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Urolithiasis and cystitis associated with Staphylococcus delphini group A and mortality in post-weaning mink kits (Neovison vison)

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ABSTRACT

Mortality of mink kits represents a significant loss to production. However, causes of post-weaning mortality in mink kits in modern Danish mink production systems are still relatively poorly documented. We performed a cross-sectional mortality study on eight Danish mink farms including 1893 post mortem examinations of mink kits found dead or euthanized. We assessed the prevalence of cystitis and urolithiasis leading to mortality. Gross pathological findings as well as animal characteristics were recorded and associations with post mortem microbiology (using culture and MaldiTof-MS Vitek MS system) were investigated.

Cystitis and/or urolithiasis were associated with death in 33% (n = 476) and 37% (n = 166) of the examined mink kits in 2015 and 2017. On farm level, the prevalence of cystitis and/or urolithiasis leading to mortality varied from 0.25% to 1.27% with a low overall mortality of 0.9–4.5%. The bacterial agent most frequently isolated in post mortem bladder swabs from mink with a post mortem diagnosis of urolithiasis and cystitis was Staphylococcus delphini group A (51/283) with a significant (p < 0.0001, CI = [19.5;4745.7]) association to gross pathological findings in the urinary tract. Staphylococcus delphini group A was cultured from 70% of the skin swabs obtained from apparently healthy mink euthanized at pelting (n = 222). In conclusion urinary tract disease (cystitis and urolithiasis) was the most prevalent post mortem diagnosis during the growth period and was associated with Staphylococcus delphini group A.

1. Introduction

While most costs of mink producers are relatively fixed, mink mortality can be highly variable and may represent a significant loss of productivity. There is, however, a dearth of published data in this area. Previously urolithiasis and cystitis have been regarded as important causes of mortality in mink (Leoschke et al., 1952; Zimmerman and Schweder, 1988), though only few reports of the prevalence in Danish farms have been published and are more than 20 years old (Rattenborg et al., 1999). Systematic investigations of causal factors and pathogenesis of urolithiasis and cystitis in mink have not been conducted for more than 30 years (Sompolinsky, 1950; Nielsen, 1956; Witte and Zimmermann, 1985).

Cystitis and urolithiasis seem to occur concomitantly in mink (Sompolinsky, 1950; Nielsen, 1956; Witte and Zimmermann, 1985), but the pathogenesis including the association between the two disease entities remains obscure. In previous investigations struvite (MgNH₄PO₄·6H₂O) stones have been the most common type of stone in mink urolithiasis (Sompolinsky, 1950; Nielsen, 1956; Witte and Zimmermann, 1985; Osborne et al., 2009). Struvite can develop in alkaline urine and in cats the formation of struvite is related to feed composition and is shown to develop in sterile urine (Osborne et al., 1989a; Lekcharoensuk et al., 2001b; Matsumoto and Funaba, 2008). In humans and dogs struvite urinary stones are known to develop following a urinary tract infection because of microbially raised urine pH (Osborne et al., 1989b; Hedelin, 2002).

One previously published study finds the most common infectious agent involved in mink cystitis to be Staphylococcus intermedius (Witte and Zimmermann, 1985). Newer findings indicate that the bacterial agents of the Staphylococcus intermedius group, including Staphylococcus delphini group A (Guardabassi et al., 2012), were previously classified as Staphylococcus intermedius by conventional identification methods and according to the taxonomic knowledge at the time.

The primary objective of this study was to estimate the prevalence

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of urinary tract disease leading to mortality in mink kits in eight Danish mink farms during the growth season (July-November). Additional objectives were to assess associations between urinary tract disease leading to mortality and potential factors related to disease development including bladder infection, urolithiasis, urolith mineral composition, animal sex, body weight, body length, BMI, relative length of os penis, and cutaneous staphylococcal colonization.

2. Methods

2.1. Study design

From July to November 2015 a cross sectional mortality study was performed in eight Danish mink farms, where all euthanized or dead mink kits from eight weeks of age until pelting in November were collected daily to uncover cause of mortality. Based on the results the cross-sectional study was continued by exploratory method in July 2017 in two of the original eight farms and one additional farm. All mink kits euthanized or died during the month of July were collected daily to conduct further investigations of urinary tract disease.

