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Morphologic and histologic effects of *Chlamydia trachomatis* infection on the reproductive tract of vaccinated and non-vaccinated gilts

Diploma thesis

University of Veterinary Medicine Vienna

submitted by

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Vienna, March 2026

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Vienna, March 2026 Robert Dylan Heseltine

Abstract in English

Genital Chlamydiosis, caused by *Chlamydia trachomatis (Ct)*, is one of the most widely transmitted sexual diseases in humans worldwide. There are around 131 million accounted new infections per year, with prevalence being higher, as 75 % of infections remain asymptomatic (1). To this end, a vaccine would help stop the infection in its tracks as the current therapy with antibiotics only treats symptoms. To test vaccine efficacy there are a few potential animal models to choose from. One of them being swine, they are among other things anatomically and immunogenetically closely related to humans.

To address this data shortfall, eighteen gilts of same origin were divided into three groups: one group was infected within the uterus with *Ct*, one group was vaccinated and infected and one acted as our control group. Day 5 or 6 of cycle following euthanasia their reproductive tracts were assessed macroscopically as well as histologically, paying attention to different parameters such as weight, length and diameter.

The study revealed only minor differences between groups which might also be individual differences. Infected and vaccinated animals showed slight increases in myometrial thickness and uterine horn dimensions, along with some mucosal changes that may stem from mild inflammation. However, glandular and epithelial structures remained largely unaffected. These findings suggest that while swine do exhibit some morphologic and histologic responses to *Ct* application, they may not replicate the clinical symptoms seen in humans.

Nevertheless, in eighteen gilts of same genetics, same age, and same time point of cycle, we performed anatomical and histological baseline measurements focusing on the structures of the reproductive tract. These data are important, as such information is currently not available in textbooks.

Zusammenfassung

Die genitale Chlamydiose, verursacht durch *Chlamydia trachomatis (Ct)*, zählt zu den beim Menschen weltweit am häufigsten vorkommenden Geschlechtskrankheiten. Jährlich werden etwa 131 Millionen Neuinfektionen gezählt, wobei die Prävalenz höher ist, da 75 % der Infektionen asymptomatisch verlaufen (1). Aufgrund dessen würde ein Impfstoff dazu beitragen, eine Infektion zu verhindern, da die derzeitige Therapie mit Antibiotika nur Symptome behandelt. Um die Wirksamkeit des Impfstoffs zu testen, stehen verschiedene Tiermodelle zur Auswahl. Dazu zählen unter anderem Schweine, die anatomisch und immunologisch eng mit dem Menschen verwandt sind.

Um diese Datenlücke zu schließen, wurden achtzehn Sauen derselben Herkunft in drei Gruppen aufgeteilt: eine Gruppe wurde intrauterin mit *Ct* infiziert, eine Gruppe wurde geimpft und infiziert und eine Gruppe diente als Kontrollgruppe und wurde daher weder infiziert noch geimpft. Ihre Fortpflanzungsorgane wurden bei der Sektion am Zyklustag 6 makroskopisch und histologisch untersucht, wobei verschiedene Parameter wie Gewicht, Länge und Durchmesser erhoben wurden.

Die Studie ergab nur geringe Unterschiede zwischen den Gruppen, die auch auf individuelle Unterschiede zurückzuführen sein könnten. Infizierte und geimpfte Tiere wiesen eine leichte Zunahme der Dicke des Myometriums und der Größe des Uterushorns sowie einige Schleimhautveränderungen auf, die möglicherweise auf eine leichte Entzündung zurückzuführen sind. Die Drüsen- und Epithelstrukturen blieben jedoch weitgehend unbeeinflusst. Diese Ergebnisse deuten darauf hin, dass Schweine zwar einige morphologische und histologische Reaktionen auf die Ct-Verabreichung zeigen, diese aber nicht die klinischen Symptome beim Menschen wiedergeben.

Dennoch haben wir bei achtzehn Jungsauen derselben Genetik, desselben Alters und desselben Zykluszeitpunkts anatomische und histologische Ausgangsmessungen mit Schwerpunkt auf den Strukturen des Fortpflanzungstrakts durchgeführt. Diese Daten sind wichtig, da solche Informationen derzeit nicht in Lehrbüchern zu finden sind.

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1 Introduction

Chlamydia trachomatis (*Ct*) is an eucaryotic, obligate intracellular Gram-negative bacterium, causing Chlamydiosis, one of the most widespread sexually transmitted diseases occurring worldwide. The highest prevalence for it is found in the global south (2,3). Around 60 % of all cases are asymptomatic in both biological human sexes (4).

Even though *Ct* infections in humans can be treated relatively easily with antibiotics, the infection itself and sequalae cannot be prevented by them (5). A vaccine against *Ct* with long term protection against sequelae and infection would be beneficial in combating this endemic sexually transmitted disease. To this end, *in vivo* testing is needed to fulfil all legal and ethical obligations to continue testing on humans in the clinical phases. The animal of choice for testing currently are rodents, which have a handful of benefits over swine but lack the genetic, anatomic and physiological similarities that swine have with humans. Immunologically the largest differences between swine and humans are the inverted lymph nodes and the placental transfer of passive immunity (6). Furthermore, the genetic similarity of *Chlamydia (C.) suis* and *Ct* make pigs more susceptible to infections with the human chlamydia species and vice versa compared to rodents (7).

To this end the scoring of the genital tract in regard to redness, oedema and unphysiological fluids and the histological examination of the uterine surface epithelium and myometrium thickness helps better understand if the infection did in fact occur. Furthermore uterine, oviduct, ovary and cervix length diameter and weight parameters were collected to complement the modest primary research that has been done in swine anatomy and to establish if there is a difference between infected and uninfected. The aim of this thesis is to determine if *Ct* infection in gilts causes changes in macroscopic and histologic parameters and to add anatomical data where not a lot has been collected.

2 Literature

2.1 Chlamydia trachomatis

Genital Chlamydiosis is the most prevalent sexually transmitted bacterial disease in humans, caused by *Chlamydia trachomatis (Ct)*. It can be divided into three biovars and further subdivided into several serovars. Cells targeted by *Ct* are basically any epithelial cells, but specifically in the urogenital tract, conjunctiva, inguinal lymph nodes, oropharynx and the anorectal region (8).

Trachoma biovar, serovars A-C (9) is responsible for non-congenital blindness and visual impairments, which is the leading cause of blindness in the developing world (10). The eye disease trachoma is caused by a chronic process that consists of two parts. A recurrent, usually subclinical inflammatory disease that generally affects children and causes chronic conjunctivitis. The second, non-communicable phase normally occurs in adolescents and adults, causing scar tissue and trichiasis that leads to clouding of the cornea and if not treated eventually to blindness (11).

Lymphogranuloma Venereum (LGV) biovar, serovars L1-L3 (9) can cause lymphadenopathy, genital ulcers, proctitis and fibrosis (10). The serovars L1-L3 infiltrate the regional lymph nodes by infecting the mucosal lining first then spreading into connective tissue and reaching the lymph vessels that lead them to the lymph nodes (12).

The third biovar, containing serovars D-K (9), primarily affects the genital tract and is associated with conditions such as salpingitis, ectopic pregnancy, Fritz-Hugh-Curtis syndrome, infertility in females and epididymitis in males (13). Transmission of this biovar occurs almost exclusively through sexual intercourse (14). The clinical presentation in the female cervix ranges from asymptomatic cases with odourless, mucoid vaginal discharge without pruritis to symptomatic cervicitis, characterized by a yellow, cloudy and mucoid discharge with a reddened mucosa that tends to bleed easier (15). Endometrial oedema and irregular uterine bleeding have also been reported in women infected with *Ct* (13). Luminal occlusion and fibrosis of the fallopian tubes can be caused by *Ct* if it ascends to the upper female genital tract (16). In both sexes, infection may cause dysuria, while men may also present with a whitish urethral discharge (13). Pathological changes observed in non-human primates resemble those seen in humans, including urethral discharge, urethral lymphocyte infiltration, urethral vesicles and swollen and reddened fallopian tubes (17). In murine models, infection has also been shown to induce mucosal oedema in the uterine horns (18).

2.2 Animal models

Animals have been used as models for the better understanding of human physiology and medical knowledge for millennia. Surgery techniques, antibiotics, vaccines and disease processes have been some of the areas animal experiments have been fundamental in their development (19). Due to their phylogenetic proximity to humans, non-human primate (NHP) research with human diseases yields a better translatability compared to other animal models (20). However, due to their advanced cognitive capacity and complex social behaviour, there are moral, ethical and legal considerations for them to be used in medical research, thereby limiting their applicability to certain areas where other species are not suitable (21). Rodents are among the most widely used animal models due to their cost-effectiveness, small size, 99 % genome similarity to humans, and a large genetic and molecular toolbox to work with. However, their translational value is limited by low predictive reliability for human outcomes, as well as significant differences in immune system, metabolism, size, longevity and physiological differences that don't capture the essentials of human diseases (22,23). Swine are more of an intermediary animal model, as they are more similar to humans in terms of physiology, immunology and genetics compared to rodents (24,25), while being less ethically controversial and financially demanding than NHP (25). Anatomical differences in the swine genital tract include but are not limited to a bicornuate uterus resulting in greater uterine length, a less acidic vaginal pH of 7, different hormonal cycles and the presence of cervical pulvini in pigs, which hinder access to the porcine uterus (24) .

