

Urinary tract infections in culled sows from Greek herds: prevalence and associations between findings of histopathology, bacteriology and urinalysis

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Research

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Abstract

Background

Urinary tract infections (UTI) of sows which include cystitis, which may progress to ureteritis and pyelonephritis affect their productivity, longevity and welfare. In this study we determined the prevalence of UTI by histopathology and bacteriology and investigated possible associations between histologically confirmed cystitis and the results of urinalysis and urine cultures in culled sows from three Greek farrow-to-finish herds.

Materials and methods

Routinely culled sows were included in the study. Their urinary bladders were collected from abattoirs and examined histopathologically. Furthermore, urinalysis and urine cultures were performed on urine samples aseptically collected from the bladders.

Results

Histologically confirmed cystitis was evident in 85/185 (45.94%) culled sows. Among those, 44 (51.76%) suffered from acute and 41 (48.24%) from chronic inflammation. The majority of the positive urine cultures were due to colonization of the urinary tract with *E.coli*, which was responsible for 55.81% of the total cases, followed by *Staphylococcus spp.* which caused 18.60% of the infections detected. Evidence of cystitis was associated with bacteriuria and sows with bacteriuria were 2.30 ($p = 0.03$, 95% CI: 1.10–4.83) times more likely to have histologically confirmed cystitis compared to sows with negative urine cultures. Bacteriuria was associated with proteinuria ($p < 0.01$), urine pH ($p < 0.01$) and presence of sediment ($p < 0.01$) in urine. Sows with proteinuria had 9.72 (2.63–35.88) times higher odds of bacteriuria than those without. Histologically defined cystitis was associated with proteinuria ($p < 0.01$) and increased urine pH ($p < 0.01$). Sows with proteinuria were 5.18 times (2.03–13.2) more likely to have histological lesions consistent with cystitis, than those without.

Conclusions

In the studied herds, UTI affected almost one out of two culled sows. Bacteriuria, which was more common among sows with UTI than those without, was mainly ascribed to members of the intestinal and environmental microbiota. Proteinuria and the existence of urine sediment which were associated with UTI may be proposed as likely on-farm predictors of UTI in live sows.

Introduction

Urinary tract infections (UTI) of sows, which include cystitis (inflammation of the urinary bladder), which may progress to ureteritis (inflammation of the ureters) and pyelonephritis (inflammation of the kidneys), is a major health and welfare problem, with economic impact to the swine industry because of sudden death, which is due to pyelonephritis¹, or sub-optimal reproductive performance²⁻⁷. UTI result from colonization of the normally sterile urinary tract with bacteria, most usually members of the fecal microbiota, which ascend from the vagina and distal urethra⁸ mainly prepartum⁹. In UTI affected sows, ureteric valves are shortened from their normal length, and in case of cystitis bacteria can reflux back to the kidney¹⁰. Bacteriuria (culture positive urine), however, may not be always detected in samples from UTI affected sows or may be detected from sows without UTI^{6,11}.

The prevalence of UTI, which was estimated in live sows¹²⁻¹⁵ as well as at abattoir^{2,11,16} by either reagent strip tests, urine culture or histopathology, vary considerably ranging from 15.8¹⁴ to 58%¹⁵. There are many reasonable explanations for this wide variation in the estimates, which challenge the comparability among study results; the most important being the different case definitions (e.g. the reagent strip tests which have been applied in some of the on-farm studies are ineffective in the diagnosis of especially chronic UTI¹¹), and the different parity structure of the studied sow populations (e.g. Madec and Leon (1992) detected UTI in 23.5% of sows of parity ≥ 5 whereas none of the first-parity sows had evidence of UTI). Earlier studies¹⁷⁻²⁰, which investigated the causes of sow mortality, reported the frequency of UTI, as diagnosis based on gross pathology and/or bacterial isolation, ranging from approximately 6^{19,20} to 19.4%¹⁷. Nonetheless, UTI remain largely under-diagnosed and therefore untreated^{6,21}, despite being associated with several periparturient diseases which may guide producers' decision for early sow culling²⁻⁵. The most frequently reported bacteria associated with cases of UTI belonged to *E. coli*, streptococci, staphylococci, and *Actinobaculum suis*, either alone or in combination²². In diagnosed cases of UTI, suggested approaches included the use of antibiotic treatment with broad spectrum antibiotics¹⁰.