2.2. Animals and specimens

The farms included in the study in 2015 housed a total of 103,755 mink kits and did not have an unusual history of infectious disease or mortality. The mink kits were housed under farming conditions according to the normal standard of Danish mink production and legislation either in pairs (standard cages) or in groups of three to four mink (double cages) after 10 weeks of age. All mink were fed with commercial mink feed. In 2015 the included farms received feed from five different feed kitchens. In the 2017 cross-sectional study included farms (n = 3) housed a total 33,011 mink kits and were supplied by the same feed kitchen. Dead animals (n = 1411 in 2015 and n = 452 in 2017) were collected daily and kept frozen (-20°C) until time of examination. Storage time for cadavers varied between one day and two months in 2015 and three to 4.5 months in 2017. Urinary bladder swab samples (n = 283) and uroliths were collected during necropsy of mink kits sampled in July 2017. In addition, bladder swabs were collected from 54 apparently healthy mink kits at pelting time in November 2017. Interdigital skin swabs (n = 222) were collected from apparently healthy mink kits euthanized at the time of pelting on four mink farms (110 of the samples originated from mink in one farm. The remaining 112 samples derived from three additional farms).

2.3. Necropsy and sampling procedures

Necropsies were performed following a standardized necropsy procedure. In 2017 the necropsy procedure was modified slightly to allow for microbiological samples to be obtained from the urinary bladder without contamination of the sample. A transverse skin incision was made from the lateral side of the thorax behind one forelimb continuing over the sternum to end behind the other forelimb. The incision was continued longitudinally from the forelimbs to the thigh continuing over the sternum to end behind the other forelimb. The interdigital skin incisions were flushed in 5 mL Heart Infusion Broth (Oxoid) supplied with 6.5 % NaCl, which was incubated for 24 h at 37 °C and streaked on blood agar (Blood Agar Base (Oxoid) supplied with 5 % sterile bovine blood). After incubation for 24 h at 37 °C the plates were observed for growth of hemolytic colonies. One hemolytic colony from each positive culture was isolated. The isolates were analyzed by MaldiTof-MS Vitek MS system was used for identification of bacterial isolates as previously described (Jaegersen et al., 2017).

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2.4. Microbiology

Swab samples obtained from the urinary bladders were spread on blood agar consisting of Blood agar base (Oxoid) supplied with 5 % sterile bovine blood and incubated for 24 h at 37 °C under anaerobic conditions using Anaerogen TM Atmosphere Generation System (Oxoid Ltd, Wade Road, Basingstoke, UK) according to the directions of the producer. Anaerobic incubation was used in order to reduce swarming of Proteus. MaldiTof-MS Vitek MS system was used for identification of bacterial isolates as previously described (Jaegersen et al., 2017).

The interdigital swabs were flushed in 5 mL Heart Infusion Broth (Oxoid) supplied with 6.5 % NaCl, which was incubated for 24 h at 37 °C and streaked on blood agar (Blood Agar Base (Oxoid) supplied with 5 % sterile bovine blood). After incubation for 24 h at 37 °C the plates were observed for growth of hemolytic colonies. One hemolytic colony from each positive culture was isolated. The isolates were analyzed by MaldiTof-MS Vitek MS system. Incompletely identified staphylococcal isolates were subsequently identified to species level by Multiplex-PCR according to Sasaki et al. (Sasaki et al., 2010).

2.5. Urolith analysis

Mineral composition of uroliths was analyzed at the Canadian Veterinary Urolith Centre using quantitative methods with optical crystallography (polarized light Microscopy), X-ray microanalysis, Fourier transformation infrared spectroscopy or scanning electron microscopy if required (Houston et al., 2016).