2.3 Animal models for genital tract chlamydia

Pig-tailed macaques have the most similar female genital tract anatomy, microflora and physiology. They have a 28–30-day menstrual cycle and are naturally susceptible to *Ct (25)*. Mice and other rodents are not naturally susceptible to *Ct*, as *Ct* has challenges overcoming murine innate immunity and fails to induce lasting pathologies in the upper genital tract (26). However, when infected cervicovaginally with the mouse specific *C. muridarum,* mice can develop pathologies analogous to those in humans including hydrosalpinx. Guinea pigs offer an advantage over mice, as their reproductive physiology more closely resembles that of humans. Both species are spontaneous ovulators, have an actively secreting corpus luteum and can acquire chlamydial infections through sexual transmission. Hormonally, they are also more similar to humans (25). Nonetheless, as with other rodent species, *Ct* is not suitable for use in guinea pigs. Instead, *C. psittaci* is employed, which causes Guinea pig inclusion conjunctivitis, a condition that closely mirrors human *Ct* infections (25). In contrast, pigs can

be intravaginally infected with *Ct* serovar E, allowing *Ct* to ascend to the upper genital tract. There, *Ct* targets the cervical epithelium cells and the superficial epithelial layer causing inflammation, hyperaemia, and oedema of the uterine wall and excess watery liquid in the lumen (27).

2.4 Chlamydia vaccines

Despite extensive research efforts, a fully developed vaccine against any Chlamydia species is still lacking. Vaccine candidates based on purified major outer membrane protein (MOMP) have shown protective effects against chlamydial genital infections in mice (28). Another vaccine candidate based on chlamydial-protease-like-activity factor (CPAF) protein has been tested on pigs and demonstrated high antibody levels along with a strong T-helper cell response (29). The first clinical trial since the 1960s was carried out in the UK on humans evaluating the CTH522 antigen combined with either the CAF01 or $Al(OH)_3$ adjuvant. Results indicated that the experimental vaccine was safe and induced satisfactory antibody titres in both serum and vaginal fluid (1). Even though this study is promising, the role of antibodies in humans in controlling an ascending infection remain uncertain (30).

2.5 Anatomy and histology of the swine reproductive tract

Anatomically the female genital tract consists of two ovaries each with the dimensions 22.3. ± 0.9 cm x 33.2 ± 1 cm (31). The fallopian tube connects the ovaries to the uterus and consists of three distinct parts, the fimbriated infundibulum, the ampulla and the isthmus. Following the fallopian tube the bicornate uterus follows with two separate uterine horns that conjoin to form the uterine body. The porcine cervix acts as a gateway between uterus and vagina, that features cervical pulvini. The vagina in turn is connected to vestibulum and vulva through the urethral orifice. Histologically, the genital tract consists of three layers: the endometrium, muscular layer and the perimetrium. The epithelium consists of simple columnar that increases during oestrus and is heavily folded as well as containing branched and coiled endometrial glands (24).

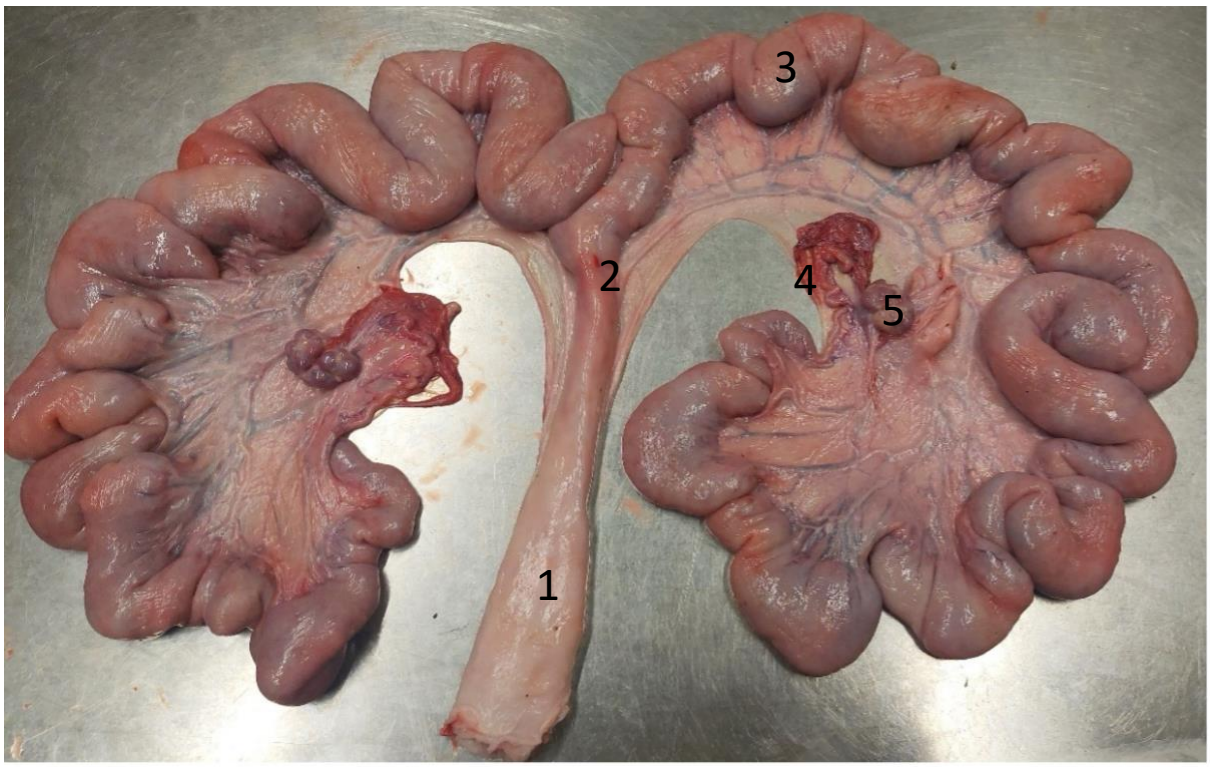


Figure 1: Illustration of the porcine uterine tract

Following the number in figure one from 1 to 5 are the cervix, the uterine body, the left uterine horn, the left oviduct and the left ovary.

2.6 Hypothesis and aim of this study

To address the data shortfall in swine reproductive anatomy, infected and uninfected reproductive tracts were measured macroscopically and histologically in size, length and further macroscopic changes that might occur. It was hypothesized that infected porcine reproductive tracts differ in morphology and histology compared to non-infected ones and vaccinated ones, potentially showing variations in size, colour, and tissue structure. The experiment aims to provide insights into potential variations in these parameters and contribute to a deeper understanding of swine as an animal model.

3 Materials and Methods

3.1 Experimental design

This animal trial was approved by the institutional ethics committee, the Advisory Committee for Animal Experiments (§12 of Law for Animal experiments, Tierversuchsgesetz-TVG) and the Federal Ministry for Science, Research and Economy (ETK-048/04/2024).

For this experiment, thirty female pigs of Porcine Reproductive and Respiratory Disease Virus (PRRSV) free origin were sourced from the University's own teaching and research farm (Vetfarm-Medau, Berndorf, Lower Austria). At the age of fifteen weeks, they were relocated to the BSL-2 facility of swine medicine in Vienna and split at random into three groups à 6 animals. Since *C. suis* was shed rectally by every single animal as detected by qPCR methods and subsequent isolation in cell culture, all gilts were successfully treated with doxycycline orally via food and tylosine via intramuscular injection to eliminate *C. suis* and any other chlamydial species prior to *Ct* experimental infection.

Eighteen gilts were included in the study: Group A, the control group, consisted of six gilts, all of them were mock vaccinated and mock challenged. Group B, the vaccine control group, consisted of six gilts, were mock vaccinated and were challenged with a *Ct* Serovar D. Group C consisted of the six gilts, all were vaccinated with a *Ct* CPAF-vaccination (adjuvans: STING) and challenged with the same *Ct* strain (table1).