Diagnosis of UTI in live sows is based on urinalysis and urine culture results, whereas histopathology, which is considered the "gold standard" for diagnosis, is the method of choice in dead animals. Compared to the "gold standard", urinalysis and bacteriology demonstrated low sensitivity but relatively high specificity in detecting UTI¹¹. Because of likely bacterial contamination during sampling in the live animal, the positive predictive value of bacteriological culture was reported as being relatively low².

To the best of the authors' knowledge, the prevalence of UTI has never been reported in multiparous sows of Greek herds, neither have been detected the microbiological agents which are most frequently involved nor their sensitivity profiles determined. This is despite anecdotal reports highlighting the significance of UTI for sow longevity and productivity, especially during the warmer season of the year extending from early May to late September. Thus, the objectives of the present study were: 1) to estimate the prevalence of histopathologically confirmed cystitis in culled sows from three Greek farrow-to-finish farms, 2) to perform urinalysis and urine culture on samples from culled sows of the herds, isolate the bacteria

involved in bacteriuria incidents and determine their sensitivity profile and 3) to investigate possible associations between cystitis and the results of urinalysis and urine culture.

Materials And Methods

Study population

Three indoor, farrow-to-finish farms participated in our study, which lasted from January 2019 until April of 2020. The owners gave their written consent for participation in the study. The farms fully complied with the EU directive 2001/88/DC on animal welfare. The herds operated 250 (Farm A), 350 (Farm B) and 370 sows (Farm C), respectively, with PIC (Farm A), Danbred (Farm B) and Topigs (Farm C) genotypes. Decision of sow culling was made by each farm's manager/owner without any influence of the investigators. The farmers promptly informed us about the scheduled culling of their sows, and we successively proceeded with the sample collection, as described in the following protocol, from the abattoirs.

Animals and slaughterhouse sampling protocol

The Council Directive 93/119/EC on the protection of animals at the time of slaughter or killing was applied for all animals included in the study.

Almost 50% of the sows routinely culled in the 3 studied herds during the aforementioned time-period were included in the study. Sample collection was performed at the abattoirs with minimum interference with the normal slaughtering process. Urinary bladders were removed before evisceration in order to minimize possible contamination from gut content. Immediately after removal, the neck of the bladder was fastened¹¹. Subsequently, the surface of the bladder was surgically scrubbed. Thereafter, mid-flow urine was collected from a small incision (approximately 1 cm), using surgical blade, made in the fundus of the bladder into sterile containers. Urine samples and bladders were refrigerated immediately after collection into thermo-insulated containers with ice packs, and transferred to the laboratory for analysis. Macroscopic and microscopic evaluation was performed on all urine samples that arrived at the laboratory in acceptable condition within 6 to 12 h after collection.

Macroscopic examination of bladders

Urinary bladders were macroscopically examined, after dissection with scissors along their sagittal axis, from the neck to the apex in order to expose the mucosa. Gross lesions of the urothelium (mucosal hyperemia and bladder content) were recorded, and concretions or calculi found, were microscopically examined. Subsequently, bladders were thoroughly cleaned, and lesions were observed. They were graded as either normal, when they had no lesions or calculi, or abnormal when substantial lesions or concretions were observed (Fig. 1).

Figure 1. *Macroscopic view of pathological (a) and physiological (b) urinary bladders*

a) Gross view of urinary bladder containing sabulous material. The mucosa is hyperemic along with subserosal hemorrhages

b) Normal urinary bladder

Microscopic and histopathological examination of bladders

Full thickness representative samples from the neck and the fundus of all urinary bladders were obtained and fixed in 10% neutral buffered formalin for minimum 48 h. Samples were further processed routinely, embedded in paraffin and sectioned at 4 µm thickness. Sections were stained with hematoxylin and eosin staining, and evaluated blindly by two pathologists. Distribution of the lesions was defined as focal, multifocal or diffuse according to the literature¹¹. Cystitis was diagnosed when at least one focal area of inflammatory infiltration was observed. Urinary bladders without inflammatory infiltration were considered histologically normal (Fig. 2).

Figure 2. Microscopic view of physiological (a) and pathological (b) urinary bladder (H&E stain x4 magnification).

a))Normal urinary bladder layers

b) Urinary bladder with lymphoplasmatic infiltration

Cystitis was coded as acute when areas with necrosis and exfoliation of urothelial cells were observed along with mild or moderate superficial hyperemia and marked lymphocytic mucosal infiltration or chronic, when lymphoplasmacytic infiltration beneath the epithelium and areas with mild submucosal fibrosis were observed (Fig. 3).

Figure 3. Chronic (a) versus acute (b) cystitis. (H&E stain x20 magnification)

a) Chronic inflammatory cell infiltration composed mainly of mononuclear cells (lymphocytes and plasmocytes) in the lamina propria.

b) Diffuse leukocytic infiltration of the mucosa along with multifocal hemorrhages.

