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Gross and histopathological evaluation of umbilical outpouchings in pigs

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ABSTRACT

Clinical presentations of umbilical outpouchings (UOs) in pigs cover a variety of pathological manifestations. Pigs with UOs often do not reach the abattoir as they die due to complications or are euthanized for welfare concerns. The primary objective was to characterize the gross appearance of UOs in pigs with respect to the different types of pathological manifestations. Also the association between the pathological manifestation and presence of a wound on the UO was evaluated. Pigs (in different age groups, $n = 444$) with an UO were sampled in Denmark from different locations (two herds and at an abattoir) and examined post mortem. Tissue samples from animals with an enterocystoma or internal umbilical proliferations were collected for histological and immunohistochemical characterization. Hernia umbilicalis was the most frequent cause (72%, $n = 320$) of the UOs. It was the only diagnosis in 57% ($n = 254$) of the pigs, and in 15% ($n = 66$) of the pigs the hernia appeared in combination with other manifestations. Thus, 28% ($n = 124$) of the pigs were diagnosed with an enterocystoma, internal umbilical proliferations, subcutaneous abscess/ fibrosis or another diagnosis, presented alone or in combination. The distribution of diagnoses varied in the different age groups. Overall, 38% (110/291) of the pigs presented a wound on the UOs post mortem. The age of the pigs confounded the relation between the pathological manifestation and the presence of a wound. The odds that an UO had a wound were lower among pigs with a subcutaneous abscess/ fibrosis compared to pigs diagnosed with an umbilical hernia or enterocystoma (OR, 0.3; 95% Cl, 0.1–0.7). The odds of wounds were higher among weaners (OR, 4.3; 95% Cl 2.3–8.3) and finishers (OR, 6.5; 95% Cl, 3.4–12.7) compared with piglets from the farrowing unit. The area of wounds ranged from 0.03 to 78.5 cm^2 and increased with age ($P < 0.001$). Histologically and immunohistochemically the enterocystomas and internal umbilical proliferations seemed to be lined with mesothelial cells and both had a content comparable with mesenchymal embryonic connective tissue. However, only the cavities of the enterocystomas were also lined with mesothelial cells. In conclusion, UOs in pigs are caused by complex pathological conditions with hernia umbilicalis as the dominating diagnosis. Knowledge clarifying the different pathological manifestations causing an UO and the presence of wounds on the UOs is essential for future prevention strategies.

1. Introduction

In recent years, umbilical hernia or other conditions causing umbilical outpouchings (UOs) have received increased attention in Danish pig production. This is in line with animal welfare being more and more a subject of national policy discussions. In 2002, the prevalence of UOs in Danish crossbreed pigs (Landrace/Yorkshire × Duroc) was reported to be 4.2% (Vestergaard and Wachmann, 2002). Major between-herd variation has been reported worldwide, from 1.5% (Searcy Bernal et al., 1994) to 10.1% (Mattsson et al., 2013). The pathological conditions primarily causing UOs are hernia umbilicalis, enterocystoma, internal umbilical proliferations, subcutaneous abscesses and subcutaneous fibrotic masses (Hovmand-Hansen et al., 2021b, 2021a), however, the distribution pattern of lesions with respect to age groups has not been determined. An umbilical hernia is characterized by an opening in the abdominal wall muscles at the umbilicus (umbilical ring), through which a protruding pouch of the peritoneum forms a hernial sack (Uzal et al., 2016). Based on a comprehensive review of outpouchings in Danish pigs, Petersen (1938) considered that umbilical cysts present as a slightly fluctuating prominence in the umbilical region, originated from

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the yolk stalk. The prominence consists of a solid, fibrotic mass of uni- or multi-cameral cyst-like sacs often containing serous or bloodstained fluid. The masses were located on both sides of the umbilical ring (hourglass-shaped) or only on the inner part of the abdominal wall (Petersen, 1938). Internal umbilical proliferations earlier mentioned as peritoneal proliferations (Hovmand-Hansen et al., 2021b, 2021a) are inadequately described in the literature. They are often observed in combination with an umbilical hernia, where the proliferations branch off from the umbilical ring or the hernial sack. The oblong shape of the proliferations is terminated by a paddle-shaped or round vesicle (Andersen, 2014). The origin of enterocystoma and internal umbilical proliferations has only been elucidated on the macroscopic level, but not characterized histologically. As a result of omphalitis, umbilical subcutaneous abscesses are common finding in UOs (Ameh and Nmadu, 2002). These lesions may in the healing process turn into a subcutaneous fibrotic mass (Andersen, 2014). UOs may have a negative effect on animal welfare and the presence of wounds located on the UOs influence the severity (Barington et al., 2016). Pain and/or discomfort is reported in pigs (Schild et al., 2015) and humans (Miller et al., 1995) with an umbilical hernia. This is especially seen in cases with adherences, strangulation or obstruction of intestinal segments (Miller et al., 1995). Moreover, animals with larger umbilical outpouchings with or without ulcerations are not suitable for transportation (Anonymous, 2005).

The objectives of the present study were to characterize the distribution pattern of pathological manifestations causing umbilical outpouchings with respect to age. Moreover, the association between the pathological manifestation causing UOs and the presence of wounds on UOs was analyzed. Finally, the enterocystoma and internal umbilical proliferations were characterized histologically and immunohistochemically.