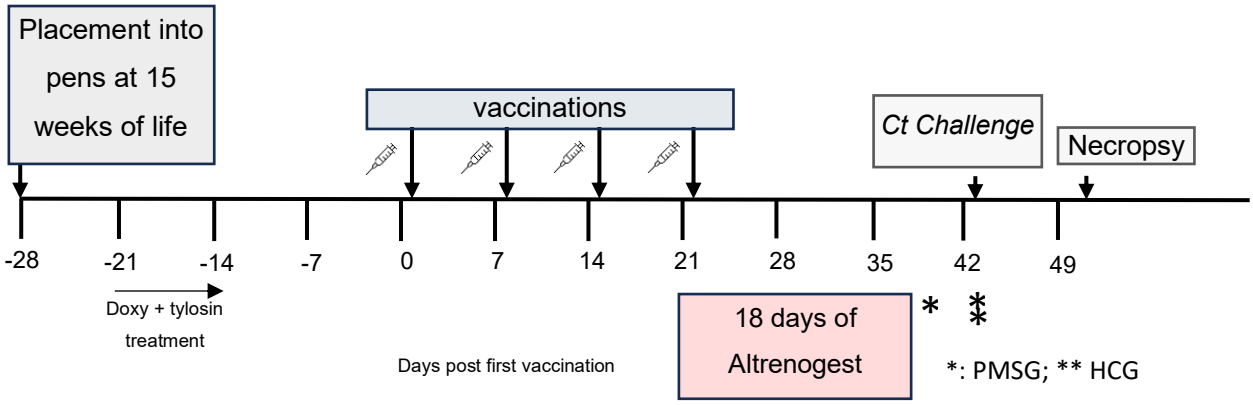


Figure 2: Experimental timeline.

Tab.1: Overview of the three different pig groups

	Number of pigs	vaccination	infection
Group A (control)	6	mock	mock
Group B	6	mock	*C.trachomatis*
Group C	6	*C. trachomatis* vaccine	*C.trachomatis*

All groups were fed ad libitum with gilt rearing feed and had constant access to fresh water through drinking nipples. Each group was housed in separate stables to reduce cross contamination *post infection*, boots and clothes were changed between groups. Mock and vaccine were both administered intramuscularly (2 mL) to each respective group at age 19 and 20 weeks old. The same vaccine was applicated intranasally (1 mL per nostril) via a LMA MAD Nasal^{TM} diffuser at age 21 and 22 weeks old. To ensure all gilts would be in oestrus at the same time, their reproductive cycle was synchronised with synthetic progestin (Altresyn 4 mg/mL Ceva Tiergesundheit, France) by an oral daily application of 20 mg per day over a 18 days period, followed by a single intramuscular injection of 1000 international units of pregnant mare serum gonadotropin (Folligon 1000 I.E., MSD Tiergesundheit) 24 hours after last altrenogest application and 500 international units of Choriogonadotropin (Chorulon 1500 I.E., MSD Tiergesundheit) 72 hours later. Pigs were in heat and ready for intracervical challenge 24 hours later. Intrauterine insemination technique was performed, but instead of semen, the insemination tube was filled with either phosphate buffered saline solution (mock) or $1x10^8$ inclusion-forming units (IFU) *Ct* serovar D in 20 mL phosphate buffered saline (infection group). To monitor infection, pigs were swabbed cervically daily. On two consecutive days, seven and eight days after the challenge, pigs were sacrificed under anaesthesia by intramuscular injection of ketamine hydrochloride (Ketamidor®, 20 mg/kg body weight) and azaperone (Stresnil®, 1.3 mg/kg body weight) following an intracardiac injection of tetracaine hydrochloride, mebezonium iodide and embutramide (T61®, 1 ml/10 kg body weight). All gilts had an age of 186 and 187, respectively. Prior to euthanasia, gilts were weighted.

3.2 Examination of the reproductive tract

The examination of the reproductive tract was done following a modified protocol created by Egli et al. (32). Directly after necropsy, the reproductive organs (ovaries, uterine horns, corpus uteri, cervix, vagina) of each pig were removed and weighed in total after trimming of excess

tissue from the ostium urethrae externum upwards excluding the broad uterine ligament. The reproductive status of the ovaries was evaluated with a scoring system modified by Egli et al. (32). Left and right ovaries were weighed separately, measured in length, width and height and functional bodies of the ovary were counted. Ovary volume was calculated using the prolate ellipsoid formula that was used by Crane et al (33). Corpora lutea of both ovaries had to be at least 0.5 cm in diameter to be counted. Corpora lutea of the left ovary were removed and weighed. Cyst-like formations larger than 15 mm in diameter were recorded as ovarian cysts as in Egli et al (32).



Figure 3: Corpora lutea count



Figure 4: Oviduct length measurement

Left and right oviduct were measured in length (cm) after removing the mesenterial tissue. Both uterine horns were separated from the corpus uteri by cutting them ten centimetres from the bifurcation. Length and diameter at the cutting place (cm) were measured, the horns were opened longitudinally, and pH paper was used to measure the pH at the cervical end. Furthermore, the mucous membrane was assessed for colour change and/or pus. In case of abnormal content or changes of surface, swab samples were taken for further bacteriological investigations. The cervix was measured in length and diameter (cm). Length measurements for ovaries, fallopian tubes, diameter of the uterine horns and cervix were carried out using a 20 cm ruler. Uterine horn length was assessed by using a one-meter measuring tape. Ovaries, corpora lutea and the reproductive tract were weighed using a set of scales.

3.3 Histological examination

For the histological examination, a 0.5 cm ring in width was cut out of each uterine horn, additionally a 1cm piece of cervical tissue as well as the whole left ovary and preserved in 10 % formaldehyde. Embedding in paraffin and making of the slides was done by staff of the institute of pathology. All histological slides were digitalized and evaluated in the university's own program (CaseViewer by 3DHistech). The assessed endometrium parameters were the thickness of the surface epithelium, the thickness of the glandular epithelium, width of the myometrium and the gland per area. For the glandular epithelium twenty locations were measured per uterine horn (Figure 1), the same was done for the surface epithelium (Figure 3). Cells, surfaces and glands that were not displayed perfectly were not evaluated. Five myometrium positions were measured with as much coverage as possible between them over the whole uterine horn (Figure 2). Disfigured and or ablated parts of the myometrium were not evaluated. Uterine glands were counted in a pre-defined circle of 306365 µm^2 which was digitally layered on top of the slide (Figure 1). Three such circles were counted per horn.

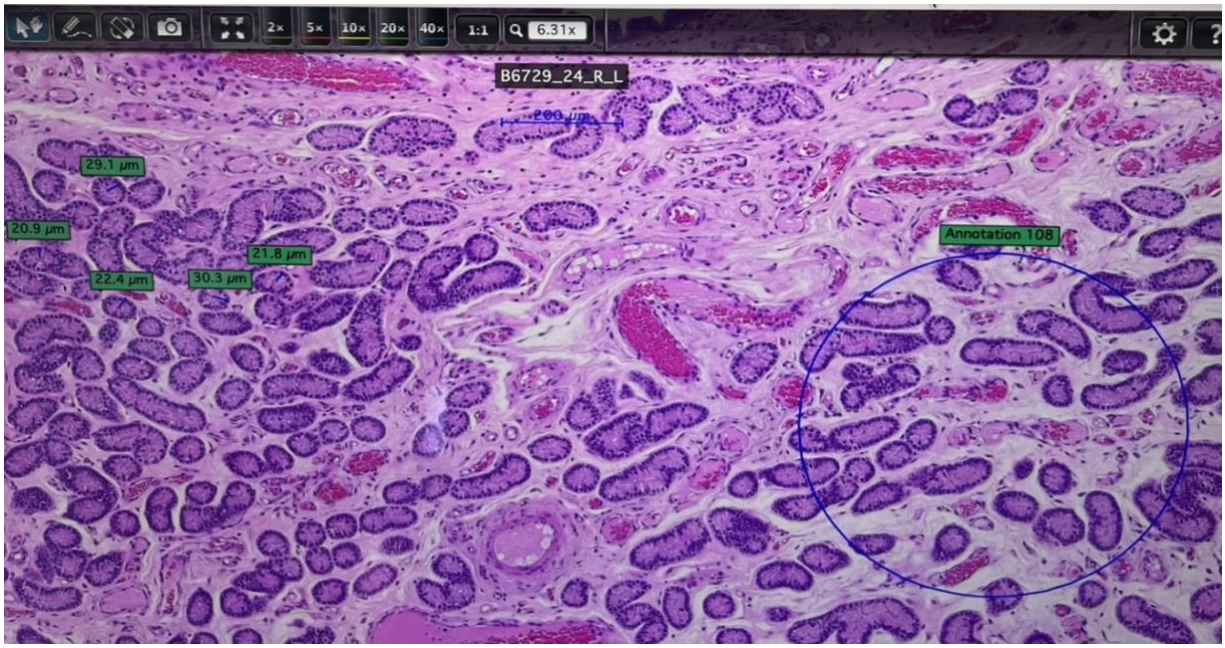


Figure 5: Histological assessment of the glandular epithelium (left) and the glands per defined area within the circle (right).