Inflammation severity was categorized according to the distribution, the intensity/degree of leukocytic lamina propria inflammation and the infiltrated layers of the bladder wall. In more detail, the inflammation was classified as mild when focal inflammatory infiltration that did not extend to deeper layers of the wall bladder was observed, moderate when bladders had multifocal or diffuse distribution of inflammatory infiltration and prominent leukocytic infiltration of the lamina propria, and severe when diffuse distribution of the inflammation, marked infiltration of mononuclear cells, and exfoliation of the epithelium with or without mucosal hemorrhages was noticed.

Urinalysis

Initially, 10 ml of urine were transferred into conical tip tubes and were evaluated for turbidity. Urine pH was measured by electric pH meter (HD2105.1® delta OHM), and protein estimation was performed using the nitric acid method²³. Then, samples were centrifuged at 1500 rpm for 5 minutes and sediment was observed under light microscope (x40 and x100). Cellular components (red blood cells and leukocytes) were recorded according to Bellino et al. (2013). Crystals were identified based on their shape size and consistency, using high power magnification (x200 and x400) and infrared spectroscopy according to bibliography^{24,25}.

Urine culture

Urine samples were inoculated onto Columbia agar containing 5% sheep blood, and onto MacConkey agar plates, and incubated at 37° C for 24 hours. Two separate samples were inoculated and incubated respectively in a CO₂ enriched atmosphere, for 24–48 hours at 37°C, and anaerobically for 48–96 hours. Pure or mixed cultures with more than 10⁵CFU/ml bacteria, were considered positive. Bacteria identification was performed using biochemical testing, and their sensitivity to commonly prescribed antibiotics, namely ampicillin, amoxicillin + clavulanic acid, enrofloxacin, tetracycline, cephalixin, colistin, ceftazidime and erythromycin, was tested.

Statistical analysis

All statistical analyses were performed using Stata 13.1 (Stata Statistical Software, College Station, Texas). Descriptive statistics of collected data were calculated. Pearson's χ^2 tests were used to compare the proportion of sows with histologically confirmed cystitis and bacteriuria, among the three herds, across parities and among season of culling. One-way analysis of variance (ANOVA) was used to compare the average urine pH values across herds, parities and season of culling. Pearson's χ^2 test was additionally used in order to test for possible associations between urinalysis parameters.

Associations between histologically confirmed cystitis and urinalysis parameters

We employed an ordered logistic regression model to assess any possible association between cystitis, classified as absent, acute or chronic, and urinalysis parameters. Cystitis, was the dependent variable, while the parameters urine turbidity, pH, presence of sediment, crystals, red blood cells, leukocytes and protein were the independent covariates. For model building we followed the procedure described by Lo Fo Wang et al (2004)²⁷. All independent variables were initially screened one by one. During this process a significance level of 0.25 was used²⁸. Then variables with $P < 0.25$ were offered simultaneously to a full model, subsequently reduced by backwards elimination²⁹ until only significant ($P < 0.05$) variables remained. Two-factor interactions were created between the remaining variables and offered one at a time to the model. Finally, we offered previously deleted variables one-by-one to the final model, in order to ensure that no variable which significantly added to the model was omitted. The proportional odds assumption of ordered logistic regression models was tested using the *oparallel* post-estimation command³⁰.

Association between macroscopical lesions in urinary bladders and histologically confirmed cystitis

The evaluation of the association between macroscopical lesions on the urothelium of the bladders and histological lesions consistent with cystitis was evaluated in a logistic regression model. The presence or absence of cystitis was the dependent variable while the presence or absence of gross lesions in the urinary bladder was the independent variable.

Associations between bacteriuria and urinalysis parameters

For the investigation of possible associations between bacteriuria and the parameters examined in urinalysis we employed logistic regression modelling. Model building followed the procedure previously described.

Association between bacteriuria and histologically confirmed cystitis

The significance of the association between bacteriuria and the presence of cystitis was tested in a logistic model. In addition, an ordinal logistic regression model was employed to investigate any association of bacteriuria with the inflammatory status of the bladder (absent, acute or chronic cystitis). Model building followed the procedure previously described.

Results

A total of 185 culled sows were sampled. Fifty-seven (57) originated from farm A, sixty-four (64) from farm B and sixty-four (64) from farm C. Their parities ranged from 1 to 9, with a median of 6.