2.6. Data analysis

Mink kits were classified as having urinary tract disease if gross pathological examination of the urinary tract included one or more of the following lesions: urethral obstruction, hematuria, pyuria, hemorrhage of the urinary bladder mucosa, urolithiasis, cystitis. Urethral obstruction was defined as a distended bladder filled with content. Cystitis was defined by macroscopic findings of pyuria and often thickening of the urinary bladder wall, with edema and/or hemorrhage.

For calculation of mink BMI and the relative os penis length the following formulas were used:

\[ BMI = \frac{\text{weight (g)}}{\left(\frac{\text{body length (cm)}}{100}\right)^{3}} \]

\[ \text{Relative os penis length} = \frac{\text{length of os penis (cm)}}{\text{body lengths (cm)}} \]

For comparing groups, the Fisher’s exact test was used for dichotomous outcome and the Welch two-sample t-test for continuous data. Statistical significance was defined at a 5 % significance level, i.e. p < 0.05.

3. Results

Urolithiasis and cystitis were identified as the primary pathological finding in 33 % (n = 476) of a total of 1441 mink kits found dead or
euthanized in eight farms during the growth period in 2015. On farm level the prevalence of mink with cystitis and/or urolithiasis leading to mortality ranged from 0.25 % to 1.27 %. Among mink kits with cystitis and/or urolithiasis leading to death or euthanasia the majority (84 %) were males. The additional primary pathological findings included: wounds (260/1441), malnutrition/dehydration (195/1441), hepatic steatosis (124/1441), enteritis (124/1441), empyma (48/1441) and pneumonia (25/1441). Occasional findings were recorded in 110 mink kits and 79 mink kits had no macroscopic pathological changes recorded. The overall mortality on the eight included farms ranged from 0.9 % to 4.5 %.

A total of 452 mink kits found dead or euthanized in July 2017 from three farms were examined by necropsy. In 37 % of these animals, gross pathological lesions in relation to the urinary tract were found (n = 166). The sex distribution was 83 % males (n = 137) and 17 % females (n = 29). It was possible to do a full post mortem assessment of the bladder in 145 mink kits with lesions in relation to the urinary tract (n = 166). For the remaining 21 mink cadavers post mortem evaluation was impaired by autolysis or post mortem abdominal damage. The macroscopic pathological findings in relation to the urinary bladder are listed in Table 1. A total of 283 urinary bladder swab samples were collected from mink kits (n = 452).

The most common microbiological finding from urinary bladder samples (n = 283) was Staphylococcus delphini group A. Staphylococcus delphini group A was cultured from bladder swabs collected from 51 mink kits of which 50 presented gross pathological lesions in the urinary tract compatible with a diagnosis of cystitis and/or urolithiasis. Among mink kits with S. delphini group A, 27 had both cystitis and urolithiasis whereas seven and 12 had either urolithiasis or cystitis, respectively. Other detected bacteria included Proteus mirabilis, Streptococcus spp. and Escherichia coli. The most common microbial findings in relation to detected urinary tract lesions are listed in Table 2.

Other Staphylococcus spp. than S. delphini group A were identified in the urinary bladder of five mink kits of which four had lesions in the urinary tract compatible with cystitis and/or urolithiasis.

Among skin samples from 222 apparently normal mink S. delphini group A was isolated from 70 % of the animals. By MaldiTof analysis these isolates were provisionally identified as belonging to the the SIG group (S. delphini, Staphylococcus pseudointermedius or Staphylococcus intermedius). By M-PCR all these isolates were differentiated to be S. delphini group A. Staphylococcus lentus was isolated from 3 %, Staphylococcus schleiferi from 1 %, Staphylococcus equorum from 4 % and Staphylococcus xylosus from 5 % of the animals. None of these staphylococcal species were isolated from 17 % of the animals.

Out of 54 urinary bladder samples from mink without pathological finding of the urinary tract 49 were sterile. The microbial findings in the remaining five samples included: one mixed culture with Proteus mirabilis, one mixed culture of Escherichia coli/ Enterococcus faecalis and three pure cultures of Streptococcus spp.