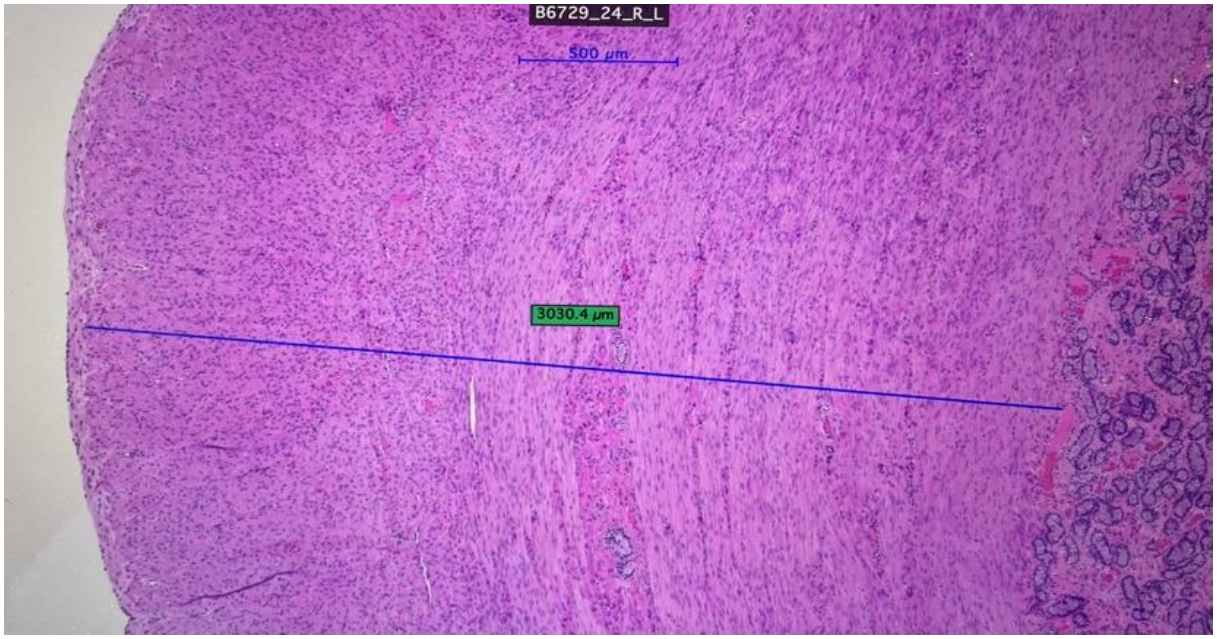


Figure 6: Histological assessment of myometrium thickness.

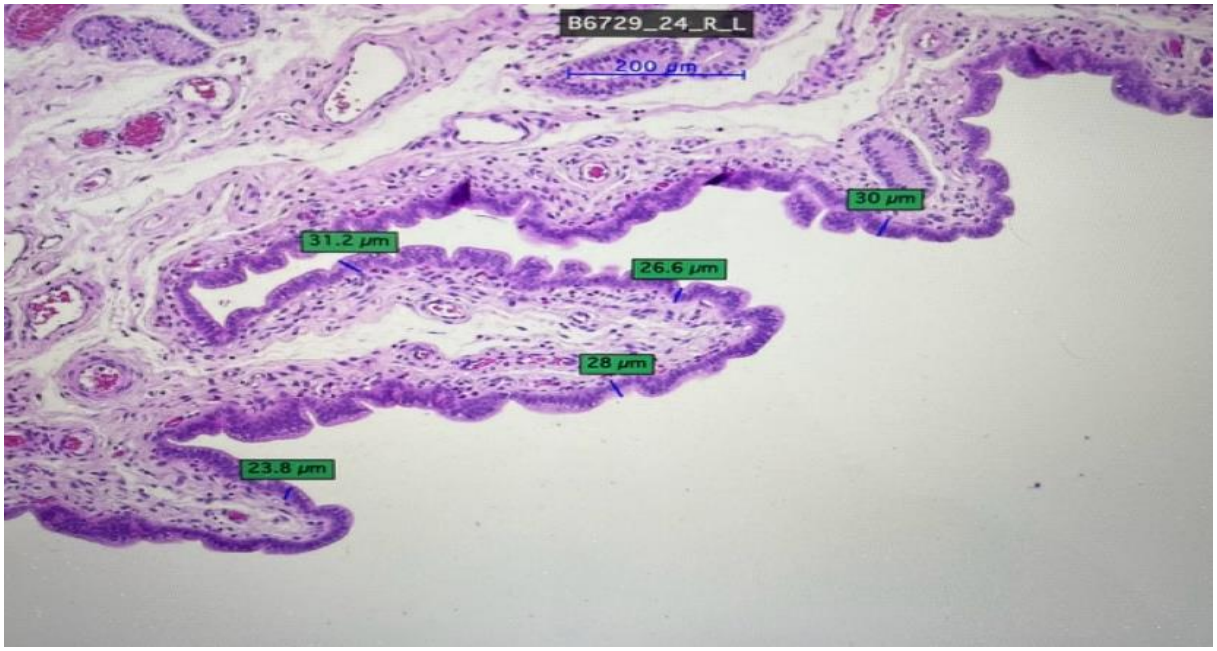


Figure 7: Histological assessment of the surface epithelium.

In the descriptive analysis, the mean ± standard deviation, median and range were presented for metric data (e.g. slaughter weight, weight of the empty urinary, weight of the reproductive system, length of the vagina, length of the cervix, the length of the right and left uterine horns, diameter of the cervix, diameter of the right and left lower uterine horn). For the categorical

data (presence of cysts, abnormalities of the mucous membrane of the uterus), the percentage of sows was presented in each case.

3.4 Statistical analysis

Statistical analyses were performed using SPSS Statistics (Version 29.0.2.0, IBM Corp., Armonk, NY, USA). The distribution of data was assessed using the Shapiro–Wilk test. For normally distributed variables, group comparisons were conducted using analysis of variance (ANOVA), followed by Tukey's post hoc test where applicable. Non-normally distributed data were analyzed using the Kruskal–Wallis test, with pairwise post hoc comparisons as appropriate. Differences between the left and right uterine tract were evaluated using the paired t-test. Categorical data were analyzed using Fisher’s exact test. A p-value < 0.05 was considered statistically significant.

4 Results

At the day of euthanasia gilts were either 186 or 187 days of age and a mean weight of 119.1 ± 4.3 kg (range 114-127 kg) for group 1, that of group 2 was 120.4 ± 4.2 kg (range 118-130 kg) and that of group 3 was 117.7 ± 5.7 kg (range 105-121.5 kg).

4.1 Macroscopic changes



Figure 8: sow 12



Figure 9: sow 13

Figure 7 (sow 12) and Figure 8 (sow 13) show the opened reproductive tract from two different uninfected and unvaccinated sows, both in group 1.



Figure 10: sow 19

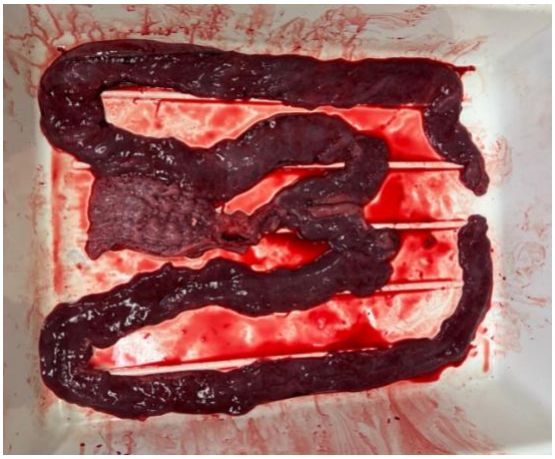


Figure 11: sow 20

Figure 9 (sow 19) and Figure 10 (sow 20) show the opened reproductive tract from two different infected and vaccinated sows, both in group 2.

