Polycystic kidney disease in the pygmy hippopotamus
(Hexaprotodon liberiensis)

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Polycystic kidney disease (PKD) was diagnosed at necropsy in a captive aged female pygmy hippopotamus (Hexaprotodon liberiensis), which presented with numerous cysts in both kidneys, liver, duodenum and one single in the pancreas. There were no premonitory clinical signs of a nephropathy observed prior to its death. Similar findings were made in the male partner animal half a year later. Both animals had been wild-caught. A literature review showed that another seven cases of PKD have been reported in pygmy hippopotamuses, and an additional screening of records available from the international studbook for the species revealed yet another six cases. In all cases, aged females were affected, and in several instances, affected animals were related to each other. These patterns suggested familiar transmission, similar to PKD in humans and other animals. The disease, and especially the presumptive bias in diagnosis towards females - the male animal of this report was to our knowledge the first case of PKD reported in a male pygmy hippopotamus -, warrant further investigation. The status of the kidneys with respect to PKD should be assessed (including histology) in every deceased pygmy hippopotamus.
1RH: Polycystic kidney disease in pygmy hippopotamus

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3POLYCYSTIC KIDNEY DISEASE IN THE PYGMY HIPPOPOTAMUS (HEXAPROTODON LIBERIENSIS)

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Abstract: Polycystic kidney disease (PKD) was diagnosed at necropsy in a captive aged female pygmy hippopotamus (*Hexaprotodon liberiensis*), which presented with numerous cysts in both kidneys, liver, duodenum and one single in the pancreas. There were no premonitory clinical signs of a nephropathy observed prior to its death. Similar findings were made in the male partner animal half a year later. Both animals had been wild-caught. A literature review showed that another seven cases of PKD have been reported in pygmy hippopotamuses, and an additional screening of records available from the international studbook for the species revealed yet another six cases. In all cases, aged females were affected, and in several instances, affected animals were related to each other. These patterns suggested familiar transmission, similar to PKD in humans and other animals. The disease, and especially the presumptive bias in diagnosis towards females – the male animal of this report was to our knowledge the first case of PKD reported in a male pygmy hippopotamus –, warrant further investigation. The status of the kidneys with respect to PKD should be assessed (including histology) in every deceased pygmy hippopotamus.

Key words: Pygmy hippopotamus; polycystic kidney disease; congenital; liver; pancreas; duodenum
INTRODUCTION

The term renal cystic disease comprises a heterogeneous group of hereditary, developmental and acquired disorders. Polycystic kidney disease (PKD) is one of the most common genetic diseases in humans and has frequently been reported in a variety of domestic, laboratory, and non-domestic species. In humans, the diagnosis of PKD is reached when at least three cysts distributed between both kidneys are identified. In domestic animals, most cases are considered to be congenital, and there is some evidence that the condition is heritable, with increased frequency in certain families of cats (Persian cats), dogs (Cairn terriers), pigs and monkeys. PKD observed in wildlife species is mostly reported in individuals that have been kept in captivity. Here, we describe a case of polycystic kidney disease in a captive pygmy hippopotamus (Hexaprotodon liberiensis), collate published as well as hitherto unpublished cases of the observation of PKD in this species, and tentatively link the observed pattern to the known female-biased sex ratio at birth and the disproportionally high male juvenile mortality in this species.

CASE REPORT

Case history I

A 41-year-old female, wild-caught pygmy hippopotamus (International Studbook No. 251) had an uneventful medical history until May of 2007 when she was noted to be weak in her hind legs. Based on her age and an inability to stand she was euthanatized two days later. At post mortem, the animal weighed 228 kg and was in good body condition. On examination the kidneys contained numerous fluid-filled cysts, 2mm to 2.5cm in diameter, which replaced the renal parenchyma, extending from the cortex into the medulla. The left kidney was moderately...
affected, but the right kidney showed a complete polycystic mesh pattern with no remaining parenchyma visible (Figure 1). A urea concentration of 19 mmol/l was measured in the aqueous humor of the eyes, using a Ecoline® Urea test kit (DuaSys Diagnostics Systems GmbH, 65558 Holzheim, Germany). The ureters and the lower urinary tract showed no lesions. Additionally, a few fluid-filled cysts were present multifocally subcapsularly and within the parenchyma of the liver (3mm to 1cm in diameter), and in the mucosa of the proximal duodenum (up to 2 x 4cm). One solitary cyst was also present in the pancreas (4cm in diameter).