2. Materials and methods

Porcine OUs were collected at three different locations in Denmark from July 2017 until December 2020. All procedures involving animals complied with the guidelines concerning animal experimentation and care of animals under study. The current study was approved by Danish Ministry of Justice Law (Licence no. 2017/ 15-0201-01372).

2.1. Animals and collection site

The pigs with UOs were collected from an abattoir (Danish Crown (DC), Ringsted) and two commercial farrow-to-finish herds. The herds had previously been selected for a longitudinally study investigating risk factors and survival of pigs with UOs (Hovmand-Hansen et al., 2021a, 2021b), and had carefully been selected in collaboration with a veterinarian based on a long-lasting problem with UOs (frequency >5%). Herd A (≈ 1050 sows) was a herd free of a number of major pathogens such as *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Brachyspira hyodysenteriae*, *Porcine reproductive and respiratory syndrome virus*, *Pasteurella multocida*, *Haematopinus suis*, and *Sarcoptes scabiei* var. *suis* (SPF-sus, 2017). Herd B (≈ 1600 sows) was of unknown health status. The pigs in both herds were cross-breed (Yorkshire/Landrace and Duroc), and a thorough description of the herds has recently been published (Hovmand-Hansen et al., 2021a).

2.1.1. Cross-sectional study at an abattoir

During a period of four days from 6 AM to 2 PM, specimens (n = 65) were independently and randomly collected. A veterinarian (Trine Hovmand-Hansen (THH)) and a technician were permitted access to two slaughter lines. Specimens were collected from pigs identified with an UO by the employees carrying out routine meat inspection. The umbilical region was cut off, numbered with blue or pink numbers according to sex and put aside in a cooler. Specimens were subsequently examined within two days.

2.1.2. Cross-sectional study in the herds

A cross-sectional study was performed in Herds A and B to assess the frequency of UOs and diagnose the different pathological manifestations (hernia, enterocystoma, abscesses, etc.) of the UOs. The routine at Herd A was to euthanize all piglets presenting an UO in the farrowing unit. For a month, the farmworkers collected spontaneously dead or euthanized piglets with an UO and kept the carcass in a freezer until examination. A veterinarian (THH) and a veterinary student visited the herd every week (four times) in the period and the veterinarian (THH) performed a gross evaluation of the collected animals (n = 162). The routine in Herd B was to group all UO-pigs at weaning; then, a herd-veterinarian made a hernia repair surgery of pigs with uncomplicated UOs, whereas pigs with complicated UOs (adherences or strangulated peritoneal/intestinal segments) were euthanized. A veterinarian (THH) and a veterinary student visited the herd at a surgery day (January 2018) and evaluated the removed UOs (n = 36) by gross inspection and the euthanized pigs (n = 7) with UOs.

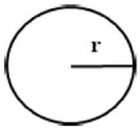
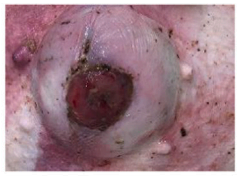
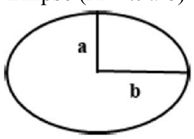
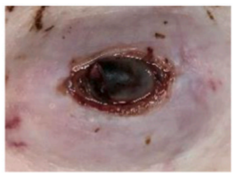
2.1.3. Cohort study in the herds

A longitudinal cohort study following pigs from birth to slaughter was designed. Within the first day of life, the piglets received an identification mark. The cohort was followed until slaughter at approximately 6 months of age. The umbilical areas of all pigs were screened monthly for the development of an UO. An UO was defined as a protrusion in the umbilical area of more than 0.5 cm in height, defined longitudinally from the base to the tip of the UO. Pigs observed with an UO received an additional identification earmark. Pigs with UOs were either slaughtered, they died or were euthanized (see below). Pigs were inspected every day by the farmworkers and every month by a veterinarian (THH) and a technician. To protect the animals from pain and discomfort they were euthanized if: 1) the general condition was affected, 2) the gait or movement were affected by the size of the UO, or 3) ulcers > 1 cm across were observed on the UO. If pigs with UOs died or were euthanized, the farmworkers made a note of date and cause of death and kept the pig in a freezer until next visit. All UOs (n = 174) were evaluated grossly by a veterinarian (THH).

2.2. Gross evaluation

The sex (male; female) and age group (piglet ≤ 28 days; weaner 29–72 days; finisher > 72 days) of the pig were registered. The UOs were evaluated with regard to size and the diagnosis. UOs were initially described from the outside by form (symmetric; asymmetric) and skin-lesions were identified; these were defined as wound(s) or superficial scratches. Wounds located ventrally on the UO were measured with a tape measure (longitudinally and across); if multiple wounds were present, only the largest wound was measured. The area of the wounds was calculated either as a circle or an ellipse depending on the shape of the wound-edges, Table 1. The size of the UO was characterized from: 1) height (cm) from basis to apex of the UO, and 2) circumference (cm) measured around the UO horizontal axis at the widest position. Reducibility was demonstrated by applying pressure at the external UO together with returning of the contents back into the abdominal cavity, which reduced the size of the UO (irreducible; partly reducible/reducible). At necropsy the following diagnoses were given: umbilical hernia, enterocystoma, internal umbilical proliferation, subcutaneous abscess, subcutaneous fibrosis or other (preputial diverticulitis/persistent urachus/hematoma). The conditions occur alone or in combination and could be located intra-abdominally, extra-abdominally or as a combination of the two. An opening in the abdominal wall was registered and the area of the umbilical ring was calculated either as a circle or an ellipse depending on the shape, Table 1. In order to state a diagnosis, manifestations were characterized according to Andersen et al. (2014), Petersen (1938) and Uzal et al. (2016). Together with the macroscopic findings, presence of ligamentum teres hepatis and ligamentum vesicae medianum was registered. Not all gross evaluation data were available,

Table 1
Calculation of the area of skin lesions and the size of the abdominal ring.

