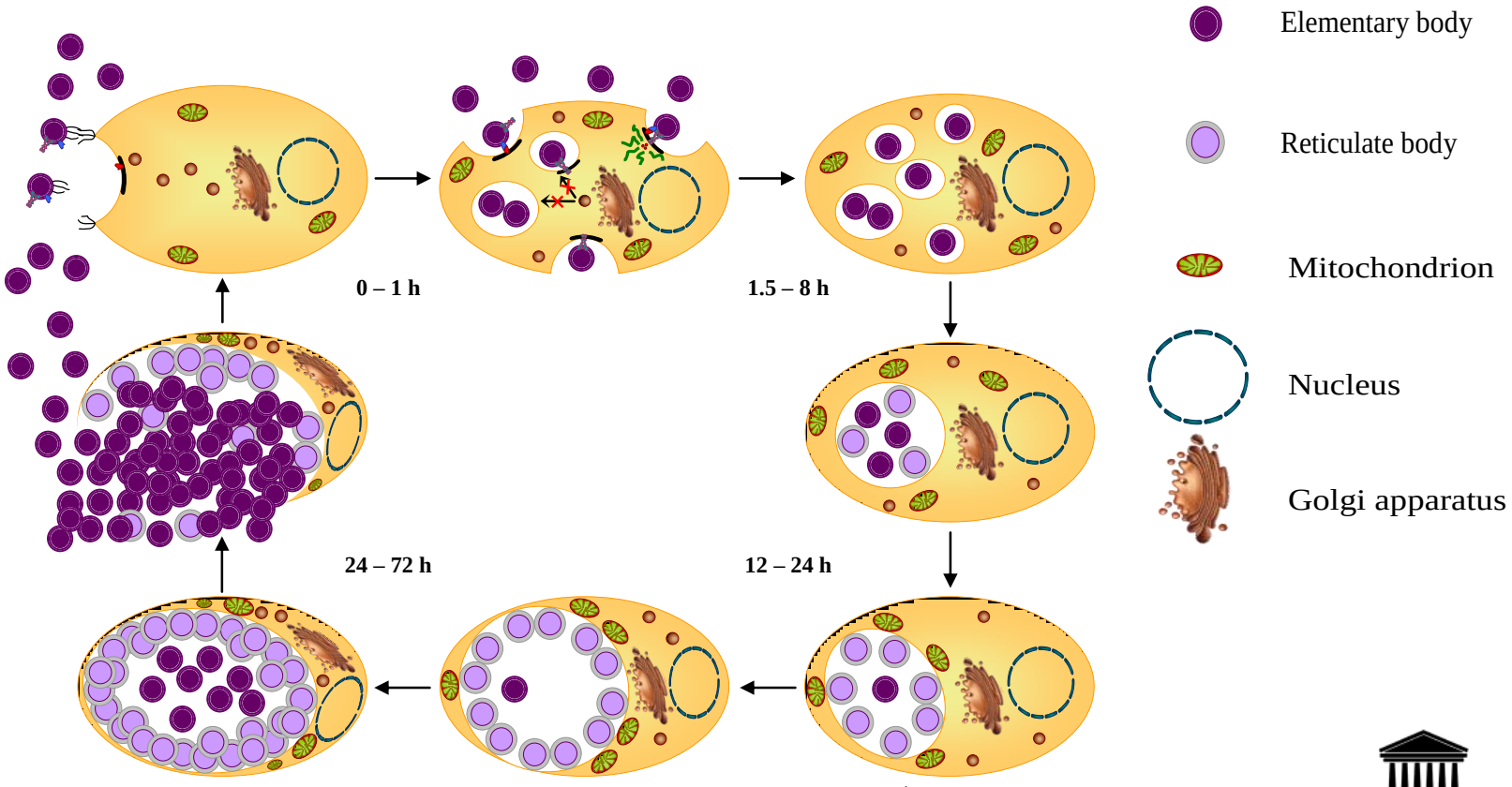
INTRODUCTION

CHLAMYDIACEAE

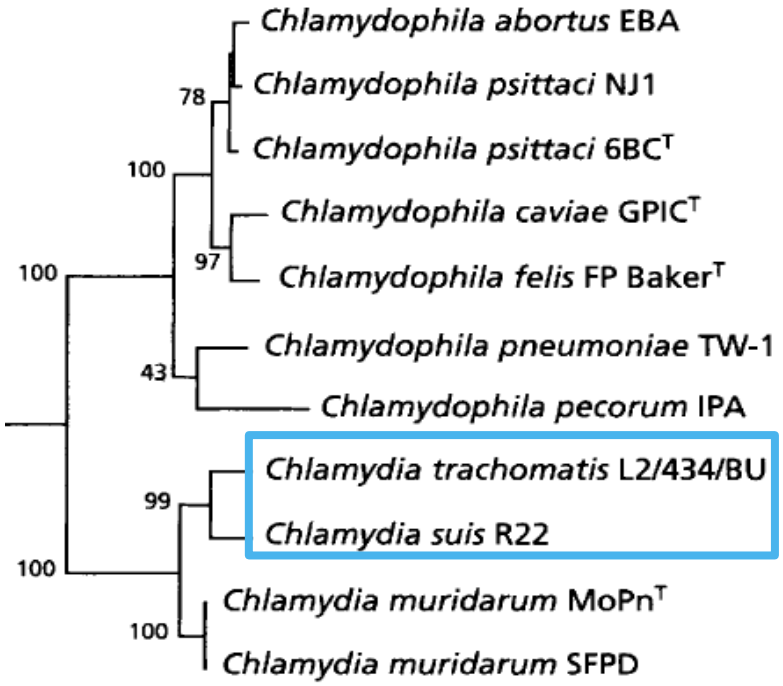
Obligate intracellular gram-negative bacteria



INTRODUCTION

CHLAMYDIA SUIS & TRACHOMATIS

- *C. suis* phylogenetically highly related to *C. trachomatis*



- *C. trachomatis* and *C. suis*
  - Sexually transmitted disease
    - Reproductive failure
  - Kerato-conjunctivitis
    - Trachoma and blindness

- *Chlamydia suis* zoonosis
  - Eye infections in humans
    - Trachoma patients in Nepal (Dean et al., 2013)
    - Swine abattoir employees in Belgium (De Puysseleyr et al., 2014)

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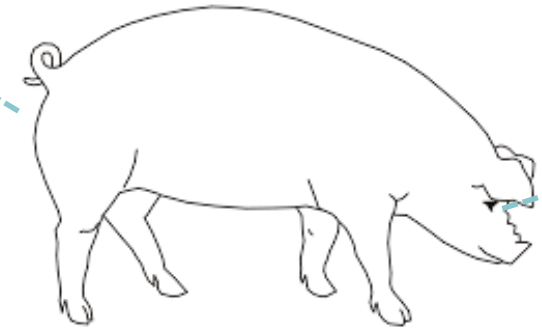
INTRODUCTION

CHLAMYDIA SUIS IN PIGS

- High prevalence of *Chlamydia suis* in the pork industry
  - Subclinical infections (as in humans)
  - Clinical infections (as in humans)
    - Economic losses

REPRODUCTIVE TRACT

- Early embryonic death
- Low number of piglets
- Non-uniform piglet weight
- Inferior semen quality