Gross examination of urinary bladders revealed that 136/185 (73.50%) had no remarkable lesions, while in the remaining, congestion was observed in 39/185 (21.60%), hemorrhages were noted in 4/185 (2.16%), concretions were present in 4/185 (3.24%), while 2/185 (1.10%) urinary bladders had simultaneously hemorrhages, congestion and concretions. Histologically, cystitis was evident in 85/185 sows (45.94%). Among those, 44 (51.76%) suffered from acute and 41 (48.24%) from chronic inflammation. Among sows with chronic cystitis, the inflammation was mild (39/41, 95.12%) or moderate (2/41, 4.88%), while among cases of acute cystitis, 27/44 (61.36%) were classified as mild, 13/44 (29.55%) as moderate and 4/44 (9.09%) as severe. There were no significant differences in the prevalence of cystitis among farms ($P = 0.59$), across different parities ($P = 0.90$) and among seasons of the year ($P = 0.09$).

Urinalysis data were available from 134 sows and are summarized in Table 1. The average urine pH differed neither among farms ($P = 0.37$), nor across parities ($P = 0.86$) nor among season of culling ($P = 0.42$). Urine turbidity was associated with the presence of sediment in urine ($p < 0.01$), which in turn was associated with the presence of crystals ($p < 0.01$). Sows with turbid urine had 8.60 ($p < 0.01$, 95% CI:

3.60-20.49) times higher odds of having urine sediment compared to sows with clear urine. Urine-culture data were available from 171 sows. Overall, the frequency of positive urine cultures was 38/171 (22.22%) and it did not differ either among farms ($P = 0.44$), or across parities ($P = 0.82$) or among seasons of culling ($P = 0.12$). Cystitis was histologically confirmed in 23/38 (60.53%) of sows with bacteriuria. The frequency of isolated bacteria species is presented in Table 2. Among the positive urine samples there were 5/38 (16.16%) with mixed infections, whereas the remaining 33/38 (86.84%) were pure cultures. The sensitivity profile of the isolated bacteria is in Table 3.

Table 1

Laboratory findings	In total (n=134)	Farm A (n=27)	Farm B (n=54)	Farm C (n=53)
Urine appearance				
Clear	99 (73.88 %)	18 (66.67%)	41 (75.93 %)	40 (75.47%)
Turbid	35(26.12 %)	9 (33.33%)	13 (24.07%)	13 (24.53%)
Proteinuria				
No	104 (77.61%)	20 (74.07%)	43 (79.63%)	41 (77.36%)
Yes	30 (22.39 %)	7 (25.93%)	11 (20.37%)	12 (22.64%)
Sediment				
Absence	81 (60.45 %)	13 (48.15 %)	32 (59.26%)	36 (67.92%)
Presence	53 (39.55%)	14 (51.85%)	22 (40.74 %)	17 (32.08%)
Leucocytes				
Absence	114 (85.07%)	24 (88.89%)	46 (85.19%)	44 (83.02%)
Presence	20 (14.93%)	3 (11.11%)	8 (14.81%)	9 (16.98%)
Red blood cells				
Absence	114 (85.07%)	24 (88.89%)	43 (79.63%)	47 (88.68%)
Presence	20 (14.93%)	3 (11.11%)	11 (20.37%)	6 (11.32%)
Crystals				
Absence	88 (65.67%)	16 (59.26 %)	35 (64.81%)	24 (88.89%)
Presence	46 (34.33%)	11 (40.74%)	19 (35.19%)	3 (11.11%)
Average pH (\pmSD)	7.27 (\pm 0.043)	7.33 (\pm 0.111)	7.23(\pm 0.620)	7.30 (\pm 0.071)

Results of urinalysis on samples collected from the urinary bladders of 134 culled sows from three farrow-to-finish Greek herds

Table 2

	In total	Farm A	Farm B	Farm C
Escherichia coli	24/43 (55.81%)	7/12 (58.33%)	12/19 (63.16%)	5/12 (41.67%)
Enterococcus spp	4/43 (9.30%)	2/12 (16.67%)	1/19 (5.26%)	1/12 (8.33%)
Enterobacter spp	4/43 (9.30%)	1/12 (8.33%)	2/19 (10.53%)	1/12 (8.33%)
Staphylococcus spp.	8/43 (18.60%)	2/12 (16.67%)	2/19 (10.53%)	4/12 (33.34%)
Klebsiella spp.	1/43 (2.33%)	0/12(0.00%)	1/19 (5.26%)	0/12 (0.00%)
Corynebacterium suis	2/43 (4.66%)	0/12(0.00%)	1/19 (5.26)	1/12 (8.33%)
Frequency of isolated bacteria species in 38 culled sows with positive urine cultures. In 5 sows, mixed infections were observed				