	Wound Area (A) (cm^2)	Abdominal ring (A) (cm^2)
Circle ($A = \pi \cdot r^2$) 		
Ellipse ($A = \pi \cdot a \cdot b$) 		

because the type of collection differs. For UOs collected at slaughter and after hernia repair surgery, the external evaluation, such as size and reducibility was missing, because the umbilical area was cut off the pig. Finally, some pigs were stored frozen at the herds, and even though they were supposed to be defrosted, the UO were still frozen at the post mortem examination.

2.2.1. *Tissue sampling*

Samples were taken from animals which were diagnosed with an enterocystoma or internal umbilical proliferations at gross evaluation. The samples were collected by a transverse section; representing the interior and exterior side of the wall. The samples were immersion-fixed in 10% neutral buffered formalin for at least 10 days.

2.2.2. *Light microscopy*

For routine microscopy, the immersion-fixed tissue samples were processed through stepwise concentrations of ethanol and xylene and embedded into a paraffin wax block (Wolfe, 2018). From each tissue block, one or two microscopic sections (4–5 μm in thickness) were cut and stained with hematoxylin and eosin (HE) (Wolfe, 2018). Three observers assessed the histological parameters of connective tissue, mesenchymal tissue, cell-density, mononuclear leucocytes, neutrophils or macrophages. Following the first histological evaluation, immunohistochemical staining was performed on selected specimens. The following specific antibodies were used; vimentin (Vimentin Mab., 1:250, DAKO Glostrup, Denmark M0725), a marker for mesenchymal cells, and cytokeratin (CK) (Cytokeratin clone AE1/AE3. Mab., 1:300, DAKO Glostrup, Denmark M3515), a marker for epithelial cells. Furthermore, the Anti-LYVE1 antibody (Anti-LYVE1 (ab33682), 1:500), which is a marker for lymphatic vessel endothelium, was used. To assess antibody binding, relevant control tissues (lung and liver) were stained.

2.3. *Data management and statistical analysis*

Data processing and statistical analyses were performed in R version 4.0.2 (R Core Team, 2020). A P-value less than 0.05 defined statistical significance. The characteristics of different pathological manifestations obtained at gross evaluation were described and the pathological manifestations within age groups are presented as a contingency table.

2.3.1. *Statistical models*

To investigate the association between the pathological manifestation and wounds on the UOs, only data from the herds were included, because no skin-lesions were present in the abattoir material because animals presenting a pathological process in general are considered unfit for transport (Anonymous, 2005). Furthermore, pathological manifestation groups including fewer than five pigs were excluded, along with pigs with more than one pathological manifestation of the UO, because it was unclear which group the pig should represent.

The pathological manifestations associated with the presence of wounds (outcome) were identified using a multivariable logistic analysis, for which the R function glm() was used. The predictor variable of main interest was the type of pathological manifestation causing the UO. The variable was categorized into: umbilical hernia, enterocystoma, and abscess/fibrosis and internal umbilical proliferation. Furthermore, the following demographic variables were offered to the model: collection site (Herd A, Herd B), sex (female, male) and age group (piglet, weaner, finisher). Previously, a positive association between age of the pig and the size (circumference and height) of the UO has been established (Hovmand-Hansen et al., 2021b); therefore, only age, was incorporated in the model. At first, a univariable logistic regression model was used to screen for the association between the explanatory variables and the presence of wounds on the UOs. If a P-value of a specific variable was ≤ 0.25, the variable was offered to the multivariable logistic model, including the presence of wounds on the UO as outcome and the variable "pathological manifestation" as the primary variable, while other variables were added to control for these. Model reduction was done using backward elimination with the likelihood ratio test (lmtest package). Furthermore, confounding was assessed by comparing the parameter estimates of the univariable and multivariable models. The variables were considered as confounders if changes above 20% in the coefficients were identified, and any confounder was kept in the model. The goodness of fit was assessed from Pearson standardized residuals plot, looking for extreme residual values. The impact of specific values was evaluated by comparing the model with and without outlying residuals.

An analysis of variance (ANOVA) (aov () – function in R) was implemented to evaluate the differences in the mean area of wounds (outcome) on the UO between the different pathological manifestations and age groups. Initially, the area of wounds was described statistically (mean, median, SD and range) and the assumption of normality was visualized for each group by density- and qq-plots and a shapiro-wilk test (shapiro.test () – function in R) of normality was applied. If data were skewed, a log transformation was implemented. To check the assumption of homogeneity of variances a Bartlett's test (Bartlett.test () – function in R) was implemented. The likelihood ratio test (lmtest package) was used to evaluate model fit. To report the effect of differences a Tukey's Honest Significant Difference test (TukeyHSD) was applied using the TukeyHSD() - function in R.