EYE

Conjunctivitis

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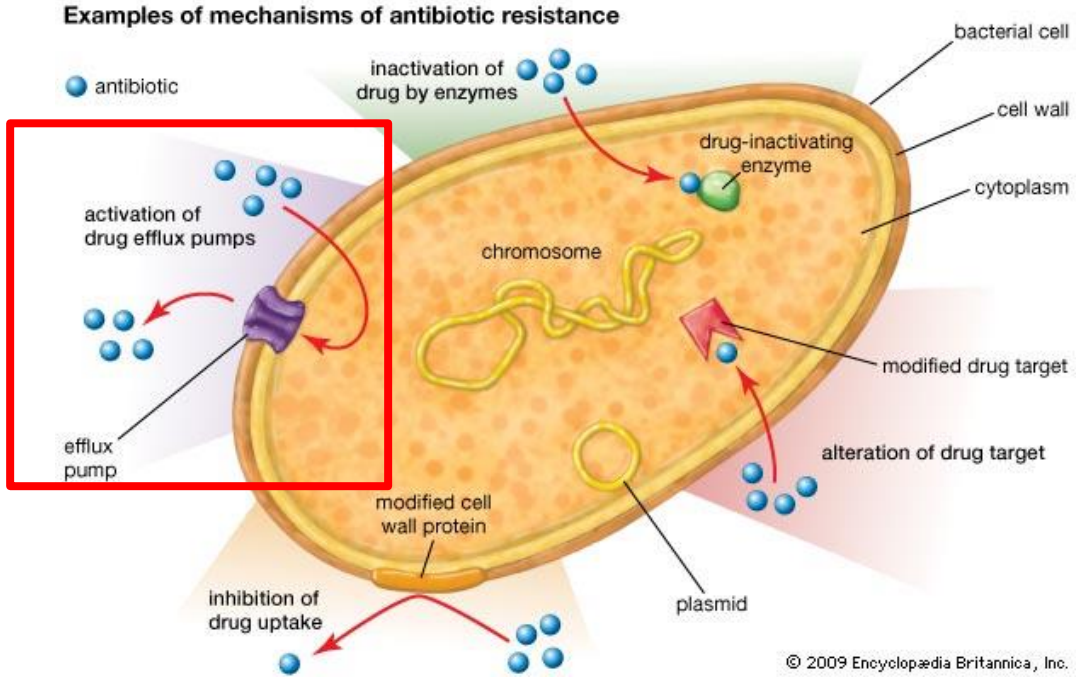
INTRODUCTION

TETRACYCLINE RESISTANCE

- Treatment of chlamydial infections
  - Tetracyclines
    - Broad spectrum, low toxicity, tissue distribution, inexpensive
- Tetracycline resistant *Chlamydia suis*
  - *tet(C)* gene
  - Many countries
    - United States
    - The Netherlands
    - Belgium
    - Switzerland
    - Italy
    - Cyprus
    - Israel
    - Germany



EXPERIMENTAL VAGINAL *C. SUIS* INFECTION AND RE-INFECTION IN GILTS

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- ***Chlamydia* vaginal experimental infection model SPF pigs**
  - Vanrompay et al., Infect. Immun, 2005; De Clercq et al., Vet. Res., 2014
- **Experimental infection and re-infection of gilts**
  - Vaginal infection (10^7 IFU of *C. suis* strain S45) using 3 groups (n = 5) of gilts:

1. Macroscopic lesions at autopsy + histopathology
2. Vaginal excretion and *C. suis* replication in tissues
3. Immune response:
   - Serum and mucosal IgM, IgG and IgA antibodies (ELISA)
   - T cell proliferation assays on PBMC, spleen and lnn MC
   - Flow cytometry on PBMC, spleen and lnn MC
   - Cytokine & chemokine ELISA's on PBMC, spleen and lnn MC

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MATERIALS AND METHODS

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GROUP TREATMENT	1 Controls (n = 5)	2 Infection group (n = 5)	3 Infection/re-infection group (n = 5)
***C. suis* S45** D13: age 9 weeks	**PBS**	**PBS**	**Primary infection**
***C. suis* S45** D69: age 17 weeks	**PBS**	**Primary infection**	**Secondary infection**
Euthanasia D90: age 20 weeks	✝	✝	✝

RESULTS: MACROSCOPIC LESIONS

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Macroscopic lesions at euthanasia

- Infection group
  - All pigs large amounts of serous fluid in the urogenital tract, strong congestion of the ligaments + mucosae and enlarged local draining lnn, sometimes with congestion
- Re-infection group
  - 4 of 5 pigs no serous fluid and all severely enlarged local draining lnn, but no congestion of the lnn

→ **Scores for macroscopic lesions significantly higher in the infection group as compared to the re-infection group**