Table 3

Isolated bacteria	Total cases isolated	% percentage of sensitivity							
		AMP	AMC	ENR	TET	CN	COL	CAZ	ERY
Escherichia coli	24	25	66.66	95.83	41.62	79.16	75	91.66	66.66
Enterococcus spp.	4	0	75	100	25	75	100	100	100
Enterobacter spp.	4	50	75	100	75	75	100	100	100
Staphylococcus spp.	8	25	62.5	87.5	25	87.5	100	87.5	62.5
Klebsiella spp.	1	0	0	0	0	100	100	100	0
Corynebacterium suis	2	0	100	100	50	100	100	100	100

Sensitivity of the isolated bacteria to ampicillin (AMP), amoxicillin + clavulanic acid(AMC), enrofloxacin (ENR), tetracycline (TET), cephalexin (CN),colistin (COL),ceftazidime(CAZ) and erythromycin (ERY).

Association between histologically confirmed cystitis and urinalysis parameters

After the screening process, five parameters, namely, urine turbidity, proteinuria, pH, presence of sediment and presence of leukocytes were eligible for the full model. After model building, proteinuria and pH were significant. Their interaction was insignificant ($p = 0.63$). The assumption of proportionality of the odds was not violated ($p = 0.15$, Brant test). The odds of acute and chronic cystitis combined, versus absence of cystitis were 5.18 ($P = 0.001$, 95% CI: 2.03–13.20) times greater in the presence of proteinuria. Likewise, for one unit increase in pH, it was 3.2 ($P = 0.006$, 0.13–0.72) times less likely for a sow to have chronic or acute cystitis compared to absence of cystitis.

Association between macroscopic lesions in urinary bladders and histologically confirmed cystitis

Urinary bladders with macroscopic lesions were 5.60 ($P < 0.001$, 2.70–11.80) times more likely to present histological lesions of cystitis, either acute or chronic, compared to those without gross lesions.

Association between bacteriuria and urinalysis parameters

In the screening process, all urinalysis parameters were eligible for inclusion in the full model. After model building, proteinuria, presence of sediment and pH were retained. The 2-way interactions examined were insignificant. Sows with proteinuria had 9.72 ($p < 0.01$; 2.63–35.88) times higher odds of bacteriuria than those without. Sows with sediment in their bladder had 6.00 ($p = 0.01$; 1.50–23.76) times higher odds of bacteriuria, compared to sows without sediment. Lastly, for one unit increase of urine pH it was 3.40 ($p = 0.01$; 1.10–10.56) times more likely for a sow to have bacteriuria.

Association between bacteriuria and histologically confirmed cystitis

Sows with bacteriuria were 2.30 ($P = 0.03$; 1.10–4.83) times more likely to have histological lesions consistent with cystitis in their urinary bladders, than sows with negative urine cultures. Moreover, an association of bacteriuria with the inflammatory status of the bladder was detected; acute cystitis was 3.55 ($P = 0.004$; 1.48–8.50) times more likely to be accompanied by bacteriuria when compared to absence of cystitis. In contrast, the odds of bacteriuria did not differ ($P = 0.39$) either between sows with chronic or no cystitis or between sows with acute or chronic cystitis ($P = 0.09$).

Discussion

The effect of diseases of the urogenital track, such as endometritis and cystitis, is considered an important determinant of farms' economic output and animals' welfare status³¹. Biksi et al. (2002) reported positive association between cystitis and endometritis, while Gmeiner et al. (2007)³² suggested the existence of a bidirectional reservoir of infections, with non-specific and opportunistic pathogens, between the uterus and the urinary tract. In our study, we estimated the prevalence of UTI in culled sows from 3 Greek swine herds through histopathological evaluation of the urinary bladder, urine culture and

urinalysis. Furthermore, we investigated possible associations between histopathologically defined cystitis^{2,11} and the results of urinalysis and urine culture.