Additional findings included bilateral symmetric lenticular cataracts, two elastic whitish nodules (2 and 3.5cm in diameter) in the wall of the forestomach, as well as multiple papillomatous proliferations of the forestomach mucosa.

Post mortem tissue samples were fixed in 4% neutral buffered formalin and processed routinely. Two micrometer tissue sections were stained with hemalaun and eosin for light microscopy. Sections of the two nodules were also stained immunohistochemically for smooth muscle actin (SMA; monoclonal mouse anti-human alpha SMA clone 1A4 and detection kit, Dako, Baar, Switzerland; chromagen hematoxylin Gill’s No. 2, Merck, Zug, Switzerland).

Histologically, the renal cysts were lined by a cuboidal to squamous epithelium, surrounded by fibrous connective tissue of variable thickness. Most cysts were empty, but some contained cellular debris and small amounts of eosinophilic material. In the severely affected right kidney, only small residues of parenchyma were present between the numerous cysts, mostly consisting of glomeruli with moderate to severe dilatation of the Bowman's capsule (Fig. 2). The left kidney was much less severely affected with scattered cysts present in both cortex and medulla. Mild interstitial inflammatory infiltrates were present, consisting of lymphocytes and plasma cells.

Hepatic cysts appeared histologically as moderately dilated bile ducts lined by cuboidal epithelium located in portal areas. The surrounding tissue was unaffected. The duodenal cysts...
were dilated duodenal glands (Brunner’s glands) and were lined by columnar epithelium and filled with seromucinous fluid and cell debris. The pancreatic cyst was also lined by cuboidal epithelium. In some lobules of the pancreas there was a focal lymphoplasmacytic inflammation with atrophy of acini.

Both nodules in the forestomach were well-demarcated and well-differentiated neoplasms in the tunica muscularis consisting of whorled spindle-shaped cells surrounded by fibrous tissue. These cells stained positive for SMA with immunohistochemistry and were diagnosed as leiomyomas. The enlarged villi of the forestomach consisted of proliferative squamous epithelium with supporting stroma and no evidence of malignancy and were diagnosed as papillomas.

Case history II

In November of 2007, the 40 year-old, wild-caught male partner animal (International Studbook No. 250) of the female presented as Case I above, was euthanatized for a fast-growing swelling on its left mandible and neck. At necropsy, the animal was in good body condition and weighed 218 kg. The swelling was a 15 x 20 cm abscess (Actinomyces pyogenes was isolated by culture). The animal had multiple fluid filled cysts present in both kidneys, the liver, and the duodenum. The kidneys were severely affected, similar to the right kidney in case I. In contrast to case I, the pancreas was not affected. The histologic appearance of the kidneys was also similar to case I. A urea concentration of 10 mmol/l was measured in the aqueous fluid of the eyes. Additional findings included a unilateral cataract, multiple papillomatous villi of the forestomach, and an old rib fracture with callous formation.
Retrospective review