3. Results

3.1. Macroscopic characterization of UOs and underlying pathological manifestations

In total, 444 pigs with UOs were examined post mortem, with 379 of the UOs sampled from pigs in herd A and B and 65 sampled at the abattoir. The pigs represented the following age groups: 32.7% (145/444) piglets from the farrowing unit (age 0–28 days), 25.2% (112/444) were weaners (age 29–72) and 42.1% (187/444) were finishers (age > 72 days).

The most common manifestation of an UO was a hernia umbilicalis. In the majority of the pigs, umbilical hernia was represented as the only pathological manifestation (57.2% (254/444)) and in 14.9% (66/444) the umbilical hernia was combined with another diagnosis, e.g.; subcutaneous abscess or fibrotic masses, enterocystoma or internal umbilical proliferation, Table 2. In 11.7% (52/444), pigs were diagnosed with an enterocystoma and in 10.1% (45/444) an abscess or fibrosis was fund at the umbilicus. Internal umbilical proliferations were represented as the only diagnosis in four pigs (0.9%), otherwise this manifestation was found in combination with other diagnoses (6.8% (30/444)). The frequency of diagnoses in different age groups according to the different collection sites is given in Table 2. From the table it appears that the frequency of umbilical hernia represents 93.8% (136/444) in piglets and decreases with age to 50.9% (57/444) in weaners and 32.6% (61/444) in finishers. Further, it is apparent from Table 2, that the frequencies differ between UOs collected at the abattoir and UOs collected in the herds. Umbilical hernia represents 18.5% (12/65) in UOs collected at the abattoir, whereas the frequency was 63.9% (242/379) in the herds. Enterocystoma was more frequently represented in UOs from the abattoir (26.2% (17/65)) compared to UOs from the herds (9.2% (35/379)). The gross evaluation of each diagnosis is characterized in the following section.

3.1.1. Umbilical hernia

Gross findings were described from 212 pigs with an umbilical hernia as the only diagnosis and with all registrations included. The distribution by sex was 72.2% (153/212) females and 26.9% (57/212) males. The age-distribution was as follows; 58.5% (124/212) piglets, 23.6% (50/212) weaners and 17.9% (38/212) finishers. In total, the median circumference of the hernial sacs was 9.0 cm ranging up to 58.0 cm and the median height was 2.3 cm ranging up to 18.0 cm, and increased with age, Table 3. The hernial sac appeared reducible in 76.9% (163/212) of cases. The size of the umbilical ring varied from 0.03 to 44.0 cm^2, with a median size of 0.8 cm^2. If the umbilical ring was wide enough, the content protruding into the hernial sac was mainly omentum and intestinal segments. In 9.0% (19/212) of the pigs, adherence was observed with the hernial sac and omentum and/or intestinal segments as the dominating site of adherence. On incision, the thickness of the hernial wall varied between 0.1 and 1.0 cm, with a median size of 0.2 cm. Depending on the age of the pigs (Fig. 1), a fibrous band from the liver (ligamentum teres hepatis) or from the bladder (ligamentum vesicae medianum) or both were often attached to the hernial sac or at the border of the umbilical ring. Ligaments were observed in 122 of the pigs diagnosed with an umbilical hernia. In 66% (80/122) of the pigs the ligamentum teres hepatis was present, in 14% (17/122) of the pigs the ligamentum vesicae medianum was present and both ligaments were present in 20% (25/122) of the pigs registered with ligaments.

3.1.2. Enterocystoma

Grossly, an enterocystoma was described in 51 pigs as the only diagnosis (see photo illustration in Table 4). The distribution by sex was 54.9% (28/51) females and 37.3% (19/51) males. The age-distribution was as follows: 9.8% (5/51) piglets, 35.3% (18/51) weaners and 54.9% (28/51) from the finisher section. The lesions showed a spherical or

Table 2
Frequency of pathological manifestation with respect to age groups and collection site of Danish pigs with umbilical outpouchings.

Pathological manifestation	Piglets, n (%)				Weaners, n (%)				Finishers, n (%)				Total, n (%)
	Abattoir; cross sectional	Herd; cross sectional	Herd; cohorte study	Total	Abattoir; cross sectional	Herd; cross sectional	Herd; cohorte study	Total	Abattoir; cross sectional	Herd; cross sectional	Herd; cohorte study	Total	
Umbilical hernia (UH)		129	7	136 (93.8)		40	17	57 (50.9)	12		49	61 (32.6)	254 (57.2)
Enterocystoma		5		5 (3.4)		14	4	18 (16.1)	17		12	29 (15.5)	52 (11.7)
UH and enterocystoma		1		1 (0.7)		3	2	5 (4.5)	2		5	7 (3.7)	13 (2.9)
Abscess /fibrosis		2	1	3 (2.1)		9	8	17 (15.2)	7		18	25 (13.4)	45 (10.1)
UH and abscess/fibrosis				0 (0)		2	8	10 (8.9)	6		18	24 (12.8)	34 (7.7)
UH and internal umbilical proliferations				0 (0)			2	2 (1.8)	13		4	17 (9.1)	19 (4.3)
Internal umbilical proliferations alone /combined[a]				0 (0)			1	1 (0.9)	7		7	14 (7.5)	15 (3.4)
Other[b]				0 (0)			2	2 (1.8)	1		9	10 (5.3)	12 (2.7)
Total	0	137	8	**145**	0	68	44	**112**	65	0	122	**187**	444 (100)