The estimated prevalence of cystitis was within the previously reported range of prevalences in culled sows from 27 to 53%^{2,11,16}. Interestingly, these studies demonstrated a high frequency of cystitis, affecting anywhere between 1/3 and ½ culled sows. Similar to the findings of Bellino et al (2013) and Grattarola et al (2010), we found that bacteriuria was less frequent, by almost 50%. Relevant to previous studies^{15,33} we also detected *E.coli* isolates as the leading cause of bacteriuria, accounting for approximately 50% of all incidents, followed by *Staphylococcus* spp.; these microbes were cumulatively responsible for over 70% of incidents. Most likely, these cases were the result of ascending infections which are deemed common because of the female's short urethra and the physiological relaxation of the sphincter muscle peripartum. These bacteria which originate from the animals' intestinal tract, populate the environment of pig houses and ascend and colonize the lower part of the urinary tract^{11,34,35}. Interestingly, we found only 2 sows infected with *Actinobacillus suis*, a specific contaminant of the urinary tract with descending significance compared to data reported in the past mainly due the wide application of artificial insemination^{11,36}

The *E. coli* and the other Gram-negative (*Enterobacter* spp.) and Gram-positive (*Staphylococcus* spp., *Enterococcus* spp.) isolates were resistant to the vast majority of antibiotics considered, a finding in line with previous relevant reports^{15,37}. Based on our findings, the narrow range of options for antimicrobial treatment of urinary tract infections in the studied herds only includes cephalosporins or polymyxins; both last-resort categories of drugs against multi-resistant bacteria strains of major significance in human health³⁸.

We found that almost 1 out of 3 tested sows had turbid urine. Importantly, among sows with bacteriuria urine turbidity was recorded in almost 2 out of 3 animals. Urine turbidity was associated with presence of sediment, which in turn, was associated with crystalluria. Urine may sometimes contain less sediment compared to the amount observed in the bladder through ultrasonographic depiction³⁹. However, our collection method of mid-stream urine after gentle shaking of urinary bladders for about a minute led to a rather homogenous distribution of urine sediment. We microscopically examined the detected sediment, and recorded all of its constituents, i.e. red blood cells, leukocytes and crystals. In our data, crystalluria was highly associated with presence of urine sediment. Crystals in urine may be either the result of small abnormalities in the mineral composition of the feed, such as imbalance in calcium and phosphorus intake⁴⁰, and/or the result of insufficient water consumption and/or, alternatively, be induced by local infections⁴¹.

Researchers postulated that higher parity predisposes both to endometritis and to cystitis^{5,26,42,43}. In our data, parity was not associated either with histologically confirmed cystitis or with bacteriuria, similarly to reports from Biksi et al. (2002) and Piassa et al. (2015). Moreover, we found no among-herd or among-season variation in cystitis or bacteriuria frequency. Sows with proteinuria were more likely to have

histological lesions consistent with acute or chronic cystitis than those without proteinuria or were more likely to have positive urine cultures than those without proteinuria. Proteinuria is the presence of abnormal protein level in urine, a consequence of either abnormal transglomerular passage of proteins, because of increased permeability of glomerular capillary wall and their subsequent impaired reabsorption by the epithelial cells of the proximal tubuli⁴⁴, or because of the breakdown of urea by bacteria, in an alkaline environment (urine Ph > 8). Grahofer et al. (2020) in their recent report of biomarkers for diagnosis of cystitis in sows identified and proposed as one of the most reliable and sensitive urinalysis biomarkers, in comparison to the gold standard of histopathology, the presence of proteinuria. In human medicine, Carter et al (2006)⁴⁶ also found an association between proteinuria and bacteriuria but the causative relationship remains undefined.

Although in previous studies^{15,35} researchers reported no correlation between the presence of cystitis and urine pH, we found that higher pH values were associated with increased odds of bacteriuria or, with decreased odds for a sow to have lesions of acute and/or chronic cystitis, compared to having a normal bladder. It has been shown that alkaline pH supported the development of certain urinary sediment and was associated with bacteriuria. Nonetheless, a causative relationship between alkaline urinary Ph and bacteriuria is difficult to establish because it is affected by several factors²². In some studies the acid-base balance of the diet correlated to the urinary pH and to the total bacteria colony forming units in urine^{43,47}. Lowering of urine pH was proposed as a mean to inhibit or control urinary bacterial overgrowth³⁶. In sows with urinary tract infection, urine alkalization may result from urea transformation into ammonia by the bacterial flora⁴⁸. Therefore, high urine pH may be the result of the presence and the metabolism of bacteria, or may preceded or even triggered the colonization of the urinary tract with bacteria. In the cross-sectional context of this study inferences about the exact role and the potential causal effect of urine pH in urinary tract infections are not justified.

Presence of sediment was associated with higher odds of bacteriuria. Kauffold et al, (2010) reported, using ultrasonography, that sows with UTI, defined by high bacterial count and macroscopic/biochemical urine abnormalities, were more likely to have high or moderate amounts of sediment in urine than those without UTI. Recently, Grahofer et al. (2020), also concluded that evaluation of presence of sediment is useful to detect sows with cystitis and bacteriuria.