Uroliths were sampled from 31 animals. Struvite was identified as the type of urolith in 28 of these animals. Urinary bladder swabs obtained from 16 of these 28 mink kits were positive for S. delphini group A, and sterile bladder swabs were obtained in three cases of struvite urolithiasis (3/28). The remaining uroliths included calcium oxalate (n = 1), struvite and calcium phosphate carbonate (n = 1) and one urolith composed of allantoin. The allantoin uroliths were sampled from a male mink kit of the color type white (BMI = 17.73) presented with urinary bladder distension including mucosal edema and hemorrhage with multiple vesical calculi by macroscopic examination.

3.1. Statistical analysis

With Fisher’s exact test and a 5 % significance level there was a significant association between sex and the occurrence of lesions in the urinary tract compatible with cystitis and/or urolithiasis in mink kits (p = 0.04, CI = [1.00;2.83]) from July 2017.

With Fisher’s exact test with a 5 % significant level there is a significant association between detection of S. delphini group A in the urinary bladder and the occurrence of lesions in the urinary tract compatible with cystitis and/or urolithiasis in mink kits (p < 0.0001, CI = [19.5;4745.7]). There was no significant relation between P. mirabilis, Streptococcus spp., E. coli and lesions in the urinary tract compatible with cystitis and/or urolithiasis. With the Welch two-sample t-test the differences between means of body weight, body length, BMI, os penis length (in males) and relative os penis length (in males) in mink kits with and without lesions in the urinary tract compatible with cystitis and/or urolithiasis were tested. There were significant differences between the means of several parameters related to size: body weight (males: p < 0.0001; females: p = 0.027), body length (males: p = 0.002), BMI (males: p < 0.0001). The difference between means of os penis lengths was significant (p = 0.047) whereas the difference between the relative os penis length was non-significant. The means and standard deviations were computed. The complete results are listed in Table 3.

4. Discussion

Urolithiasis and/or cystitis were diagnosed in 33 % of mink (n = 1441) in the period July – November 2015 and 37 % of the mink (n = 452) collected in July 2017. The results are comparable with previous findings by Rattenborg et al. (1999) identifying urinary tract infection and urolithiasis as the third most frequently identified post mortem diagnosis (13.1 %) in four project farms when investigating the mortality throughout the production year. The results of the present study indicate that urolithiasis and/or cystitis remains one of the most important causes of mortality during the growth period in modern mink production systems. Compared to previous studies, we found a higher prevalence of urolithiasis and cystitis, however this difference in prevalence may be due to differences in study design. Rattenborg et al. (1999) record mortalities throughout an entire year and therefore their prevalence of urinary tract disease could be expected to be lower compared with our result, as our investigation focused on the season when urinary tract disease is most frequently identified as a cause of death (Zimmermann and Schweder, 1985). In a more recent study of mortality in mink from North America a much lower prevalence (5/373) of urinary tract disease was found (Wilson et al., 2015). The investigation by Wilson et al. (2015) was based on a limited number of animals submitted from farms over a five-year period, whereas in our study all dead or euthanized minks from the study farms were

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary of gross pathological findings in mink kits with urinary tract disease leading to mortality (n = 145).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mink kits</td>
<td>Macroscopic pathological findings</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Mucosal edema, urinary bladder</td>
</tr>
<tr>
<td>N</td>
<td>72</td>
</tr>
<tr>
<td>%</td>
<td>50 %</td>
</tr>
</tbody>
</table>

* Defined as a distended urinary bladder filled with content.
systematically included. In previous studies of cats and dogs, struvite
NS = Non-significant (p > 0.05).
BMI = Body Mass Index.

mink kit gross pathological findings of the urinary tract. 

Table 3
Mean with standard deviation (SD) of specific animal parameters related to mink kit gross pathological findings of the urinary tract.