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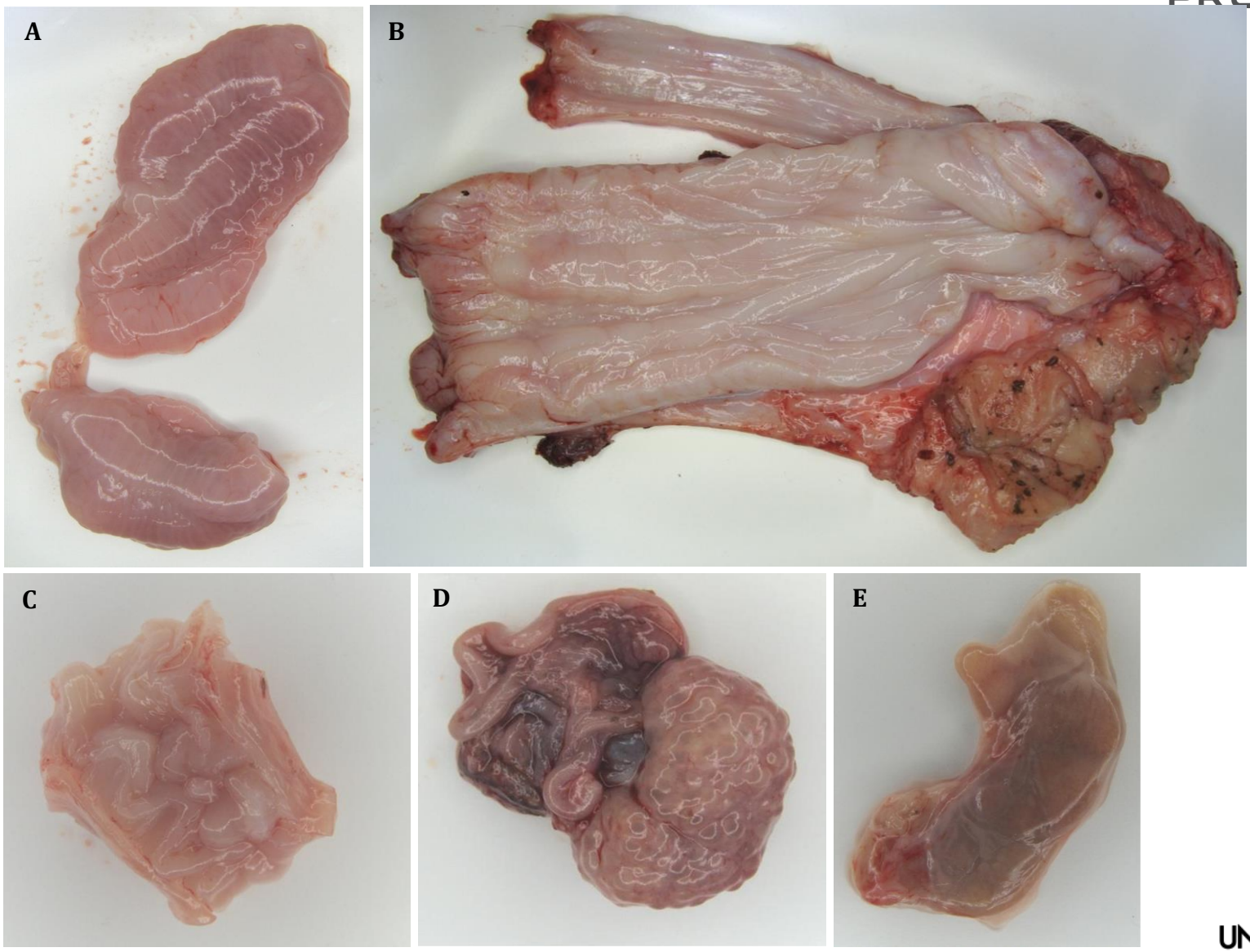
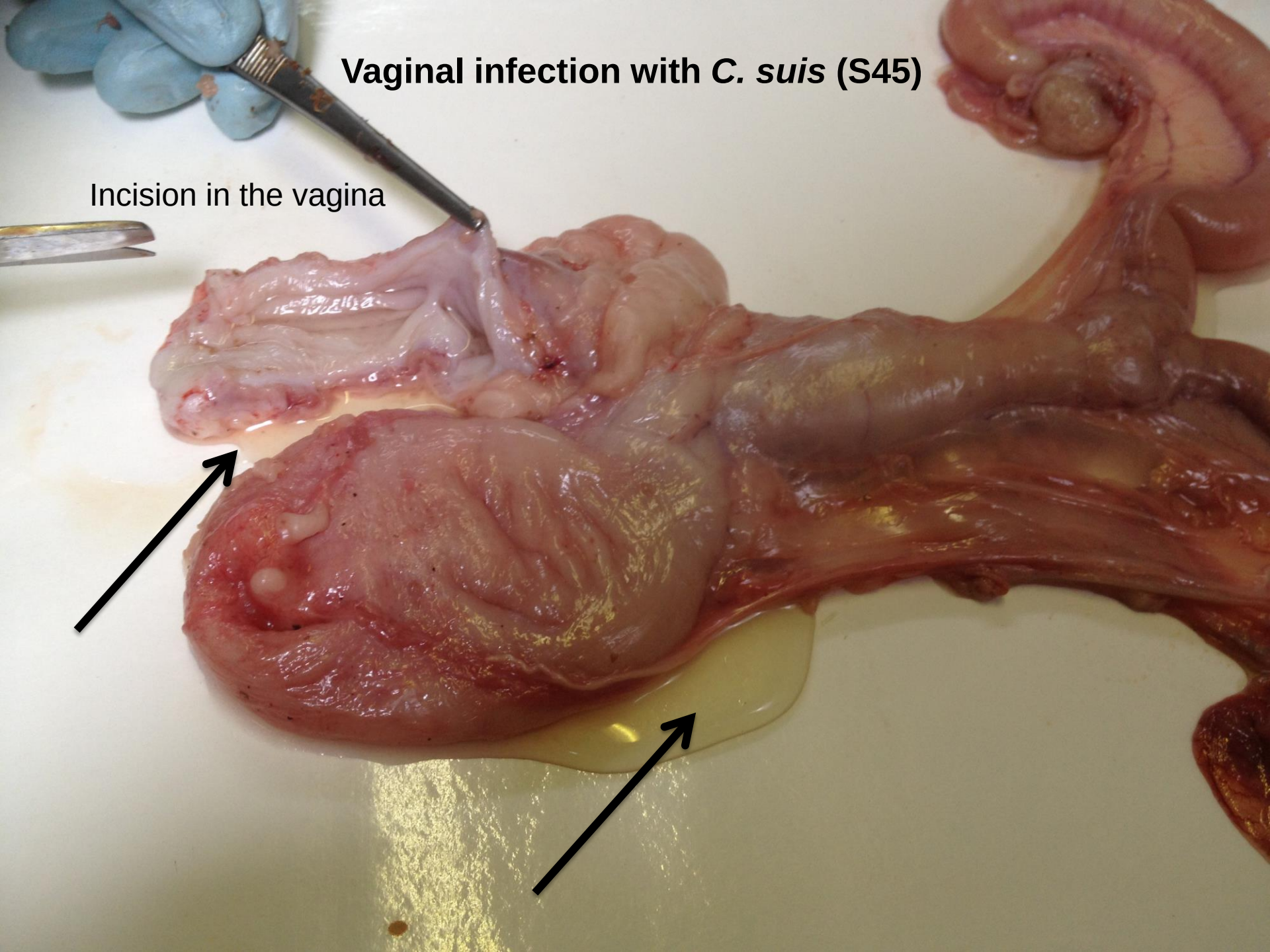