In conclusion, in this study in culled sows from three Greek farms we found that cystitis was quite frequent affecting almost half of the animals tested, while more than half animals with bacteriuria had histologically confirmed cystitis. We found that in most cases microbiota isolated from urine was mainly part of the normal intestinal flora which points to environmental and managerial involvement in the causality of UTI. Proteinuria was associated with both cystitis and bacteriuria. Furthermore, urine turbidity, existence of sediment and crystalluria were highly associated, with almost 2 out of 3 sows with bacteriuria having turbid urine. Therefore, urine turbidity and proteinuria appear as valuable diagnostic tools for UTI of sows.

Declarations

Ethics approval

This study was conducted in farms that complied with the current laws concerning the protection of animals kept for farming in the European Union⁵¹. Approval of the study protocol by an animal care committee was not required because taking part in the study was in no way painful or invasive for the animals.

Consent for publication

Not applicable

Availability of data and materials

Available from senior author upon request

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

MC trained the employees, visited the farms and collected the data, performed the statistical analysis and drafted the manuscript. VS assisted in the statistical analysis and assisted in drafting the manuscript. VP together with CM pathologically examined the urinary bladders collected. FC together with SC and GP assisted with data collection from farms and drafting the manuscript. EP performed urinalysis and microbiological testing of urine samples. LL attracted funding, designed the study, selected the herds which participated in the study, assisted with the employees' training, guided the statistical analysis and critically revised the manuscript. All authors read and approved the final manuscript.

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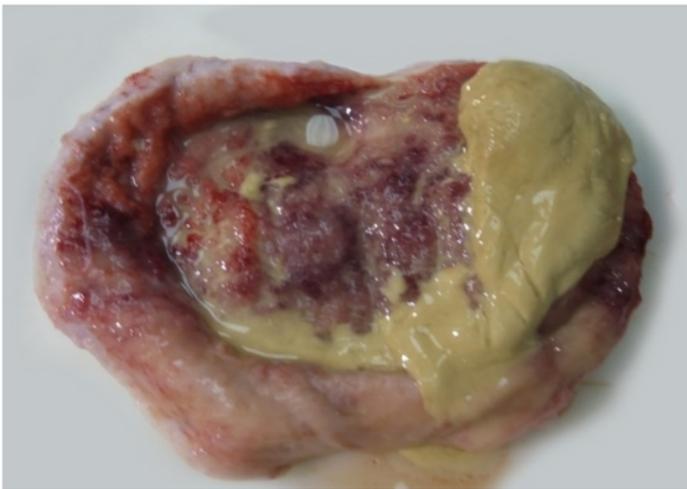
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Figures

a)



b)



Figure 1

Macroscopic view of pathological (a) and physiological (b) urinary bladders a) Gross view of urinary bladder containing sabulous material. The mucosa is hyperemic along with subserosal hemorrhages b) Normal urinary bladder