[a] Peritoneal proliferation alone/combined: alone (n = 4), combined with enterocystoma (n = 6), combined with abscess/fibrosis (n = 3), combined with hernia and abscess/fibrosis (n = 2).
[b] Other: hematoma (n = 3), edema (n = 3), edema (n = 2), patent urachus (n = 6), peritoneal invagination (n = 1).

Table 3
Descriptive statistics of size (circumference and height (cm; median (min;max)) of umbilical outpouchings from 308 pigs in different age groups.

Pathological manifestation	Size Median (cm) (min;max)	Total	Age groups		
			Piglets	Weaners	Finishers
Umbilical hernia					
	Circumference	9.0 (3.5;58.0)	7.5 (3.5;22)	10.0 (4.0;42.5)	40.5 (13.0;58.0)
	Height	2.5 (0.3;18.0)	2.0 (0.3;5.0)	2.75 (0.3;10.5)	7.0 (2.5;18.0)
Enterocystoma					
	Circumference	13.9 (4.0;50.0)	6.0 (4.0;10.0)	7.8 (4.5;21.3)	25.0 (12.6;50.0)
	Height	4.0 (1.0;13.0)	1.5 (1.0;4.0)	2.25 (1.0;5.0)	7.0 (3.0;13.0)
Abscess/fibrosis					
	Circumference	9.3 (1.5;47.0)	8.0 (3.1;9.0)	8.0 (5.0;18.5)	13.3 (1.5;47.0)
	Height	2.3 (1.0;13.0)	2.5 (1.0;2.5)	2.0 (1.0;5.7)	3.5 (1.0;13.0)
Total	Circumference	10 (1.5;58.0)	7.3 (3.1;22.0)	9.0 (4.0;54.5)	31.5 (1.5;58.0)
	Height	3 (0.3;18.0)	2.0 (0.3;5.0)	2.5 (0.3;10.5)	6.0 (1.0;18.0)

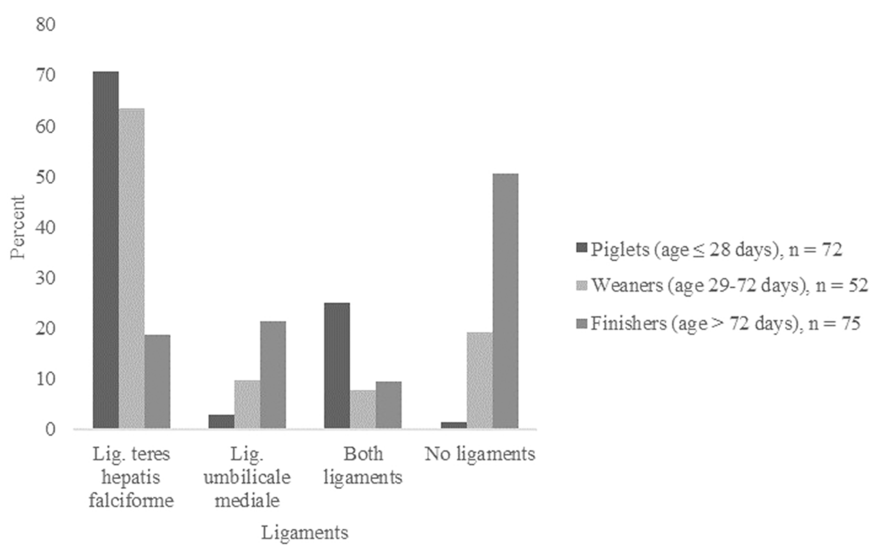
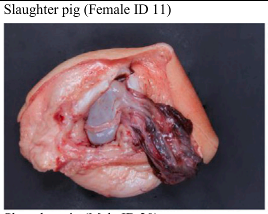
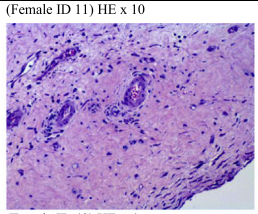
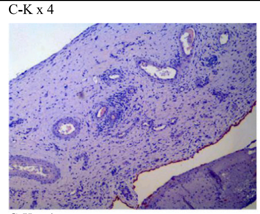
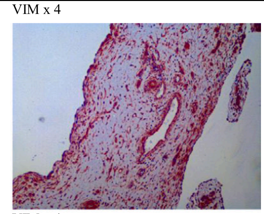
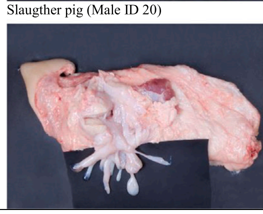
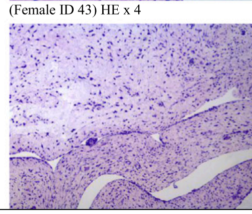
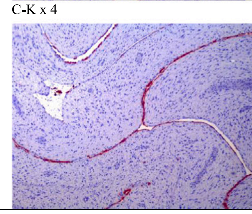
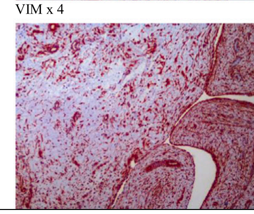


Fig. 1. Remnants of fetal circulatory ligaments registered in different age groups of pigs with an umbilical hernia.