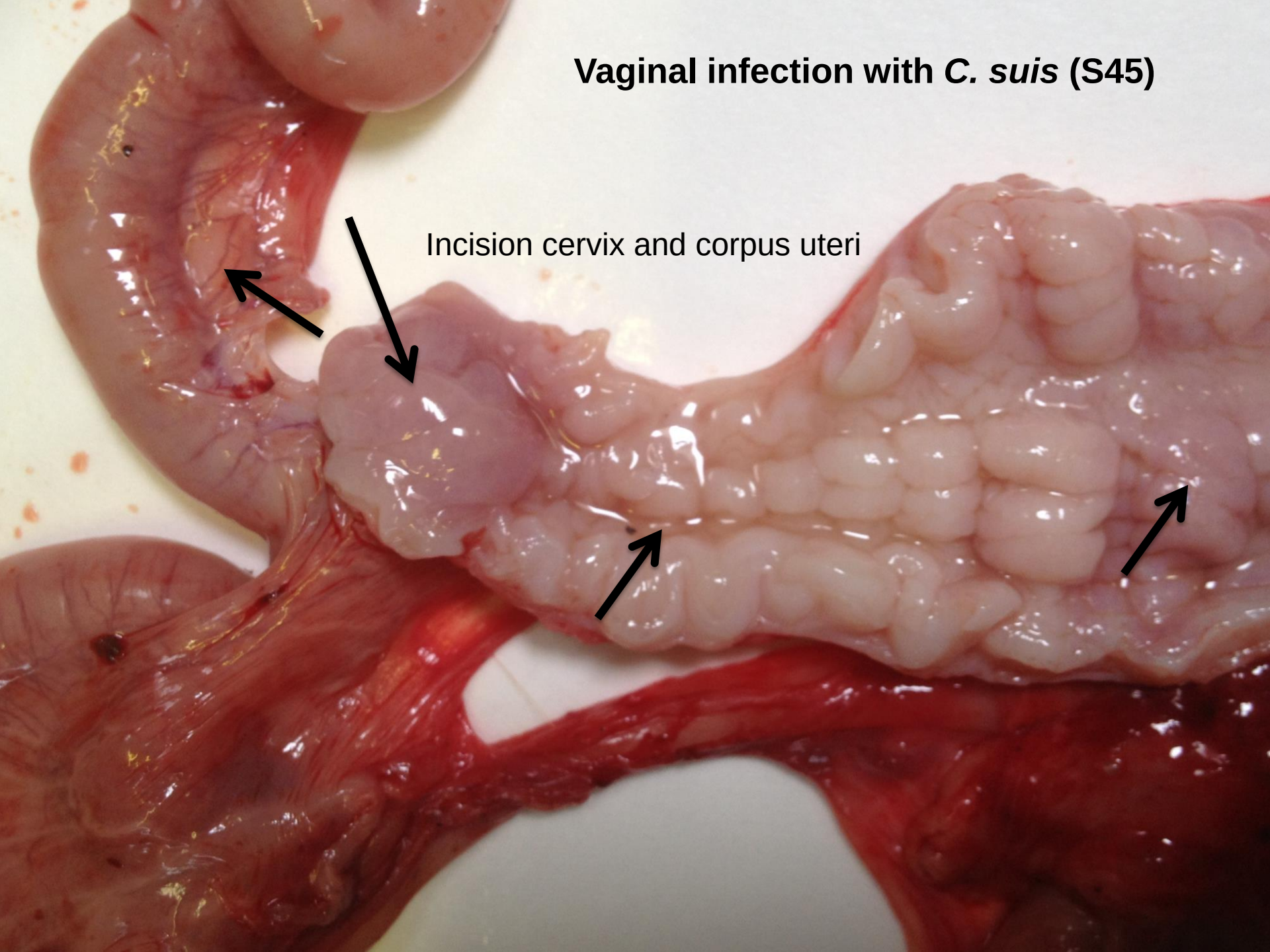
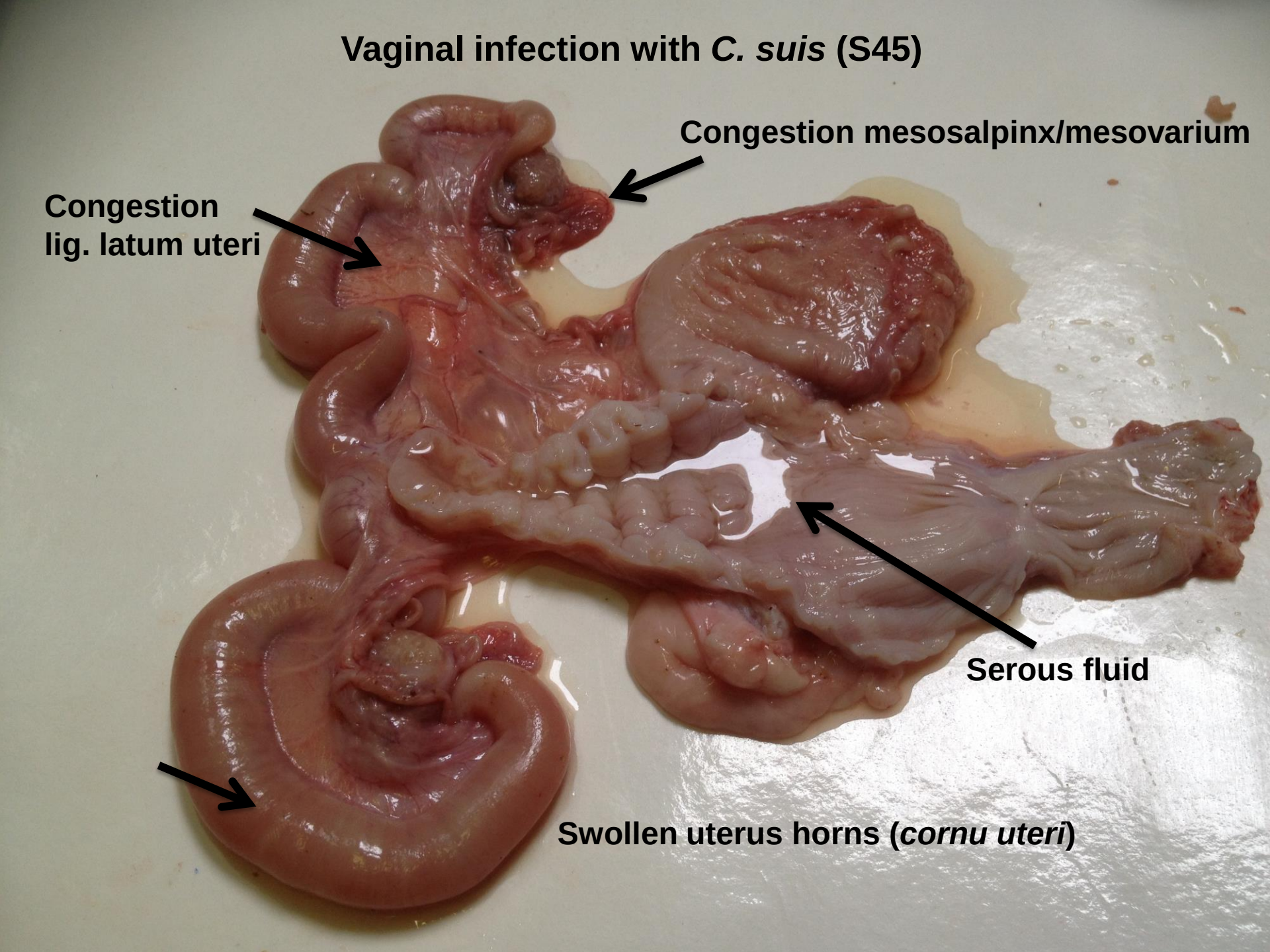
Non infected control gilt