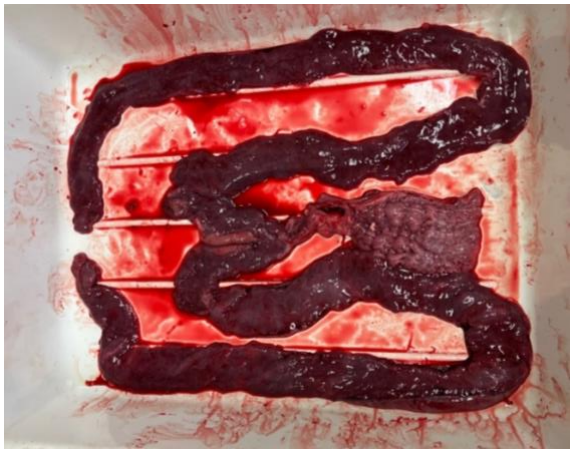


Figure 12: sow 23

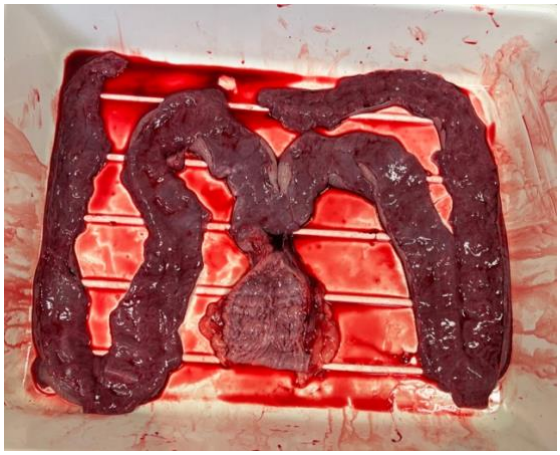


Figure 13: sow 28

Figure 11 (sow 23) and Figure 12 (sow 28) show the opened reproductive tract from two different infected and vaccinated sows, both in group 3.

After removal of the ovaries, the cervix and uterine horns were longitudinally opened for examination. Sow 12 showed marked hyperemia of the cervical and uterine horn mucosa. In group 1, two thirds of sows displayed no visible macroscopic changes, whereas this proportion decreased to one-third in groups 2 and 3. Mucosal redness was seen in one third of group 1, half of those in group 2 and two thirds in group 3. The presence of pus, mucus, or other exudates was recorded in one third of group 1 and 17 % of group 3. No such findings were noted in group 2. Increased intrauterine fluid was identified in 17 % of group 2.



Figure 14: Sow 16 example for mucosal redness.

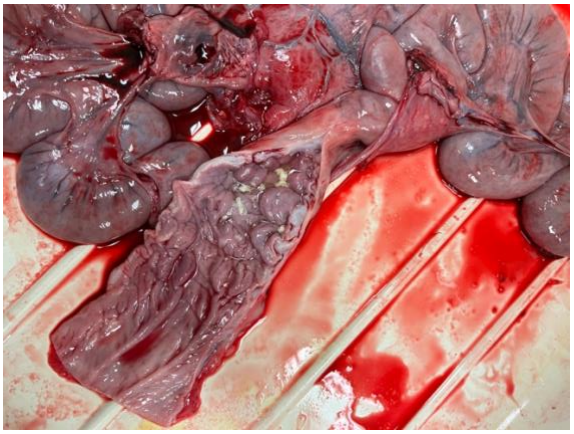


Figure 15: Sow 12 example for pus, mucus and other exudates.



Figure 16: Sow 19 example for increased uterine fluid.

4.2 Analysis of uterine pH

The uterine pH was numerically higher in the challenged groups: group 2 being the most basic with a mean pH of 7.6 (SD 0.5, range 7-8) and group 3 with a mean pH of 7.3 (SD 0.7, range 6-8). In the control group the pH was most acidic (mean 7.1, SD 0.4, range 6.5-8).

4.3 Uterine horns morphology

Group 1 had the numerically shortest uterine horns (mean 129 cm, SD 12.6 cm, range 110-152 cm). Group 2 had roughly similarly long uterine horns (mean 136 cm, SD 21.6 cm, range 96-179 cm) than group 3 (mean 136 cm, SD 17.8 cm, range 102-169 cm). There is a noticeable length disparity between left and right uterine horn in all groups with the left uterine horn being on 4 to 12 cm longer (Figure 6). Group 2 had the numerically smallest diameters of horns (mean 1.6 cm, SD 0.34 cm, range 1-2.3 cm) whereas group 3 had the numerically largest

diameter (mean 1.9 cm, SD 0.26 cm, range 1.2-2.5 cm). In between was group 1 (mean 1.8 cm, SD 0.29 cm, range 1.4-2.3 cm) (Figure 17).

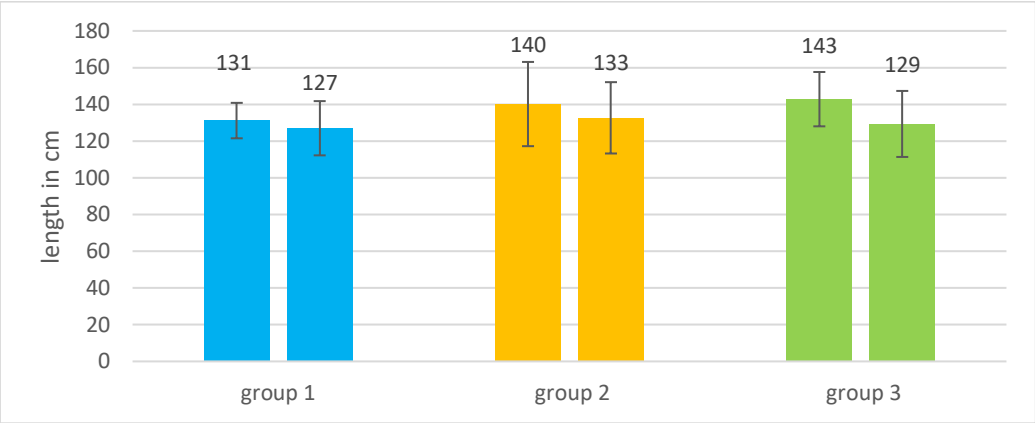


Figure 17: Left (left column) and right (right column) uterine horn length in cm, shown for each group. Mean and standard deviation for each group and horn.

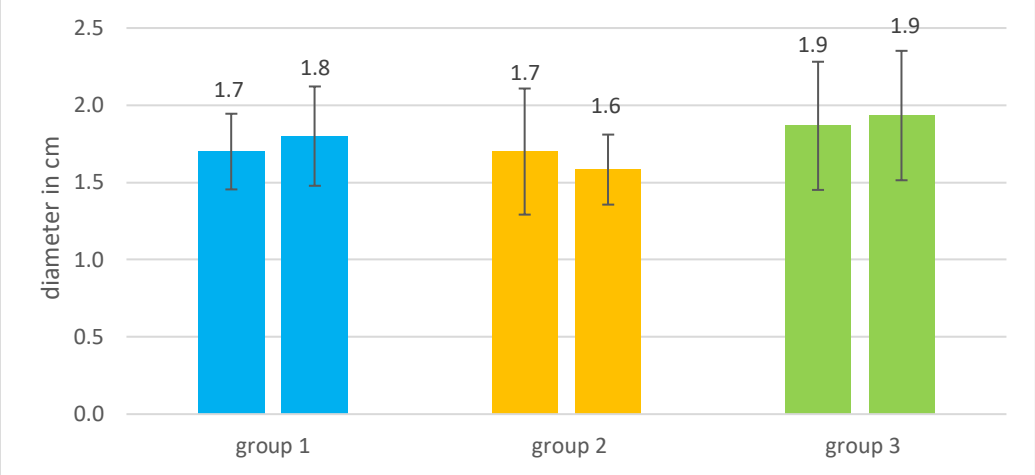


Figure 18: Left (left column) and right (right column) uterine horn diameter in cm. Mean and standard deviation for each group and horn.

4.4 Cervix morphology

The vaccinated groups 2 and 3 are numerically similar in cervix length with a mean of 8.5 cm (SD 11 cm, range 6-11 cm) in group 2 and a mean of 8.5 cm (SD 11 cm, range 5-11 cm) in group 3. The mean cervix length is numerically longer in group 1 (mean 9.6 cm, SD 13.5 cm, range 6-13.5 cm) (figure 18). Group 1 and 2 are numerically similar in diameter and group 3 is

numerically larger by a factor of 16 %. Average cervix diameter was numerically largest in group 2 (mean 3 cm, SD 0.7 cm, range 2.5-3.5 cm), in the control group (mean 2.5 cm, SD 0.4 cm, range 2-3 cm) and group 3 (mean 2.5 cm, SD 0.3 cm, range 1.5-3.5 cm) the mean was identical.

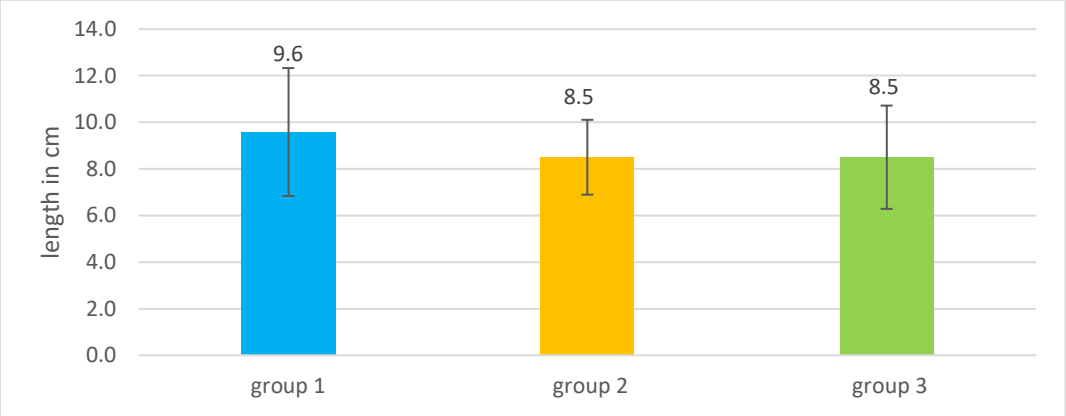


Figure 19: Cervix length in cm. Mean and standard deviation for each group.