Occasional cases of PKD in pygmy hippopotamuses are described in the literature. Jarofke and Klös evaluated necropsy and clinical history reports of 147 cases of diseased pygmy hippopotamuses. In one of these pygmy hippopotamuses, polycystic kidneys were found at necropsy; however, negative findings (absence of lesions) were not documented. Raymond et al. reported six cases in which PKD was identified at necropsy in pygmy hippopotamuses from the National Zoological Park in Washington DC. Prior to death, these animals displayed the following clinical signs: anorexia (four animals), lethargy (three animals), weight loss (one animal), polydipsia (one animal) and polyuria (one animal). One animal had been without clinical signs prior to death. Two pygmy hippopotamuses had BUN values of 107 and 89 mmol/l at time of death; one of these animals also had a creatinine value of 866 µmol/l. All animals were females in the late second to early fourth decade of life; two were wild caught and four were captive born at the National Zoological Park, Washington DC. All except one were related to each other. As in the case presented here, the kidneys of all animals showed small to large numbers of cysts, which contained clear, light yellow to dark brown fluid, and which effaced the cortices and medullae to varying degrees. Interstitial fibrosis, primarily within the medullae, was seen in histologic examination. The collecting ducts and renal tubules were ectatic and some of the latter were lined by hyperplastic and dysplastic epithelium. Other renal findings were membranous glomerulopathy and renal arteriosclerosis. Whereas these histological features indicated, according to the authors, an acquired polycystic disease, the animals` pedigree could also imply a familial pattern.

A set of historical necropsy reports was kindly provided from the studbook office of the Zoological Garden of Basle, Switzerland. Based on the minimum requirement for a diagnosis of
PKD in humans - three cysts distributed between both kidneys\textsuperscript{5}, we identified six additional cases of pygmy hippopotamuses with polycystic kidneys and one case with a single renal cyst (Table 1). The six animals with polycystic disease were all aged females. Four of these were wild caught and three were captive born (two at the Zoological Garden of Basle, one at the National Zoological Park, Washington DC; unfortunately, it could not be determined whether this animal was related to any of the animals with PKD reported by Raymond et al.\textsuperscript{15}). Two of the pygmy hippopotamuses were mother and daughter and shared the same wild caught sire. The wild caught dam of the mother also had PKD (Table 1). The only male pygmy hippopotamus in the historical case collection had only one single renal cyst at necropsy (Table 1). Most likely, this finding is not correlated to PKD. In many species, simple renal cysts occur sporadically with an augmented prevalence in age.\textsuperscript{12}

Only five necropsy reports of descendents of the couple described in this report were available. The animals had produced at least 7 male and 8 female calves. Only 5 out of these calves survived longer than 14 days, and only 3 of the surviving 5 reached an age over two years. These three (studbook no. 455, 506 and 718) were all females; one died at the age of approximately 8 years and one at the age of 23 years. The third animal (studbook no. 506) reached an age of at least 7 years; she died but unfortunately the date of her death is unknown. PDK was not diagnosed in any of these animals. Similar to the case described in detail here, a leiomyoma of the forestomach wall was reported for two of the cases, potentially a clinically irrelevant finding of old age.
DISCUSSION

This case report strongly supports the previous suspicion of Raymond et al.\textsuperscript{15} that PKD is an important disease in captive pygmy hippopotamuses, and presents the pathological findings as an example for other veterinarians performing necropsies in this species. In our view, the most problematic issue to date is the lack of reporting of negative findings, which means that the available information does not allow a calculation of the prevalence of the problem or a conclusive investigation of hereditary patterns so far. In future necropsies of individuals of this species, the presence or absence of PKD should be clearly stated.

Captive pygmy hippopotamuses show a set of very peculiar demographic traits:\textsuperscript{24} they have a highly female-biased sex ratio at birth, with only 41\% of newborns being males; those males that are born have a higher juvenile mortality than the females (35.8\% vs. 28.8\%), and the average interbirth interval before the birth of daughters is longer (21.7 months vs. 19.4 months before sons), suggesting selective abortion of males. We repeated the counts made by Zschokke,\textsuperscript{24} extending them to the most recent data: up to the end of 2006, 1012 offspring of known sex had been born, of which 430 (42.5\%) were males and 582 (57.5\%) females; of these animals, 180 males (41.9\%) and 182 females (31.3\%) did not survive into their second year of life.