<table>
<thead>
<tr>
<th></th>
<th>Mink kits with lesions* in the urinary tract</th>
<th>Mink kits without lesions* in the urinary tract</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 120</td>
<td>n = 160</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
<td>0.027</td>
</tr>
<tr>
<td>1157.27 ± 371.23</td>
<td>mean ± SD</td>
<td>915.61 ± 387.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>39.89 ± 4.11</td>
<td>38.34 ± 4.20</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI</td>
<td>17.72 ± 3.22</td>
<td>15.57 ± 3.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Os penis length (cm)</td>
<td>3.35 ± 0.58</td>
<td>3.21 ± 0.55</td>
<td>0.047</td>
</tr>
<tr>
<td>Relative os penis length</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>n = 26</td>
<td>n = 58</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
<td>0.027</td>
</tr>
<tr>
<td>929.77 ± 327.78</td>
<td>mean ± SD</td>
<td>763.33 ± 249.36</td>
<td></td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>37.46 ± 4.08</td>
<td>36.10 ± 3.75</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>17.02 ± 3.21</td>
<td>15.88 ± 3.23</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI = Body Mass Index.
NS = Non-significant (p > 0.05).
* Relative os penis length = (os penis length (cm))/body length (cm).
b Displaying the results of the five most frequent microbial findings.
* Comparable with urolithiasis and/or cystitis defined by gross pathological findings.

We demonstrated Staphylococcus spp. in skin swabs from 83 % of the apparently healthy mink (n = 222), the predominant species (79 %) being S. delphini group A. This finding supports a previously published study by Guardabassi et al. (2012) suggesting that the mink is a natural host for S. delphini group A. In this previous study 118 SIG isolates from mink with 86 originating from the skin of diseased mink, were all identified S. delphini group A (Guardabassi et al., 2012). S. delphini group A seems to be an important part of the normal skin microbiota of mink. Our results, identifying S. delphini group A as the most frequent bacterial agent associated with urolithiasis and cystitis, as well as part of the skin microbiota of healthy mink, suggest a role as an opportunistic pathogen and raises the question of what factors are leading to the potentially harmful colonization in the bladder of the mink kits.

Staphylococcus delphini group A as a part of the mink skin flora raises the question whether our detection of S. delphini group A in bladder specimens may be a result of contamination during sampling. Several arguments opposes sample contamination: 1) our refined sampling method were used for all bladder specimens cultured, 2) the strong association found between S. delphini group A and urinary tract lesions, 3) 49 of 50 cultures presenting S. delphini group A from mink with urinary tract lesions were pure cultures, and 4) when examining bladder specimens from 54 healthy animals at pelting S. delphini group A was not detected in any of the samples.

The main urolith type obtained from mink kits in this study was struvite (28/31) with three other urolith types identified: calcium oxalate (n = 1), mixed struvite and calcium phosphate carbonate (n = 1) and allantoin (n = 1). Allantoin is the end product of hepatic purine catabolism (Byers et al., 1947; Leser et al., 1989) in most animals. Allantoin can crystalize in the urine of African cricetidae (rodents) in response to deprivation of water for several weeks (Buffenstein et al., 1985), but to our knowledge allantoin has not previously been reported as a mineral of uroliths in production animals. The animal was found to be in good body condition with distention of the urinary bladder, indicating acute mortality associated with lower urinary tract obstruction. The data obtained in this study did not reveal the cause of allantoin urolith formation in this mink.

Our identification of struvite as the primary urolith type in mink kits is in agreement with previously published studies (Sompolinsky, 1950; Leoschke et al., 1952; Nielsen, 1956; Osborne et al., 2009). Staphylococcus delphini group A was the only bacterial agent significantly associated with uroliths and cystitis. S. delphini group A has been reported to be urease producing (Sasaki et al., 2007) and may thereby generate a rise in urine pH, which creates an environment favoring struvite formation (Olson et al., 1989). This could indicate an ascending infection with subsequent struvite formation as the pathogenesis of urolithiasis and cystitis in mink.

However, among 28 mink with gross pathological lesions combined
with sterile urinary bladder swabs three had struvite urolithiasis. This indicates that struvite stone formation is possible in a sterile environment as reported in ferrets (Nwaokorie et al., 2011) and in cats where effect of diet on urine pH and mineral composition in addition to specific proteins in the urine can facilitate struvite stone formation (Osborne et al., 1989a; Lekcharoensuk et al., 2001b; Matsumoto and Funaba, 2008). Hence, the detection of S. delphini group A could be explained by secondarily infected urolithiasis (de Cógain et al., 2014). Feed alteration as lowering magnesium and/or phosphorus content or adding urinary acidifiers (e.g. ammonium chloride) might prevent occurrence of sterile struvite urolithiasis (Lekcharoensuk et al., 2001b).