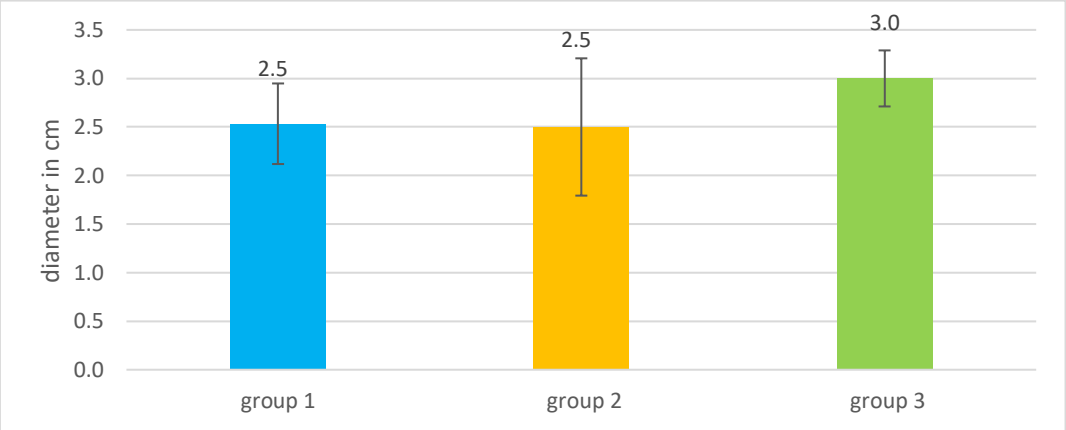


Figure 20: Cervix diameter in cm. Mean and standard deviation for each group.

4.5 Uterus weight

Mean uterus weight of group 1 was 1.5 kg (SD 0.12 kg, range 1.3-1.7 kg), there were no differences in average uterus weight of group 3 (mean 1.4 kg, SD 1.6 kg, range 1.3-1.55 kg) and group 2 (mean 1.4 kg, SD 3.4 kg, range 1.35-1.6 kg) (Figure 20).

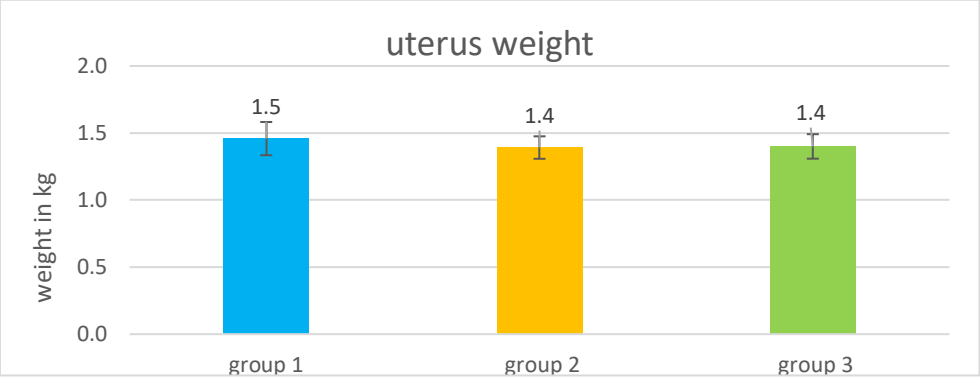


Figure 21: Average uterus weight in kg. Mean and standard deviation for each group.

4.6 Oviduct length

On average, group 1 oviducts were 18.9 cm (SD 3.5 cm, range 14-28 cm), group 2 oviducts were 18.9 cm (SD 1.6 cm, range 16- 21 cm) and group 3 oviducts were 18.7 cm (SD 3.4 cm, range 10-25 cm) in length (Figure 21). All measured oviducts were unobstructed and intact.

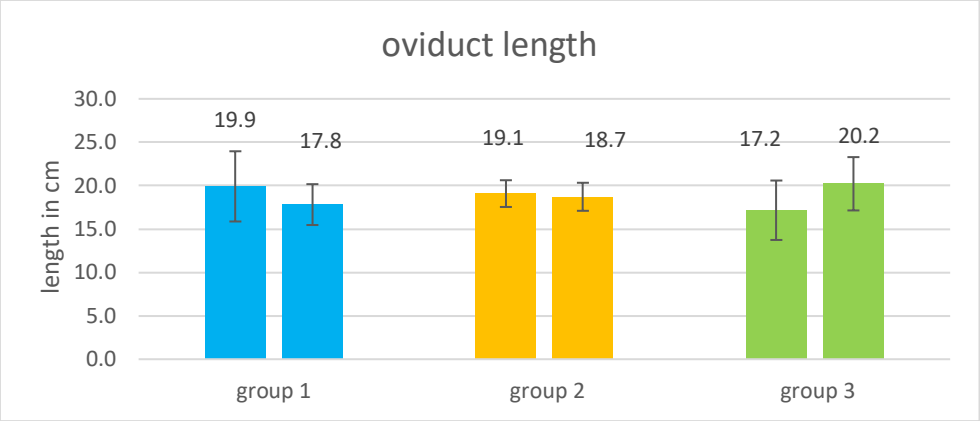


Figure 22: Average oviduct length in cm. Mean and standard deviation for each group and oviduct. Left oviduct (left column) and right oviduct (right column).

4.7 Ovarian weight and volume

All sows and therefore their ovaries were in the luteal phase. Mean ovary weight was 15 g (SD 8.0 g, range 4-32 g) in group 1, 11 g (SD 3.4 g, range 6-18 g) in group 2 and 19 g (SD 20.1 g, range 4-76 g) in group 3 (figure 12). Group 1 ovaries were 15 mL (SD 8.4 mL, range 5-37 mL), group 2 ovaries were 16 mL (SD 4.7 mL, range 5-25 mL) and group 3 ovaries were 19 mL (SD 17.8 mL, range 6-73 mL) on average in volume. Average ovary length was 4.2 cm (SD 0.8 cm, range 3-55 cm) in group 1, 4 cm (SD 0.5 cm, range 3-5 cm) in group 2 and 4.1 cm (SD 1.2 cm, range 3-7 cm) in group 3. Mean ovary width was 2.9 cm (SD 0.7 cm, range 1.5-4.5 cm) in group 1, 2.8 cm (SD 0.6 cm, range 2-3.5 cm) in group 2 and 3 cm (SD 0.8 cm,

range 2-5 cm) in group 3. Mean ovary hight was 2.4 cm (SD 0.6 cm, range 2-3.5 cm) in group 1, 2.2 cm (SD 0.5 cm, range 1.5-3 cm) in group 2 and 2.5 cm (SD 0.7 cm, range 1.5-4 cm) in group 3.

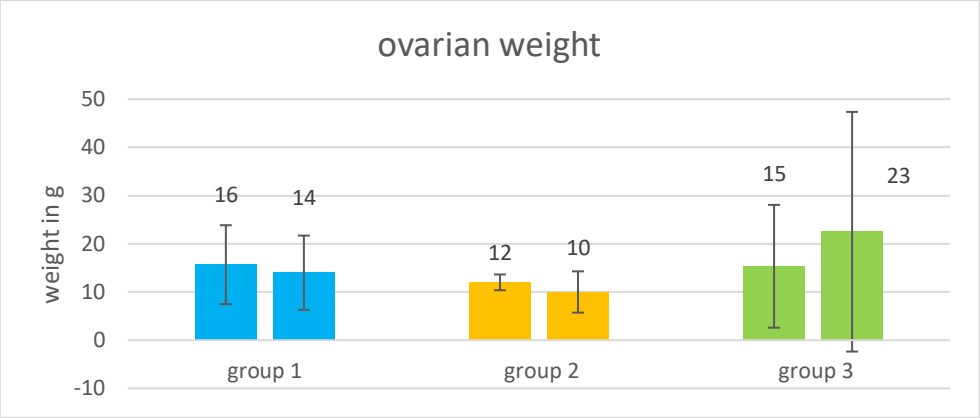


Figure 23: Ovarian weight in g. Mean and standard deviation for each group and ovary. Left ovary (left column) and right ovary (right column).