This disproportionately high fetal, neonatal and juvenile mortality in males of the species warrants an explanation. As long as conclusive necropsy reports on aborted fetuses, neonates and juveniles are lacking, a disease that is diagnosed mainly in old females - with little clinical relevance up to a very progressed stage -, offers an appealing explanation for the pattern: it could be hypothesized that the disease is of no clinical relevance in females, but incompatible with survival in males already at very early stages of life.
Whether PKD could offer such an explanation must remain highly speculative to date. On the one hand, the bias of reported cases towards females (13 out of 14 reported cases) appears too high to be a result of chance alone; but as long as negative results cannot be allocated with security, prevalences cannot be tested statistically. On the other hand, the finding that the male described as case II did reach a very high age contradicts the idea that PKD is incompatible with old age in all male pygmy hippopotamuses.

In humans, at least two different genetic patterns of PKD transmission are known - adult/autosomal dominant PKD (ADPKD) and infantile/autosomal recessive PKD (ARPKD).\textsuperscript{22} The autosomal dominant form is characterized by steady and presumably lifelong growth of cysts in both kidneys. While the cysts cause overall enlargement of the kidneys, there is a loss of functional parenchyma and a progressive decrease in renal function. Extrarenal manifestations in liver and pancreas are often seen but have minimal clinical significance. ADPKD is compatible with life as long the remaining parenchyma can compensate the loss of kidney function.\textsuperscript{22} The less common autosomal recessive form, in contrast, develops rapidly and is often diagnosed in early infancy by massive nephromegaly; it often manifests as stillbirths or death within the first few weeks of life.\textsuperscript{23}

Thus, the description of ADPKD matches the observations made in pygmy hippopotamuses, with detection of the disease at old age and little clinical relevance. In the animal investigated in detail in this study (Case I), the BUN value of the eye fluid could indicate renal dysfunction to an extent that could not be compensated anymore; in contrast, the BUN value of the male animal (Case II) did not suggest impairment of renal function. From the limited pedigree information there is, the occurrence of PKD in three successive generations (Table 1) is compatible with an autosomal dominant heredity pattern. Similarly, the presumed absence of PKD in the offspring of
the couple from Zurich Zoo investigated here does not contradict an autosomal dominant heredity pattern, and one could speculate that more offspring of this couple would have manifested PKD had they survived longer. The only feature in which the pygmy hippopotamus would differ from the classical ADPKD is the presumable gender bias of the diagnosis, which is unusual for an autosomal hereditary pattern. The fact that PKD has been observed in a total of 8 animals that were considered “wild caught” is remarkable and suggests that the disease should be prevalent in the free-ranging population.

The description of ARPKD, with fetal or neonatal death, could match the observed pattern of fetal or neonatal death in pygmy hippos – again with the reservation of the gender bias. The gender bias could hypothetically be explained by a gonosomal heredity pattern. To the authors’ knowledge, PKD has not yet been reported to be gonosomal-dominant inherited in any species. However, the case of the aged male animal presented here rules out a gonosomal pattern that leads to early death. Whether PKD in pygmy hippos plays a role in the gender-biased fetal and neonate mortality, whether surviving females of the species are indeed affected more often by PKD, and whether different forms of PKD occur in the species, needs to be investigated in further studies.

There is some indication that in genetically susceptible individuals, environmental factors may trigger the initiation and perhaps progression of PKD. Werder et al. observed that the prevalence of PKD developing in genetically susceptible mice seemed to be correlated to the degree of exposure to environmental microbes. The presence of bacterial endotoxin and fungal β-D-glucans in fluid from renal cysts in humans with PKD supports the hypothesis that microbial agents play an important role in the pathogenesis of the disease. As such, investigations into environmental conditions for pygmy hippos and other animal species that develop PKD
may be warranted. If we accept the hypothesis that PKD is a reason for the observed
demographic abnormalities in the captive pygmy hippopotamus population, then the fact that
Zschokke\textsuperscript{24} found a difference in sex ratio distortion between individual zoos that was not related
to inbreeding or other genetic factors, could be an indication that environmental factors might be
involved as well. In humans, the reduction of dietary omega-6 fatty acids is assumed to have a
prophylactic effect;\textsuperscript{19} in this context, a reduction in any grain-based dietary compounds and an
increase in roughage components would be a simple supportive measure.\textsuperscript{3,4} The low metabolic
rate of hippopotamuses\textsuperscript{18} makes the use of energy-dense, grain-based concentrate feeds in these
species questionable anyhow.