In our study, a significant majority of mink kits with urinary tract disease leading to mortality were males in consistency with previous investigations (Sompolinsky, 1950; Nielsen, 1956; Zimmermann and Schweder, 1985). Due to anatomical differences ascending cystitis in dogs and cats is more prevalent in females than males and often caused by Escherichia spp. (Dunning and Stonehewer, 2002). Staphylococcus delphini group A is non-motile (Varaldo et al., 1988) and the mechanisms leading to infection remain unknown. In humans Staphylococcus saprophyticus cause urinary tract infections in young male individuals (Abrahamsson et al., 1993; Adeghate et al., 2016) and this is related to obstructive uropathy (prostatic) and urinary catheters (Hovelius et al., 1984). In a similar way, sterile struvite urolithiasis causing urinary obstruction may be creating opportunity for infection by S. delphini group A in the male mink kits. Further research is needed in order to clarify the pathogenesis.

Demonstration of Proteus mirabilis, Streptococcus spp. and Escherichia coli in urinary bladder swabs was not found to be significantly associated with urinary tract disease in this investigation.

Mortality in male kits with urolithiasis and cystitis is known to be associated with urethral obstruction (Nielsen, 1956). In wild mink, variation in the length of the os penis has been shown to be affected by environmental contamination with persistent organic pollutants (Elliott et al., 2018). In farm mink, variation in length of the penile bone has been suggested to affect fertility, to cause obstructions of the lower urinary tract and to be associated with urinary tract disease. In our investigation the relative os penis length was systematically recorded, but we found no association between penile bone length and urinary tract disease.

The higher male mortality in our study may be explained by anatomical differences in the urinary tract. The male urethra is more narrow and longer than the female urethra thereby enhancing the risk of plugs especially in the pelvic flexure or behind the os penis (Sompolinsky, 1950). It is also reported that female cats have a reduced risk of urethral obstruction compared to male cats (Lekcharoensuk et al., 2001a). We found significantly higher body weights among both male and female mink kits with urolithiasis and/or cystitis compared to mink found dead or euthanized with other lesions in July. Moreover, male kits had higher BMI and a longer body compared to other male kits found dead during the same period. These findings may indicate that high bodyweight and fast growth may be risk factors of development of urolithiasis and cystitis in mink. As feed composition is related to struvite urolithiasis in cats (Osborne et al., 1989a; Lekcharoensuk et al., 2001b) one could speculate that large and more feed-consuming mink males are at higher risk of urolithiasis. Additionally, selection for growth is shown to compromise immune function in poultry (van Der Most et al., 2011), if applicable for mink, the faster growing male kit might be predisposed to urinary tract infection. Additionally, mortality in animals of good fitness indicates an acute progress of urinary tract disease. In order to further investigate this, the study material should be expanded to also include growth, body score and disease occurrence in the remaining living population on the farm.

5. Conclusion

In conclusion, cystitis and urolithiasis were identified as the most prevalent cause of mortality in mink kits during the investigation period. Importantly, the finding that urinary tract disease remains the most important cause of death in mink kits during the growth period in modern mink production systems emphasizes the need for improved preventive measures targeting this disease. Being a part of the skin microbiome of healthy mink and the only bacterial agent found significantly associated with cystitis and urolithiasis on the investigated farms, Staphylococcus delphini group A is suggested as an opportunistic pathogen in mink. Further research must be conducted in order to identify factors affecting the prevalence of cystitis and urolithiasis in mink farms, including factors facilitating S. delphini group A colonization of the bladder of mink kits. Genetics of the animals and management procedures could be factors of interest in further investigations of prevention of cystitis and urolithiasis in mink kits from July to pelting.

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Declarations of interest

None.

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