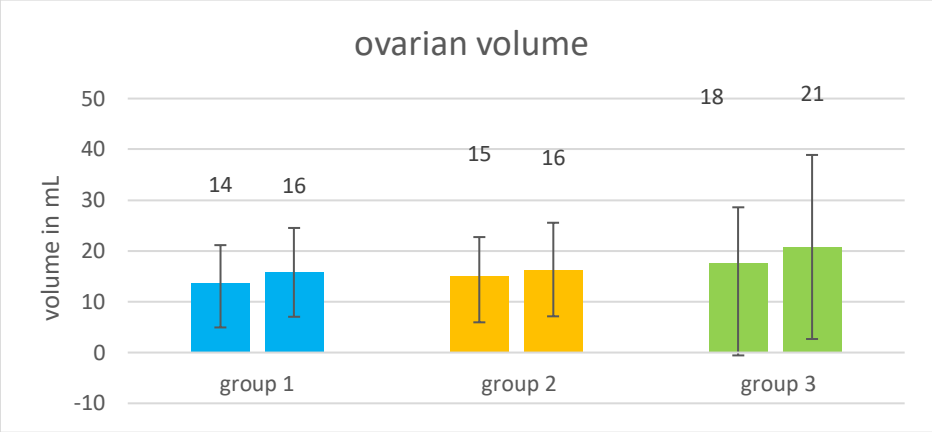


Figure 24: Average ovarian volume in m. Mean and standard deviation for each group and ovary. Left ovary (left column) and right ovary (right column).

4.8 Number of corpora lutea and total corpora lutea weight

The mean number of corpora lutea in group 1 was 12 (SD 3.9, range 4-16), in group 2 11 (SD 5, range 4-18) and in group 3 9 (SD 2.9, range 4-13) on average. Group 1 corpora lutea were 3.2 g (SD 1.2 g, range 1.4-4.7 g), group 2 corpora lutea were 2.8 g (SD 1.1 g, range 1.6-4.8 g) and group 3 corpora lutea were 3.1 g (SD 1.0 g, range 1.7-5.1 g) on average in weight.

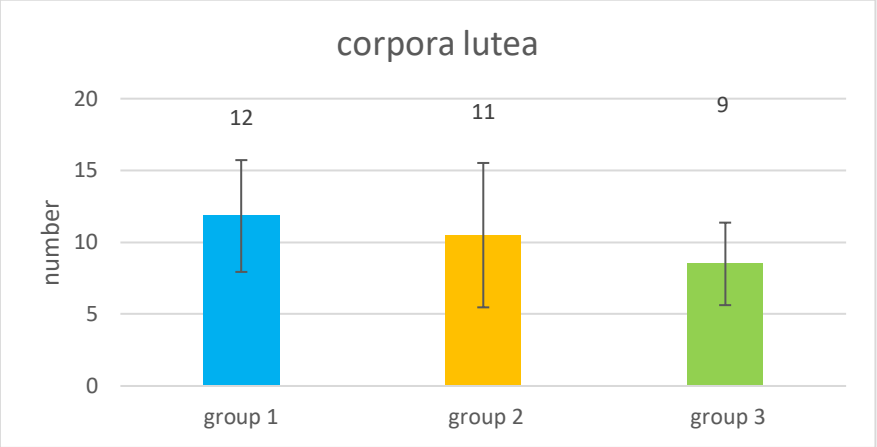


Figure 25: Number of visible corpora lutea. Mean and standard deviation for each group.

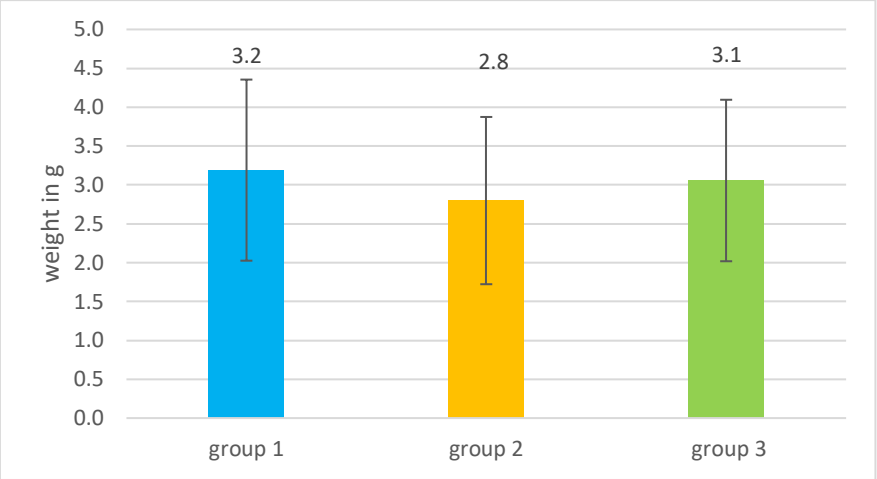


Figure 26: Total luteal weight. Mean and standard deviation for each group.

4.9 Ovarian cysts

Ovarian cysts were found on one pig in group 3 (16,6% of the group). In the other groups there was no occurrence of ovarian cysts.

4.10 Histological assessment of the uterine glands

Group 1 uterine gland epithelium thickness (UGET) mean in width was 23 µm (SD 3.7, range 15-38 µm), group 2 UGET was 24 µm (SD 4.3, range 15-38 µm) and group 3 UGET was 24 µm (SD 3.9, range 17-38 µm) (figure 16).

The mean number of uterine glands in group 1 was 38 (SD 6.9, range 26-55), in group 2 40 (SD 9.6, range 23-61) and in group 3 38 (SD 7.7, range 24-59) on average (figure 17).

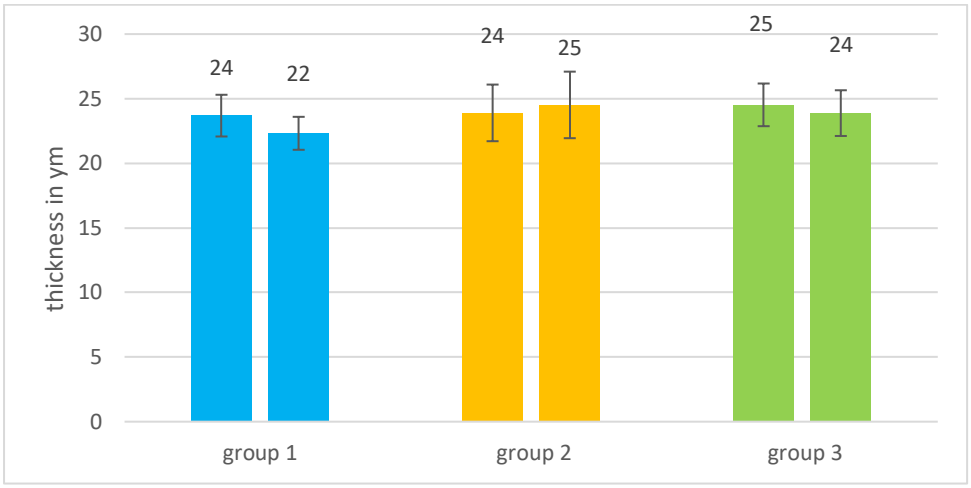


Figure 27: Uterine gland epithelium thickness. Mean and standard deviation for each group and uterine gland epithelium. Left uterine horn (left column) and right uterine horn (right column).

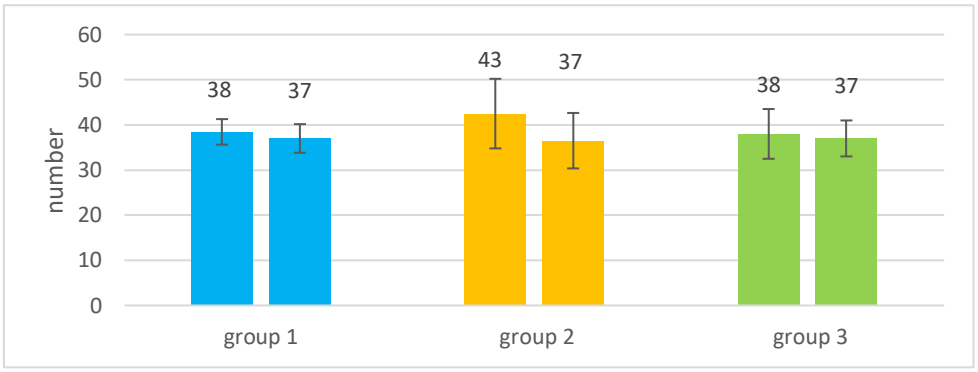


Figure 28: Number of uterine glands. Mean and standard deviation for each group and uterus horn. Left uterine horn (left column) right uterine horn (right column)

4.11 Histological assessment of the myometrium and epithelium

Group 1 myometrium thickness (MT) was 1767 µm (SD 573, range 966-4095 µm) group 2 MT was 2075 µm (SD 426, range 1266-3416 µm) and group 3 MT was 2143 µm (SD 2143, range 1073-3587 µm) on average in width.