Thus PKD seems to be a multifactorial disease with a complex pathogenesis. There are several
diagnostic approaches to detect PKD. Clinical signs shown by affected hippopotamus with PKD
are non-specific, but may include those suggestive of renal disease. Hind limb lameness\textsuperscript{1,17} and
abdominal tension\textsuperscript{11} was observed in other species with PKD. When these symptoms occur the
possibility of PKD should be considered. Blood urea nitrogen (BUN) or creatinine or phosphorus
might be elevated. In susceptible rat strains, the serum BUN level is used as a predictor of
diseased individuals.\textsuperscript{2} The use of renal ultrasonography would be a high sensitive and specific
method to diagnose PKD in pygmy hippopotamus if imaging of the kidneys is accomplished.

Further case reports of kidney disease in pygmy hippopotamuses may help to elucidate not only
the presence of lesions in different organs, but also the nature of inheritance of PKD in this
species. This collation of case reports and the ensuing epidemiological speculations demonstrate
that the relevance of linking extensive, high-quality pathological information to studbook data
cannot be underemphasized.
Acknowledgments: Our appreciation goes to Manfred Studer from the Zoological Garden of Zurich for his excellent care of the pygmy hippos.
Figure Legends.

Fig. 1: Left and right kidney of a female aged pygmy hippopotamus (*Hexaprotodon liberiensis*); left kidney with multiple small, medium and large sized cysts involving both cortex and medulla; right kidney with severe cystic mesh pattern involving both cortex and medulla.

Fig. 2: Histology from the right kidney of a female aged pygmy hippopotamus (*Hexaprotodon liberiensis*) with multiple cysts originating from both tubules and glomeruli (H&E stain).
Figure 1
Table 1. Pedigree relationships and necropsy findings in captive pygmy hippopotamus

(*Hexaprotodon liberiensis*) with renal cysts as reported in the International studbook collection for the species.

<table>
<thead>
<tr>
<th>Stud # (Sire/Dam)</th>
<th>Year of Death</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Kidney lesions</th>
<th>Extrarenal cysts</th>
<th>Other findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 (wild/wild)</td>
<td>1967</td>
<td>36-40</td>
<td>f</td>
<td>numerous cysts, fibrosis, arteriosclerosis, tracts of inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>87 (29/42)</td>
<td>1976</td>
<td>33</td>
<td>f</td>
<td>numerous cysts, glomerulo- and tubulosclerosis, interstitial fibrosis, lymphocytic infiltration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>184 (wild/wild)</td>
<td>1978</td>
<td>&gt; 15</td>
<td>m</td>
<td>single renal cyst</td>
<td>thyroid, skin</td>
<td></td>
</tr>
<tr>
<td>112 (29/87)</td>
<td>1979</td>
<td>28</td>
<td>f</td>
<td>numerous cysts, fibrosis, CaCO\textsubscript{2}-concrements in tubuli</td>
<td></td>
<td>focal pulmonary anthracosis, multifocal ulcerative dermatitis</td>
</tr>
<tr>
<td>257 (wild/wild)</td>
<td>1983</td>
<td>~16</td>
<td>f</td>
<td>disseminated cysts</td>
<td>ovaries</td>
<td></td>
</tr>
<tr>
<td>144 (wild/wild)</td>
<td>1985</td>
<td>~27</td>
<td>f</td>
<td>few cysts (3-5mm), glomerulonephritis</td>
<td>thyroid, pylorus, duodenum, bladder</td>
<td>hyperplastic thyroids, uremic abomasal calcification, ascites, hydrothorax</td>
</tr>
<tr>
<td>96 (26/77)</td>
<td>1988</td>
<td>41</td>
<td>f</td>
<td>numerous cysts</td>
<td>ovaries, thyroid</td>
<td>pyometra, hyperplasia of the right adrenal gland, forestomach neoplasm (leiomyosarcoma?)</td>
</tr>
</tbody>
</table>