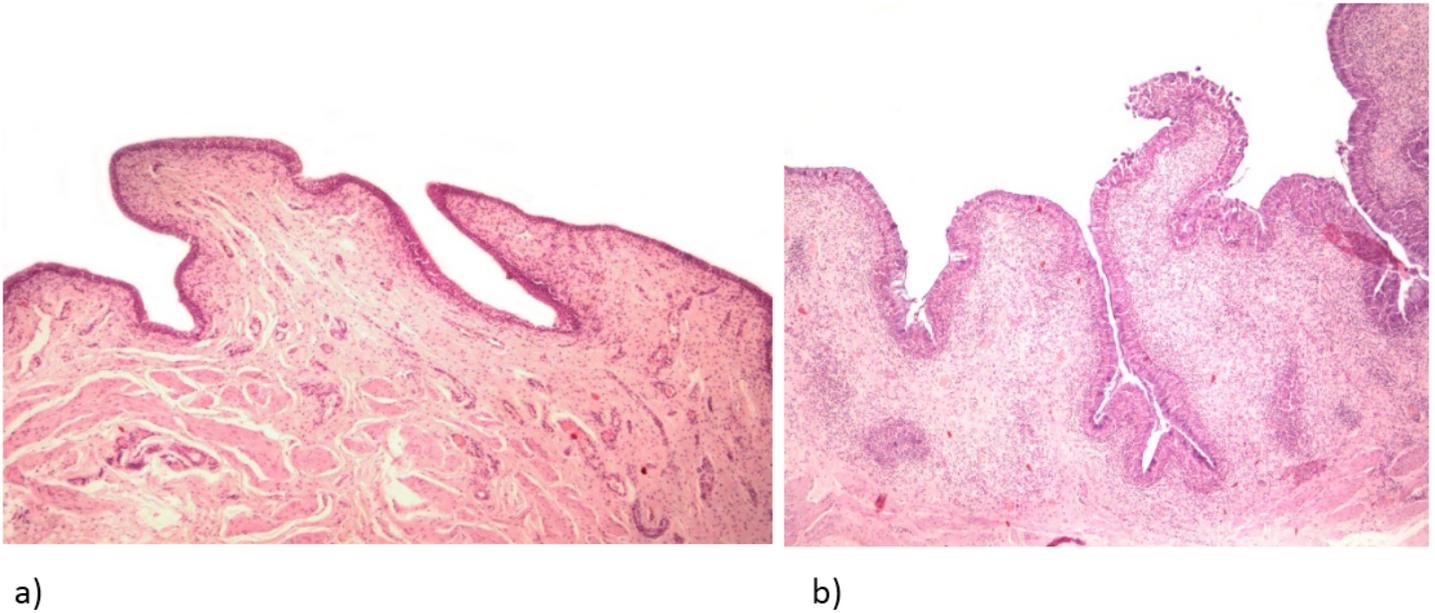


Figure 2

Microscopic view of physiological (a) and pathological (b) urinary bladder (H&E stain x4 magnification).
 a) Normal urinary bladder layers b) Urinary bladder with lymphoplasmatic infiltration

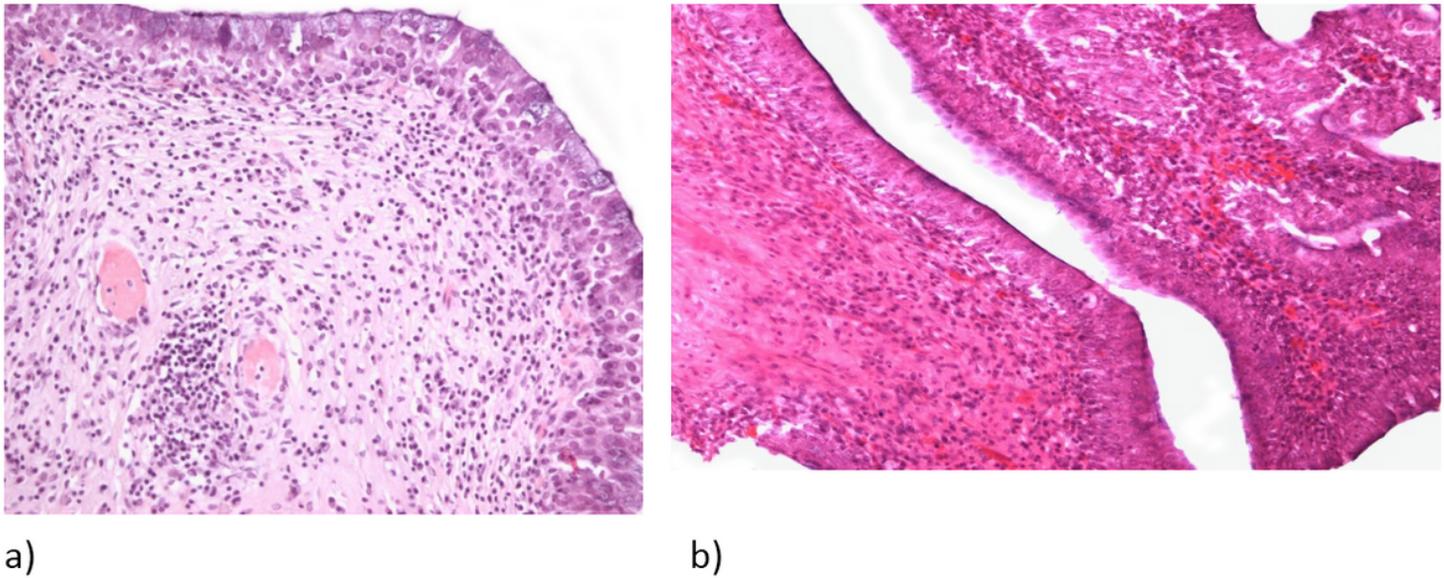


Figure 3

Chronic (a) versus acute (b) cystitis. (H&E stain x20 magnification) a) Chronic inflammatory cell infiltration composed mainly of mononuclear cells (lymphocytes and plasmocytes) in the lamina propria.
 b) Diffuse leukocytic infiltration of the mucosa along with multifocal hemorrhages.