Group 1 mean epithelium thickness (ET) was 29 µm (SD 7.5, range 13-58 µm) group 2 ET was 30 µm (SD 7.4, range 13-57 µm) and group 3 ET was 30 µm (SD 6.7, range 14-50 µm) on average in width.

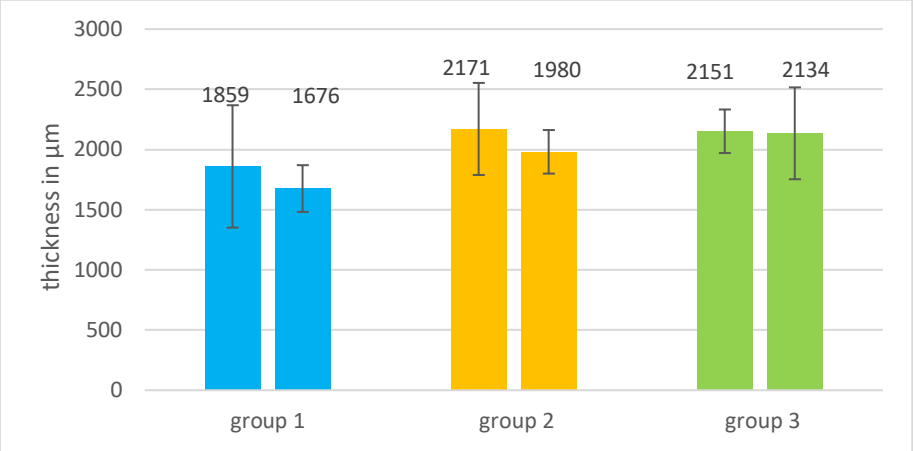


Figure 29: Myometrium thickness. Mean and standard deviation for each group and uterine gland epithelium. Left uterine horn (left column) and right uterine horn (right column).

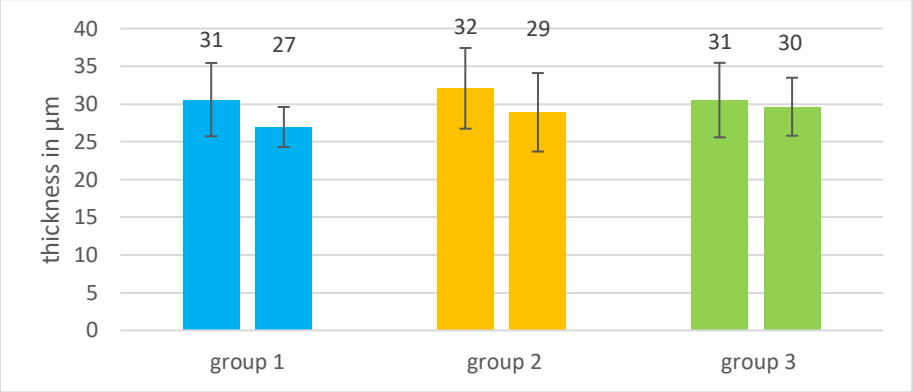


Figure 30: Uterine epithelium thickness. Mean and standard deviation for each group and uterus horn. Left uterine horn (left column) and right uterine horn (right column).

4.12 Results of the statistical analysis

Only three parameters had *p*-values lower than or exact 0.05. Body weight with group 3 being lighter than group 2 and group 1 being the intermediary has a p value of 0.005. The left uterine horn being longer than the right horn with a significance of p = 0.026 and the surface epithelium of the left uterine horn being thicker than that of the right horn with a p value of 0.001. All other parameters were statistically non-significant.

5 Discussion

Genital Chlamydiosis is one of the most widely sexually transmitted disease worldwide and affects large swathes of the population, especially in less developed countries. As with all diseases, vaccination is superior to treating the disease symptomatically. To this effect, efforts have been made to develop a vaccine using a plethora of animal models. This study aimed to investigate the morphological and histological differences in the reproductive tract of gilts following an intracervical *Ct* infection and to assess whether vaccination with a CPAF-based vaccine had a mitigating effect. Although porcine models are increasingly recognized for their anatomical and immunological similarities to humans, their suitability for modelling uterine *Ct* infection remains unclear.

The overall macroscopic findings indicated that both infection and vaccination had a negligible visible effect on the reproductive tract. Notwithstanding that the infection did not fester and that Chlamydial activity was not detected by swab tests post infection. While mucosal redness and hyperaemia were evident in vaccinated and infected gilts (groups 2 and 3), they also appeared in the control group (group 1). Redness and inflammation might have been caused by the process of challenging all sows intracervically with a catheter. Another source for mucosal redness and hyperaemia might be the oestrus cycle in which the uterine lining is physiologically hyperaemic and redend. In contrast to slaughtered pigs euthanized pigs have large amounts of blood remaining in the body and therefore have redder organs and tissue, this would be of importance when comparing porcine genital tracts of slaughtered and euthanized animals. Discharge and exudate were seen in one third of the control group and in one sow of group 3 and intrauterine fluid was only observed in one sow of group 2. This is the opposite to Käser et al.(34), were the findings showed that most of the mock inoculated pigs had no abnormal histotrophic changes however these were seen in more than halve of the *Ct* inoculated pigs. This difference is caused by the fact that the inoculation was largely successful in Käser et al. trial. These parameters would suggest a mild inflammatory response. As this inflammatory response was seen in all groups, it seems to be more likely a result of intracervical challenge in some, but not all sows, than a consequence of the experimental treatments. Pathologies were absent in the oviducts as these were all unobstructed. A basic uterine luminal pH over 7 suggests an infection took place whereas inflammation without infection leads to a more acidic milieu (35), all groups were more basic with the control group being the most acidic with an average pH of 7.1. This might suggest that an infection other than that by chlamydia might

have taken place. Morphologically, uterine horn lengths and diameters were slightly increased in infected gilts, particularly in group 3, but again, without any statistically significant differences. Such changes might reflect subclinical inflammation or fluid accumulation. The cervix of vaccinated pigs (group 3) also showed a modest increase in diameter, potentially due to localized mucosal oedema. The left uterine horn was on average longer, this has also been shown by Egli et al (32). According to Egli et al., the mean length and standard deviation of left uterine horn was 148.7 cm ± 55.1 cm and of the right uterine horn 143.5 cm ± 55.4 cm these were all measured in adult pigs whereas the gilts in this experiment had a mean length and standard deviation of the left uterine horn of 138 cm ± 17.4 cm and the right uterine horn of 130 cm ± 17.7 cm. This anatomical difference in length across all groups and the findings of Egli et al. are interesting to say the least. Reasons for this length disparity remain unknown and require further examination to determine the reason.

Shortfalls in this experiment that could be changed in future is the use of pH electrodes instead of pH paper and the use of water displacement to better calculate the ovarian volume and density instead of relying on the prolate ellipsoid formula. Similarly to the macroscopic finding between left and right uterine horn length, the left uterine surface epithelium is significantly thicker than the right uterine surface epithelium. Hyperaemic and redend uterine horns do not correlate to thicker uterine surface epithelium or myometrium. A slight numerical increase in uterine myometrium thickness was seen in groups 2 and 3 when compared to the control group. This might support the hypothesis that *Ct* infection and or contact with cPAF induces myometrial remodelling, possibly due to chronic low-grade inflammation or muscular hyperplasia. These results are shown in murine and non-human primate studies, which have reported thickening of uterine tissues following infection (17,18). Interestingly, glandular epithelium thickness and gland density hardly differed among groups, suggesting that endometrial gland structure might be less affected by short-term infection or that the infection had not yet progressed to stages where deeper structures were affected. Hyperplasia and hypertrophy of the uterine glands can be seen when mucus secretion is increased (36).

Overall, while the study revealed some minor measurable morphologic and histologic differences post *Ct* contact, the changes were slight, inconsistent and not statistically significant. This raises questions about the porcine genital tract's sensitivity and reproducibility as a model for *Ct* infection compared to rodents or non-human primates. As noted in recent literature, pigs may not fully replicate the ascending infection or chronic sequelae seen in

human *Ct* cases, although their immunological and anatomical parallels remain advantageous (24). To see the differences and better understand the viability of the reproductive tracts of pigs as a model for *Ct* infections, a similar trial with *C. suis* would be helpful and to establish a swine reproductive protocol for macroscopic and histologic sampling and data collection. To this end, the data and findings in this thesis can serve as a reference and valuable source for future work, but also for pathologist who can incorporate this basis data into their routine diagnostic assessments, as corresponding textbook knowledge is currently still lacking.

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7 List of abbreviations

Ct - Chlamydia trachomatis

SD - standard deviation

MOMP - major outer membrane protein

cPAF - chlamydial-protease-like-activity factor

PRRSV - Porcine Reproductive and Respiratory Disease Virus

BSL-2 - bio- safety level 2

UGET - uterine gland epithelium thickness

MT - myometrium thickness

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