Table 4
Illustrations of internal umbilical proliferations and enterocystoma in pigs, macroscopically and histologically.

	Macroscopically	**Histologically**		
	Slaughter pig (Female ID 11)	(Female ID 11) HE x 10	C-K x 4	VIM x 4
Enterocystoma				
	Slaugther pig (Male ID 20)	(Female ID 43) HE x 4	C-K x 4	VIM x 4
Internal umbilical proliferation				

hourglass-shaped prominence, with a median circumference of 13.9 cm ranging up to 50 cm and a median height of 4.0 cm raging up to 13 cm in the umbilical region. The size of an enterocystoma increases with age, Table 3. The cyst appeared often combined with an opening in the abdominal wall (hernial ring) and was located intra- (5.7%), or extra- (51.4%) abdominally or both (42.9%). Enterocystomas were in all cases irreducible. The sectioned surface had a uni- or multi cystic appearance with 1 to more than 10 cysts, which could be described as a honeycomb or microcystic appearance. The sacs often contained serous, blood-stained fluid in varying amount. Previous hemorrhages present as blood clots could also be present. The walls of the sacs were often edematous and fibrotic with prominent vascularity and the thickness of the cyst-wall varied from 0.1 to 2.0 cm with a median thickness of 0.4 cm. The intra-abdominal part of the cyst could be adherent to the ileum or surrounding structures such as omentum majus by fibrous strands (7.8% (4/51)). Ligaments (ligamentum teres hepatis / ligamentum vesicae medianum) were observed in 17.6% (9/51) of the pigs diagnosed with an enterocystoma.

3.1.3. Subcutaneous abscess/fibrosis

A subcutaneous abscess or subcutaneous fibrotic mass was found as the only diagnosis in 10.1% of the pigs (45/444), whereof 57.8% (26/45) were females and 42.2% (19/45) were males. The age-distribution was as follows: 6.7% (3/45) piglet, 37.8% (17/45) weaners and 55.6% (25/45) finishers. The abscess/fibrosis was mainly spherical and symmetric, with a median circumference of 9.3 cm ranging up to 47.0 cm and a median height of 2.3 cm raging up to 13 cm in the umbilical region, Table 3. The subcutaneous abscesses or fibrotic masses were irreducible. The subcutaneous abscess/fibrosis was located extra-abdominal and appeared in some pigs (n = 8) combined with an opening in the abdominal wall, ranging from 0.03 to 1.8 cm^2 in size. The abscess-wall varied from 0.5 to 2.5 cm with a median thickness of 0.8 cm.

3.1.4. Internal umbilical proliferation

The internal umbilical proliferations were seldomly the only diagnosis (0.9% (4/444)) (see photo illustration in Table 4). Often they were present together with an umbilical hernia (4.7% (21/444)). In total (21 +4), 80% (20/25) was females and 20% (5/25) was males. The proliferations were attached to the umbilical ring or arose from the hernial wall. The pathological findings of paddle-like fibrous tissue were often present in multiple numbers. No adherences were observed in pigs diagnosed with internal umbilical proliferations.

3.2. Association between the pathological manifestations and wounds on the UO

To investigate the association between the pathological manifestation and wounds on the UOs, data were reduced as described in Section 2.4.1. After the reduction, the data included 291 pigs whereof 37.8% (110/291) had a wound on the UO. Only four pigs were diagnosed with internal umbilical proliferations as the only pathological manifestation for which reason the pathological manifestations were reduced to three categories; umbilical hernia (234), enterocystoma (26) and abscess/fibrosis (31). Presence of wounds was not associated with pathological manifestations in a univariable model ($P = 0.1$), but when age groups was taken into account, a significant association was found. The results of the multivariable logistic model together with number and distribution of wounds are presented in Table 5. The odds of presenting a wound on the UOs were significantly lower among pigs diagnosed with an abscess/fibrosis (OR, 0.3; 95% Cl 0.1–0.7) compared to pigs with an umbilical hernia (OR, ref.) or an enterocystoma (OR, 1.1; 95% Cl 0.5–2.7). The predictor variable age confounded the relation between the pathological manifestation and presence of wounds, and was consequently included. The odds of having a wound on the UO were significantly higher among finisher pigs (OR, 6.5; 95% Cl 3.4–12.7)) and weaners (OR, 4.3; 2.3–8.3) compared to piglets from the farrowing unit (OR, ref.). No multicollinearity was observed in the model (VIF < 2), the model assumptions were found to be fulfilled, and no outliers with influential impact were observed.