Non infected control pig

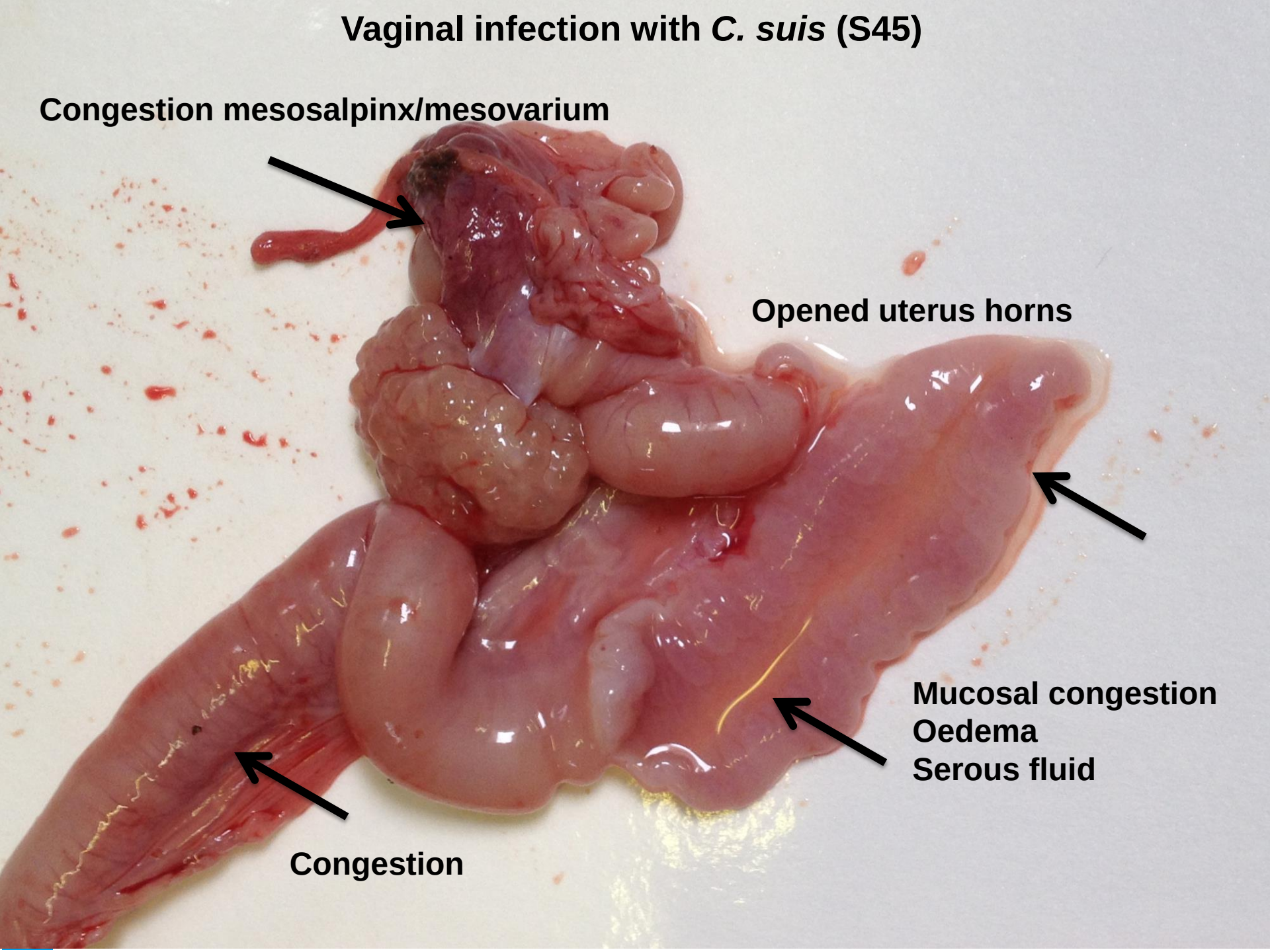



Vaginal infection with *C. suis* (S45)
Incision in the vagina

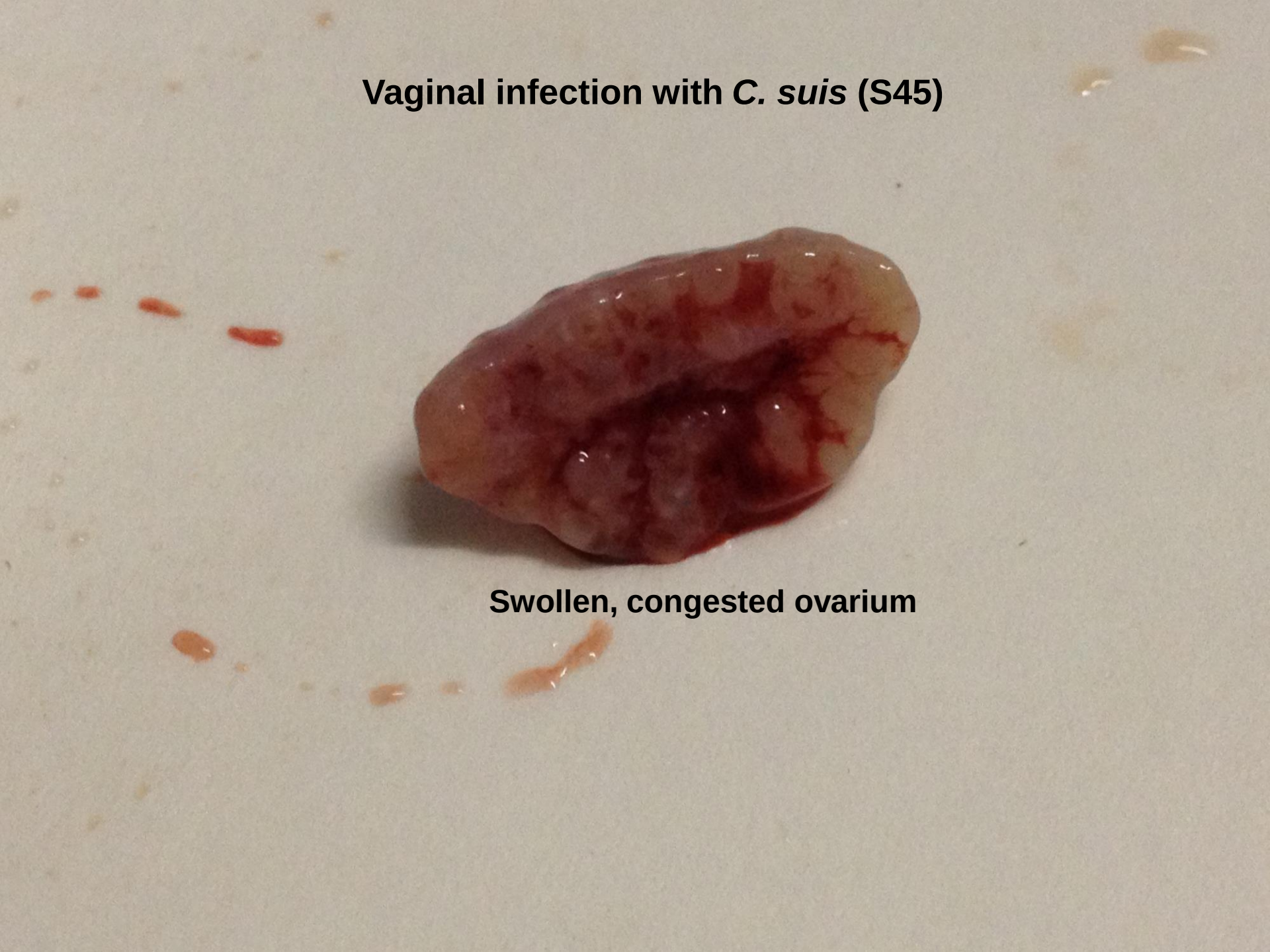

Vaginal infection with *C. suis* (S45)
Incision cervix and corpus uteri


Vaginal infection with *C. suis* (S45)
Congestion mesosalpinx/mesovarium
Congestion
lig. latum uteri
Serous fluid
Swollen uterus horns (*cornu uteri*)

Vaginal infection with *C. suis* (S45)

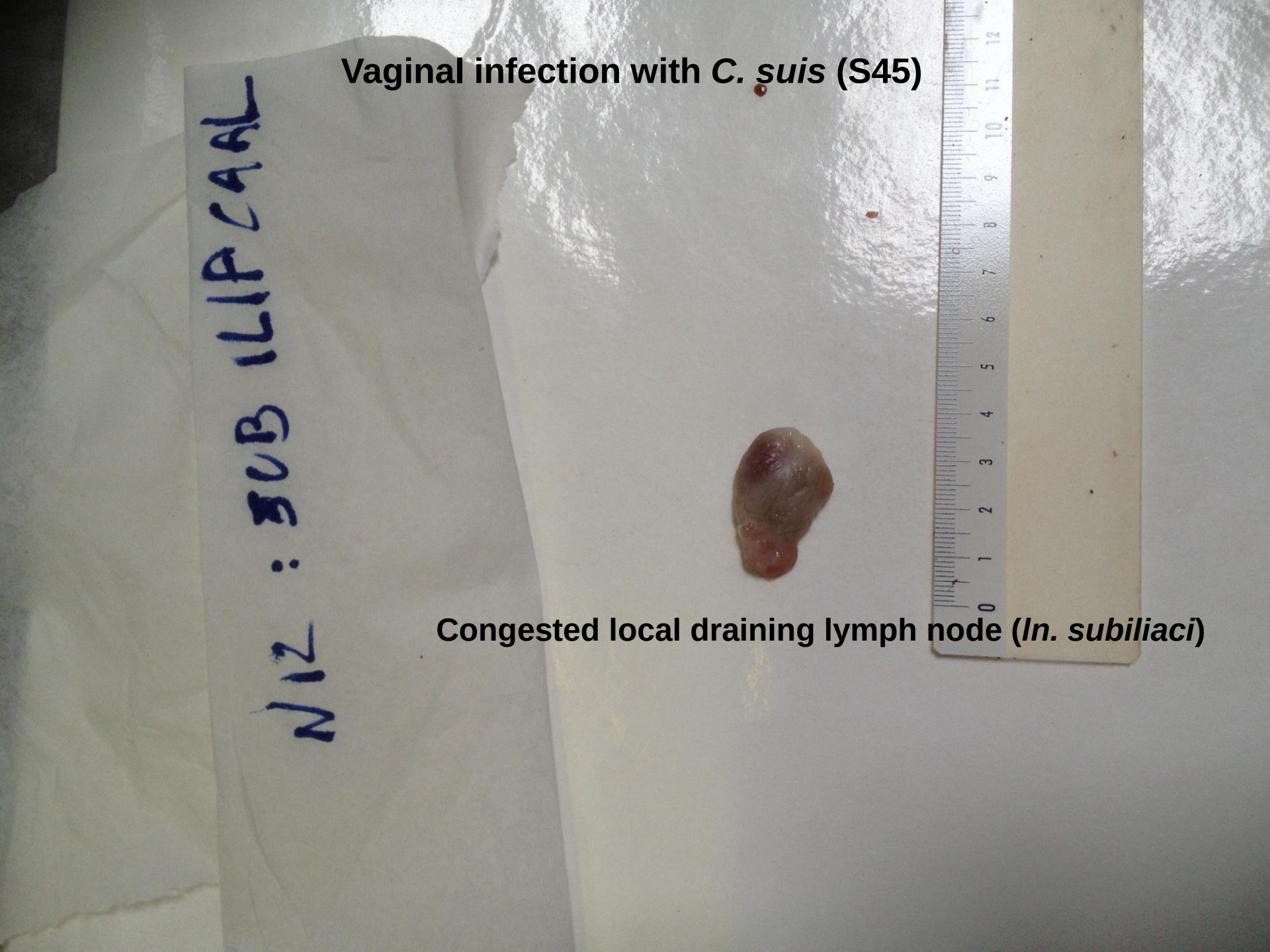


Vaginal infection with *C. suis* (S45)

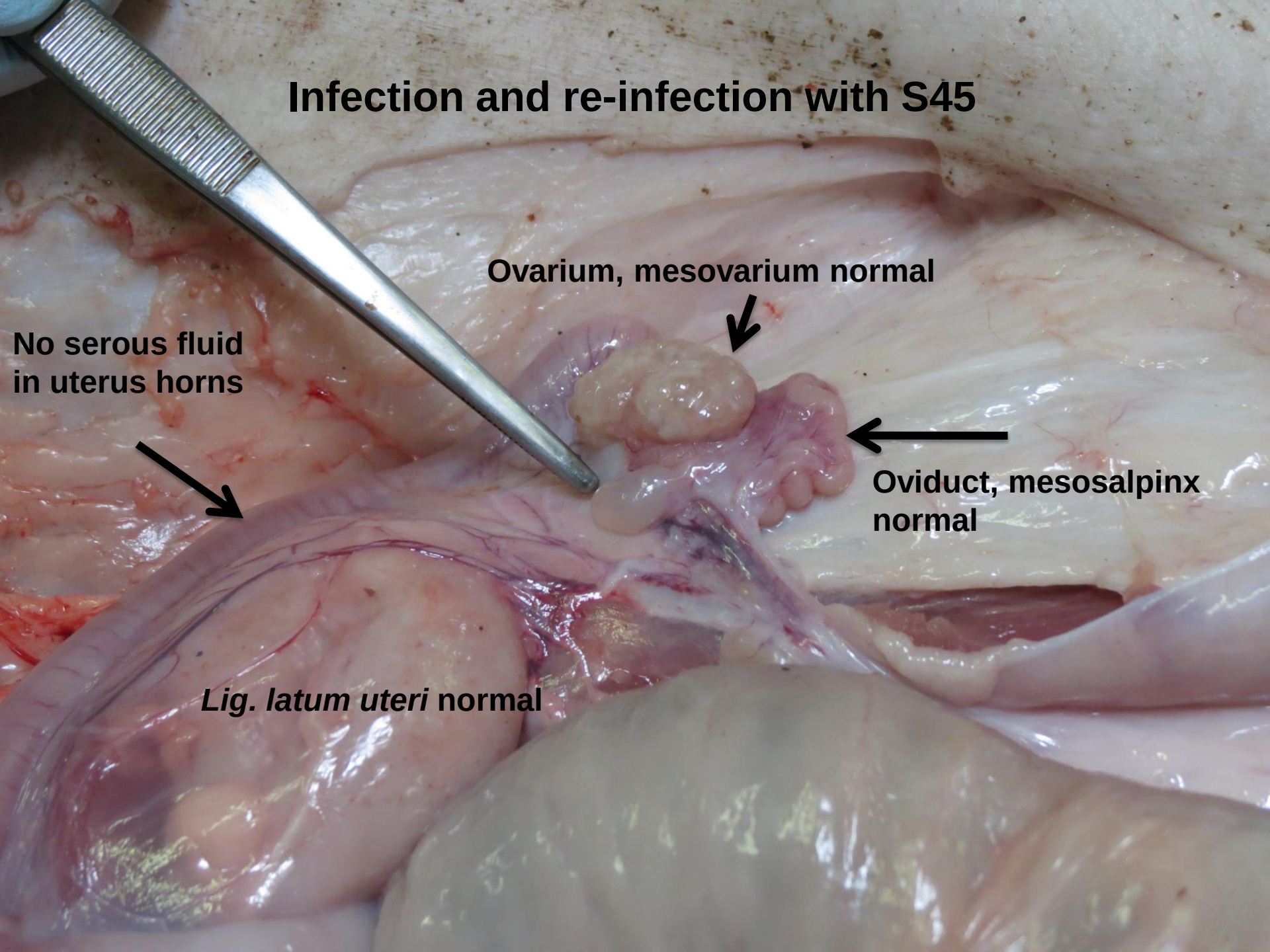


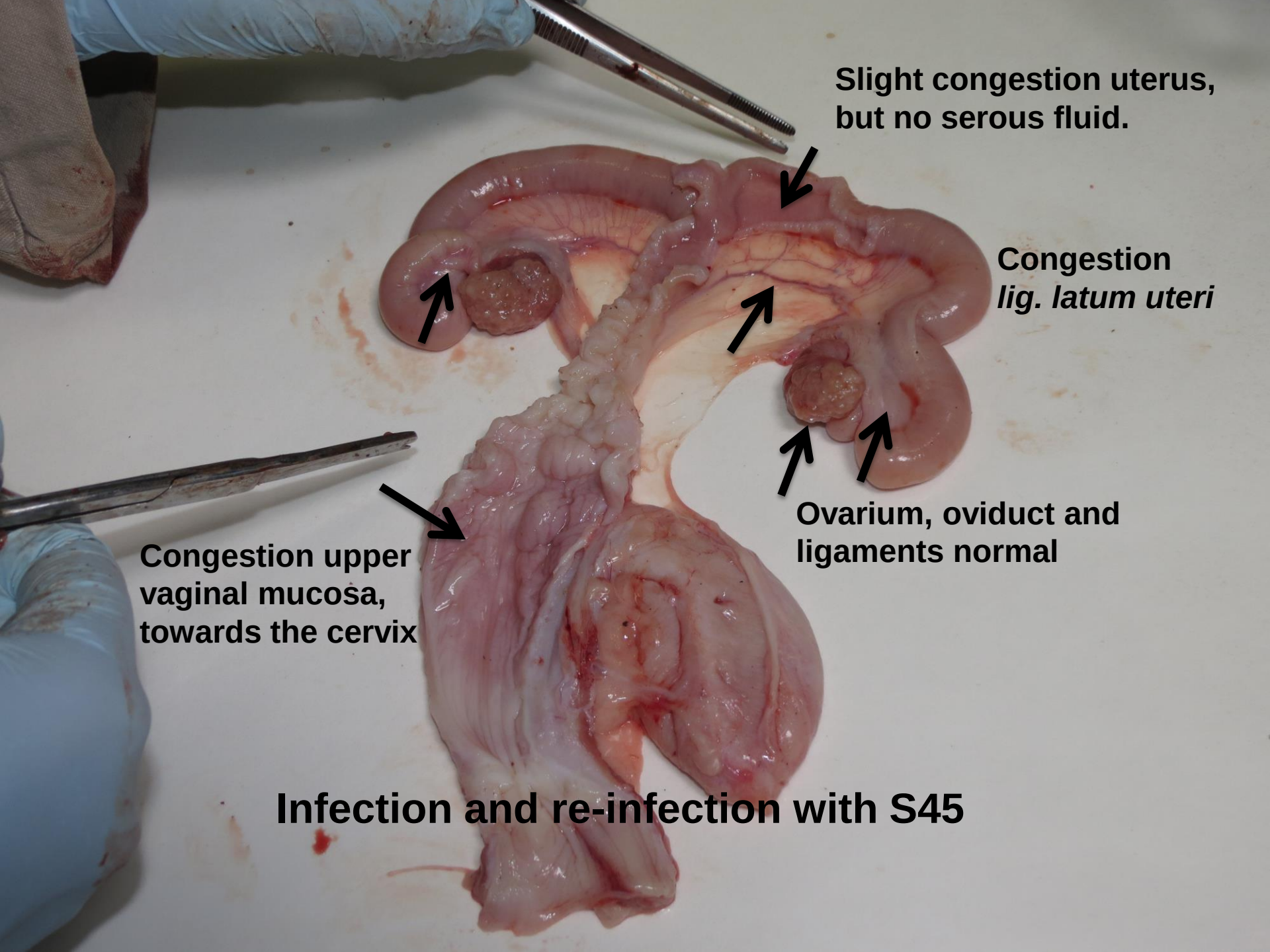
Swollen, congested ovarium

Vaginal infection with *C. suis* (S45)

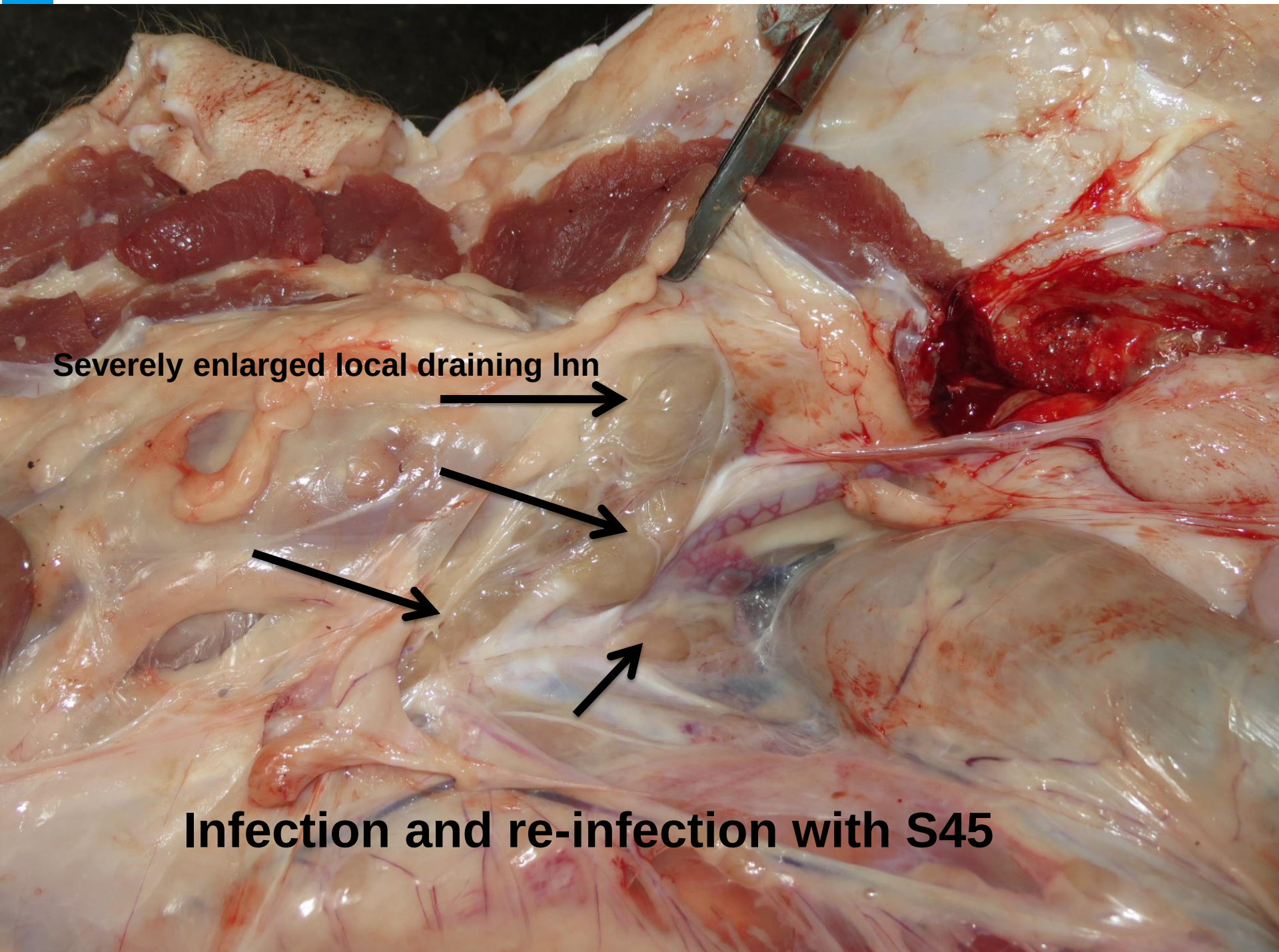


Congested local draining lymph node (*ln. subiliaci*)