The descriptive statistics of the area of wounds on the UO were presented in Table 5. In general, the mean area of wounds on the UO was 6.2 cm^2 and 50% of the wounds were less than 2.1 cm^2. The area of the wounds ranged from 0.03 to 78.5 cm^2. Because of the distribution of the data (Shapiro-Wilk test $P < 0.05$), a log transformation was used prior to analytical statistics. The results of the analysis of variance (ANOVA) showed no differences in the mean area of wounds between the different pathological manifestations ($P = 0.8$), although a significant difference in area of wounds was observed between different age groups ($P < 0.001$), suggesting that wound area increases with age. The assumption of equal variance between the different age groups ($P = 0.47$) and different pathological manifestations ($P = 0.52$) was met.

3.3. Histological and immunohistochemical features of enterocystoma and internal umbilical proliferations

3.3.1. Enterocystoma

The histological findings in the enterocystomas revealed loose connective tissue often with a high number of fibroblast and were well vascularized with perivascular cellular infiltrations. Hemorrhage and macrophages with hemosiderin could be observed as well as granulomas with necrotic centers and sporadic mineralization. Granulation tissue could be observed, presented by neovascularization and fibroblast proliferation. The external layer was lined with elongated mesothelial cells, which showed immunoreactivity for vimentin and sporadic immunoreactivity for cytokeratin. The internal layer was covered with cells, which were immunoreactive for both vimentin and cytokeratin, Table 4. In general, features of the cystic walls were comparable with mesenchymal embryonic connective tissue.

3.3.2. Internal umbilical proliferation

The histology of the internal umbilical proliferation showed also loose connective tissue, with fibrocytes of varying size. In some areas, the cell density of the fibroblasts was reduced compared to the enterocystoma, Table 4. The tissue contained large blood and lymphatic vessels, and was vascularized to varying degrees. Often, sporadic granulation tissue formation was present with neovascularization and fibroblast proliferation.

The external layer of the internal umbilical proliferations was lined with mesothelial cells, which were vimentin positive and sporadically cytokeratin positive. The cavities in the termination of the internal

Table 5
Descriptive statistics for the presence of wounds, pathological manifestations and ages in pigs with an umbilical outpouching, and the estimates from analytical statistics using a multivariable logistic model on the association between the pathological manifestations and wounds present on UOs.

	Wound on the UO		Size of the wounds (cm^2)			Estimate (β_x)	Std. error	OR (exp (β_x))	95% CI	P - value
Fixed effect	Yes (n (%))	No (n (%))	Mean (max; min)	Median	SD		Coefficient (β_x)			
Intercept						-1.3	0.2			
Age										
– Piglets	29 (10.0)	111 (38.1)	0.4 (0.03; 0.8)	0.4	0.5	Baseline	–	1	–	< 0.001
– Weaners	36 (12.4)	36 (12.4)	2.0 (0.1; 11.0)	0.8	3.2	1.5	0.3	4.3	(2.3;8.3)	
– Finishers	45 (15.5)	34 (11.7)	9.0 (0.16; 78.5)	3.5	16.2	1.9	0.3	6.5	(3.4;12.7)	
Pathological manifestation										
– Umbilical hernia	87 (29.9)	147 (50.5)	5.8 (0.03; 78.5)	2.5	11.9	Baseline		1	–	
– Enterocystoma	14 (4.8)	12 (4.1)	4.3 (0.2; 25.1)	0.8	7.6	0.1	0.4	1.1	(0.5;2.7)	0.02
– Abscess/fibrosis	9 (3.1)	22 (7.6)	12.6 (0.2; 70.7)	3.1	25.8	-1.2	0.5	0.3	(0.1;0.7)	
	$N_{pig} = 291$	$N_{wound} = 110$								

umbilical proliferations were visible and some filled with amorphous material. Elongated cells were found covering the caverns on the luminal side consistent with epi- or endothelial cells, which were vimentin positive and sporadically LYVE 1 positive. However, they were negative for cytokeratin.