Infection and re-infection with S45
Ovarium, mesovarium normal
No serous fluid
in uterus horns
Oviduct, mesosalpinx
normal
Lig. latum uteri normal



Infection and re-infection with S45



Infection and re-infection with S45

RESULTS: HISTOLOGY AND IMMUNOHISTOCHEMISTRY

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Histology

Caecum, liver, spleen, ovaria, oviducts, uterus horns, corpus uteri, cervix, vagina and urethra

Reinfection group: significantly higher infiltration of lymphocytes, plasma cells, monocytes/macrophages in the mucosa of the urogenital tract as compared to the controls and the infected group.

Mobilization of memory cells in response to re-infection?

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RESULTS: VAGINAL *C. SUIS* EXCRETION

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Mean scores per group ± standard deviation for vaginal *C. suis* shedding, at euthanasia, 21 days post infection 2.

Age	Day p.i.	Infection group	Infection / reinfection group
6 weeks	0	0,00 ± 0,00	0,00 ± 0,00
	3	0,00 ± 0,00 ^{a}	3,40 ± 0,55 ^{b}
	7	0,00 ± 0,00 ^{a}	3,40 ± 0,55 ^{b}
	10	0,00 ± 0,00 ^{a}	3,20 ± 0,45 $^{b\,*}$
	14	0,00 ± 0,00 ^{a}	2,60 ± 0,89 $^{b\,*}$
	21	0,00 ± 0,00 ^{a}	2,60 ± 0,89 ^{b}
	28	0,00 ± 0,00 ^{a}	3,40 ± 0,55 ^{b}
	35	0,00 ± 0,00 ^{a}	2,00 ± 1,15 ^{b}
	42	0,00 ± 0,00 ^{a}	1,40 ± 0,89 ^{b}
	49	0,00 ± 0,00 ^{a}	1,00 ± 0,00 ^{b}
17 weeks	56 (= infection 2)	0,00 ± 0,00 ^{a}	0,75 ± 0,50 ^{b} ↑
	59	3,20 ± 0,45 ^{b}	2,60 ± 0,55 ^{b}
	63	3,40 ± 0,55 ^{b}	3,40 ± 0,55 ^{b}
	66	3,60 ± 0,55 ^{b}	2,80 ± 0,45 $^{c\,*}$ ↓
	70	1,80 ± 1,10 ^{b}	3,00 ± 0,00 $^{c\,*}$ ↑
20 weeks	77	2,75 ± 0,50 ^{b}	2,75 ± 0,50 ^{b}

Control group negative throughout the experiment. All animals per group excreted *Chlamydia*.
Mean scores with a different superscript within a row are significantly different ($p < 0.05$).
* Statistically the same within a column.

Re-infection had no marked influence on *C. suis* excretion

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RESULTS: *C. SUIS* IN TISSUES

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Mean scores per group ± standard deviation (% positives/group) for the presence of *C. suis* in tissues, at euthanasia, 21 days post infection 2 at the age of 20 weeks.

Tissue	Infection group	Infection / reinfection group
Vagina	1,80 ± 1,10 (100)	2,00 ± 1,41 (100)
Cervix	2,00 ± 1,87 (60)	0,40 ± 0,55 (40)
Corpus uteri	0,80 ± 0,45 (80)	0,20 ± 0,45 (20)
Uterine horn R	1,40 ± 0,89 (100)	0,40 ± 0,55 (40)
Uterine horn L	0,20 ± 0,45 (20)	0,40 ± 0,55 (40)
Oviduct R	0,80 ± 1,30 (40)	0,80 ± 1,30 (40)
Oviduct L	1,40 ± 0,89 (100)	0,50 ± 0,58 (40)
Ovary R	1,60 ± 1,34 (80)	0,20 ± 0,45 (20)
Ovary L	0,80 ± 1,30 (40)	0,00 ± 0,00 (0) *
Urethra	1,20 ± 1,30 (60)	1,20 ± 1,10 (80)
Caecum	0,00 ± 0,00	0,00 ± 0,00
Spleen	0,00 ± 0,00	0,00 ± 0,00
Liver	0,00 ± 0,00	0,00 ± 0,00

Mean scores with * within a row are significantly different ($p < 0.05$).

Infection: 100% pigs with an ascending infection up to the ovaries

Re-infection: 80% pigs with an ascending infection

Number of pigs with *C. suis* in tissues generally lower in re-infection group

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RESULTS: SERUM ANTIBODIES

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Table 4 Mean *C. suis* S45-specific IgM, IgG and IgA serum titers of the infection (I) and the re-infection (R) group ± standard deviation*

Dpi	Procedure	Serum IgM		Serum IgG		Serum IgA
		Infection group	Re-infection group	Infection group	Re-infection group	Re-infection group
0	Infection R	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
7		0 ± 0.00^{c}	30 ± 0.00^{b,c}	0 ± 0.00^{c}	48 ± 16.43^{b,c}	0 ± 0.00
14		0 ± 0.00^{c}	168 ± 98.59^{b,c}	0 ± 0.00^{c}	240 ± 0.00^{b,c}	0 ± 0.00
21		0 ± 0.00^{c}	60 ± 0.00^{b,c}	0 ± 0.00^{c}	144 ± 90.99^{b,c}	0 ± 0.00
28		0 ± 0.00	0 ± 0.00	0 ± 0.00^{c}	36 ± 13.42^{b,c}	0 ± 0.00
35		0 ± 0.00	0 ± 0.00	0 ± 0.00^{c}	48 ± 16.43^{b,c}	0 ± 0.00
42		0 ± 0.00	0 ± 0.00	0 ± 0.00^{c}	36 ± 13.42^{b,c}	0 ± 0.00
49		0 ± 0.00	0 ± 0.00	0 ± 0.00^{c}	54 ± 39.12^{b,c}	0 ± 0.00
56	Infection I & R	0 ± 0.00	0 ± 0.00	0 ± 0.00^{c}	48 ± 16.43^{b,c}	0 ± 0.00
63		30 ± 0.00^{a}	30 ± 0.00^{b}	45 ± 21.21^{a,c}	264 ± 131.45^{b,c}	0 ± 0.00
70		216 ± 53.67^{a,c}	42 ± 16.43^{b,c}	120 ± 0.00^{a,c}	336 ± 131.45^{b,c}	30 ± 0.00^{b,c}
77	Euthanasia	72 ± 26.83^{a}	60 ± 0.00^{b}	195 ± 178.12^{a,c}	816 ± 321.99^{b,c}	60 ± 0.00^{b,c}

1. Controls remained negative. Primo-infection induced serum IgM and IgG (both Dpi 7), but no IgA.
2. IgG still present at re-infection time point (Dpi 56).
3. Seven days after re-infection, mean serum IgM and IgG titers increased again and kept on raising till euthanasia indicative for a secondary antibody response. IgA appeared

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RESULTS: MUCOSAL ANTIBODIES

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Table 5 Mean *C. suis* S45-specific IgM, IgG and IgA mucosal (vaginal) titers of the infection (I) and the re-infection (R) group ± standard deviation*