4. Discussion

The main type of pathological manifestation causing UOs was umbilical herniation as the only manifestation or in combination with another diagnoses (72.1%), when pigs from all collection sites where accounted for. In piglets, the frequency of umbilical hernia as the only pathological manifestation was 94%, which was reduced to 51% in weaners and to 33% in finisher pigs. An explanation may be, that some umbilical hernias disappear spontaneously between the 4th and 14th week of life (Hovmand-Hansen et al., 2021a). Spontaneous resolving of umbilical hernias is also reported in children, mainly within the first three years of life (Cilley and Shereef, 2004). Another explanation for the reduction is the high risk of fatal complications in pigs with an umbilical hernia, compared to pigs with other types of pathological manifestation (Hansen, 2014; Mattsson et al., 2013). In the present study, the frequencies differed between UOs collected at the abattoir and in the herds. In comparison, Hansen (2014) collected 148 pigs in 26 Danish herds and found umbilical hernia (42%) as the dominating pathological manifestation, whereas Andersen et al. (2014) collected UOs from a Danish abattoir and reported enterocystoma (28%) as the dominating pathological manifestation, which correspond with the present study, in which an enterocystoma was found in 26% of the UOs collected at the abattoir. In general, these findings support the hypothesis that pigs having an umbilical hernia often are removed before scheduled slaughter. The gross appearance of an enterocystoma was comparable with that of Petersen (1938); showing a single- or multi cystic, cavernous masses, located intra-, extra-, or intra- and extra abdominal in the umbilical region. The odds of presenting a wound ventrally on the UO were significantly higher among weaners and finishers compared to piglets from the farrowing unit, which suggests that the risk of having a wound on the UO increases with increasing age. A positive linear correlation between age or weight of the pig and size of the UOs have earlier been described (Hansen, 2014; Hovmand-Hansen et al., 2021b); therefore, it is expected that the odds of having a wound on an UO also increase when the size of the UO is growing. These findings are comparable with those of Hansen (2014) who grossly evaluated 148 pigs of different ages with an UO, and described a significant relation between UOs with a diameter greater than 15 cm and the presents of wounds on the UO. We also previously described a higher risk of developing a wound on larger (circumference greater than: 7 cm in piglets, 10 cm in weaners and 29 cm in finishers) UOs compared to small ones and we described reducibility (content could be reduced by hand and returned to the abdominal cavity) of the UO as a protective factor for wound development on the UO (Hovmand-Hansen et al., 2021b). In the present study, 77% of the UOs caused by an umbilical hernia were reducible, whereas the remaining 23% umbilical hernias were irreducible due to adhesions between hernial content or a narrow umbilical ring. UOs of other pathological manifestations (cyst-complexes, abscesses and fibrosis) were irreducible by nature. Despite of the protective factor with regards to wounds described in UOs caused by reducible umbilical hernias (Hovmand-Hansen et al., 2021b), the odds of presenting a wound on the UO were significantly lower among pigs diagnosed with a subcutaneous abscess or fibrosis compared to pigs with an umbilical hernia or an enterocystoma. This is in contrast to Hansen, 2014, who found a significantly higher number of wounds present on UOs diagnosed with an enterocystoma. In both studies, UOs caused by a subcutaneous abscess or fibrosis had the lowest proportion of wounds (Hansen, 2014; Hovmand-Hansen et al., 2021b). A hypothesized explanation for the difference between the manifestations could be a higher pressure in UOs caused by an enterocystoma or an umbilical hernia. If a wound is applied by tread from other pigs or caused by abrasion at stable equipment or floor, it is likely that the pressure increases the tension on the wound edges, consequently reducing microperfusing and lowering oxygen delivery to the wound, which subsequently impairs wound healing (Guo and DiPietro, 2010). Another explanation could be the larger size of UOs in finishers diagnosed with an enterocystoma or umbilical hernia compared to a subcutaneous abscesses or fibrosis. The larger size of the UO seems to expose the UO to injuries and lesions. The mean area of the wound did not differ statistically between the different pathological manifestations causing an UO.

Petersen (1938) hypothesized that the enterocystoma originated from the yolk sac, but no details on histological features have hitherto been available. The external layer was lined with elongated mesothelial cells, which were vimentin positive and sporadically cytokeratin positive. The internal layer was covered with vimentin and cytokeratin positive cells. The immunohistochemical markers vimentin and cytokeratin are well known markers for mesothelial cells. The findings support the theory of a yolk sac origin, since the inner appearance of the yolk sac is lined by a single cell layer that is of mesodermal origin and shows immunereactivity for both cytokeratin and vimentin (Carter et al., 1998). Yolk sac tumors are also described in humans, mainly reported in testicular and ovarian cancer and commonly observed in young children (Kattuoa and Kumar, 2021). The ovarian yolk sac tumor is described as large and oval with defined margins and a mixed solid/cystic nature, intratumoral hemorrhage and enlarged intratumoral vessels (Li et al., 2015). The cystic areas are histologically comparable to the mesenchyme of the primitive yolk sac (Kattuoa and Kumar, 2021). The similarities between the yolk sac tumors reported in humans and enterocystomas reported in pigs support the theory of a yolk sac origin. Internal umbilical proliferation was histologically characterized by an external layer lined with mesothelial cells showing immunostaining for vimentin and sporadically cytokeratin. As mentioned, the mesothelial cells are found in peritoneal cavities of the body where they line the serous membranes (Carter et al., 1998), and also the internal umbilical proliferations. Visible caverns inside of the proliferations were covered with elongated epi- or endothelial cells showing immunostaining for vimentin and sporadically staining for LYVE 1 but not cytokeratin. Therefore, the internal umbilical proliferations seem to be unrelated to the enterocystomas. Morphology and immunostaining seem to be comparable to peritoneal mesothelial cysts found in humans (Karpathiou et al., 2021). In humans, the changes more often occur in women (75%) compared to men (Karpathiou et al., 2021). The same seems to be the case in piglets diagnosed with internal umbilical proliferations, were 80% of the pigs were females.

5. Conclusion

In conclusion, hernia umbilicalis was the most frequent cause of UOs, especially in piglets (< 28 days). Wounds present on the UOs increased significantly with age, and the odds of presenting a wound on the UOs were lower for subcutaneous abscesses or fibrosis compared to umbilical hernia and enterocystoma. Histological similarities were present between enterocystoma and a human yolk sac tumor, which support the hypothesis that the manifestation are originating from the yolk sac. This was not the case for the internal umbilical proliferations, which are similar to peritoneal mesothelial cysts found in humans. Further investigations are needed in order to elucidate the cause of enterocystoma and internal umbilical proliferations, which might be heritable.

Declaration of Competing Interest

The authors declare that there are no existing conflicts of interest according to research and authorship of this article.

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