Dpi	Procedure	Mucosal IgM		Mucosal IgG		Mucosal IgA
		Infection group	Re-infection group	Infection group	Re-infection group	Re-infection group
0	Infection R	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
7		0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
14		0 ± 0.00^{c}	60 ± 0.00^{b,c}	0 ± 0.00^{c}	54 ± 13.42^{b,c}	0 ± 0.00
21		0 ± 0.00	0 ± 0.00	0 ± 0.00^{c}	30 ± 0.00^{b,c}	0 ± 0.00
28-49		0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
56	Infection I & R	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
63		0 ± 0.00^{c}	30 ± 0.00^{b,c}	0 ± 0.00^{c}	48 ± 16.43^{b,c}	0 ± 0.00
70		48 ± 16.43^{a,c}	24 ± 13.42^{b,c}	60 ± 0.00^{a,c}	96 ± 32.86^{b,c}	24 ± 13.42^{b,c}
77	Euthanasia	0 ± 0.00	0 ± 0.00	96 ± 32.86^{a,c}	48 ± 16.43^{b,c}	30 ± 0.00^{b,c}

1. Controls remained negative. Primo-infection induced vaginal IgM and IgG (Dpi 14), but no IgA.
2. No vaginal antibodies present at re-infection time point (Dpi 56).
3. Seven days after re-infection, mean serum IgM and IgG titers again present and one week later IgA also appeared.

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RESULTS: T CELL PROLIFERATION ASSAY

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1. **The *C. suis* primary infection did not induce a significanlty higher S.I. for PBMCs**
   - No statistical differences between the control group and the infected group.

2. **Re-infection did induce a significanlty higher S.I. for PBMCs**

Mean stimulation index (SI) ± standard deviation of PBMCs, 7 days post re-infection (Dpi 63).

Stimulation	Control group	Infection group	Infection / re-infection group
***C. suis* S45**	2,03 ± 1,42[a]	2,15 ± 1,23[a]	8,80 ± 5,46[b]

Mean scores with a different superscript within a row are significantly different ($p < 0.05$).

3. **At euthanasia (Dpi 77): proliferative responses of PBMC and of MC from the spleen and the lymph nodes were statistically the same for all groups.**

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CONCLUSIONS C. SUIS NFECTION AND RE-INFECTION IN PIGS

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Conclusions

- ***C. suis* causes an ascending infection with pathology (cfr. *C. trachomatis*)**
- **Re-infection induces "partial" protection - significant reduction of:**
  - macroscopic lesions, number of pigs still positive in the genital tract at euthanasia
- **Re-infection ➔ partial protection with significant higher:**
  - IgG and IgA titers in sera and vaginal secretions
  - Proliferative responses (S.I.) of PBMC's
  - Flow cytometry
    - Percentages of B cells, monocytes and $CD4^{-}CD8^{+}$ T cells in PBMC
    - Percentages of $CD4^{+}CD8^{-}$ T cells and $CD3^{-}CD4^{+}CD8^{-}$ spleen MC (contain pDC)
    - Percentage of $CD4^{-}CD8^{+}$ T cells in pelvic lnn
  - Production of IL-10 and IFN-γ by PBMC's

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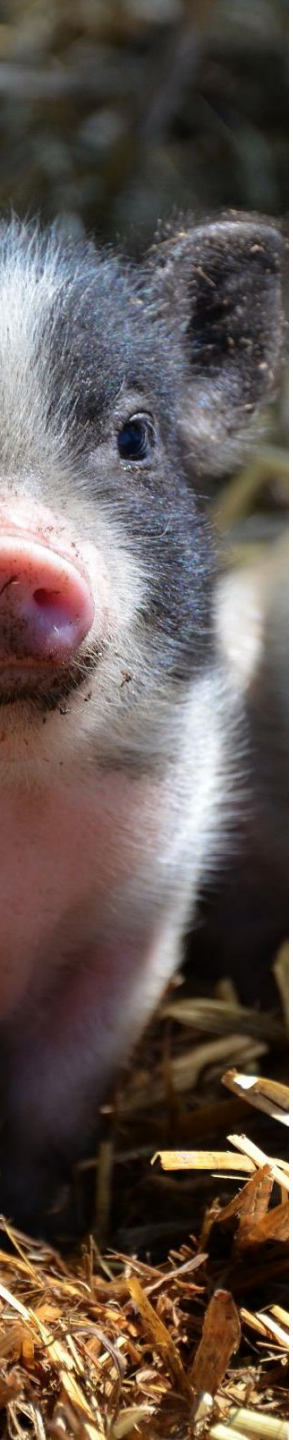

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Future research & perspectives

- Development of a vaccine (mucosal vaccine)
  - *C. suis*
  - *C. trachomatis* using the vaginal infection model in pigs
- Genomics - proteomics of *C. suis*
- *C. suis* bacterium-host cell interactions

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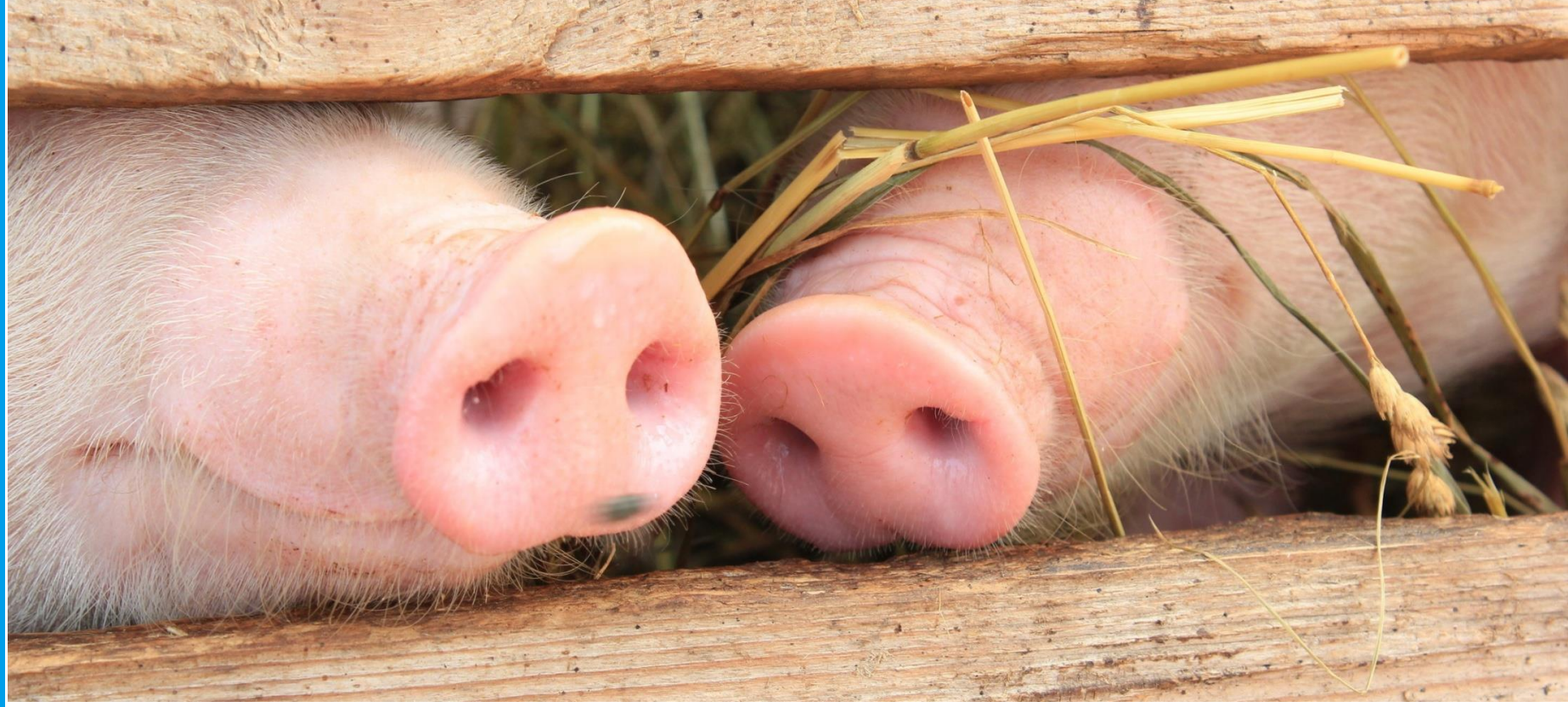

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