First report of Polycystic kidney disease occurrence in Persian cats in Serbia

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Summary
Polycystic kidney disease (PKD) is an inherited autosomal disorder in cats, mostly diagnosed in Persian cats. Renal cysts can be diagnosed by ultrasound, but cats must be at least 16 weeks old. The goals of this study were to assess the occurrence of PKD in Serbia using a randomly selected group of Persian cats, to compare the diagnostic efficacy of ultrasound and genetic tests, and to measure haematological and selected biochemical parameters. We examined 70 cats of Persian breed, between 4 months and 8 years of age. Complete blood count and selected biochemical parameters were measured, renal ultrasound was performed. Swabs of the oral cavity were obtained for genetic testing. Percentage of PKD positive cats identified by genetic testing was 48.6%, whilst only 18.6% were detected through ultrasound. Animals that were polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) positive and ultrasound negative ranged from 4 months to 3.5 years. All haematological and biochemical parameters were within the the normal range values in all examined cats. Genetic methods proved to be the most effective for reliable and early diagnosis of PKD in Persian cats. DNA analysis can be used right after birth, and excludes the need for other diagnostic procedures, such as ultrasound.

Prima segnalazione sulla presenza della Malattia policistica renale in gatti di razza persiana in Serbia

La Malattia policistica renale è una malattia autosomica ereditaria del gatto riscontrata per lo più in quelli di razza persiana. Le cisti renali possono essere rilevate con esame ecografico su gatti a partire dalla 16ª settimana di vita. Questo studio ha avuto 3 obiettivi: verificare se la malattia è presente nei gatti persiani serbi, valutare l’efficienza diagnostica dell’ecografia confrontandola con i test genetici, monitorare i parametri ematologici e biochimici. Settanta gatti di razza persiana, di età compresa tra 4 mesi e 8 anni, sono stati sottoposti ad esame ecografico renale, emocromo completo e analisi dei parametri biochimici. La PCR-RFLP effettuata su tamponi del cavo orale ha permesso di rilevare la presenza della mutazione sull’esone 29 caratteristica della malattia nel 48,6% dei gatti esaminati, a differenza dell’esame ecografico che ha rilevato positività solo per il 18,6% dei felini. Il range di età degli animali risultati positivi alla PCR-RFLP e negativi all’esame ecografico varia da 4 mesi a 3,5 anni. In tutti i gatti esaminati i valori dei parametri ematologici e biochimici non si sono discostati dai valori normali. Il test genetico è risultato il più attendibile per la diagnosi precoce della malattia nei gatti persiani. L’analisi del DNA può essere impiegata fin dalla nascita escludendo, di conseguenza, il ricorso ad altre procedure diagnostiche.
Introduction

The first published data about Polycystic kidney disease (PKD) appeared in 1969 (Barrs et al. 2011). Polycystic kidney disease is one of the most frequent causes of death in Persian cats (Egenvall et al. 2009). Similar clinical and morphological characteristics between Persian cats and humans suggest that cats could be useful models for the examination of the same ailment in human beings (Eaton et al. 2009, Aleksic-Kovacevic et al. 2010).

Polycystic kidney disease is an inherited disease in Persian cats and related breeds (Exotic Shorthairs and Himalayan cats) (Grahm et al. 2004, Lyons et al. 2004, Helps et al. 2007, Lyons 2010), the disease has a global prevalence of 37% (Di Bartola 2000). The genetic mutation causing PKD was identified in the PKD1 gene. This gene encodes a protein called polycystin-1, located on the 16th pair of autosomes. Mutation consists of a transversion of C→A, which induces a stop codone at the position 3284 on the exon 29, leading to the loss of 25% of the C-terminus of polycystin-1 (Lyons et al. 2004). This is a dominant autosomal nonsense point mutation, which causes foetal (Lee Ya-Jane et al. 2010) and neonatal (Barrs et al. 2011) death in homozygous cats, hence the disease can be clinically manifested only in heterozygous animals. Polycystic kidney disease is characterized by the formation of cysts of different sizes (from 1 mm to more than 1 cm) in cortex and medulla of kidneys, liver, and pancreas (De Cock et al. 2007). The number of cysts in a kidney may vary from 1 to more than 200 (Domanjko-Petrič et al. 2008). Cats with PKD usually show clinical signs of disease after the age of 7 years (Domanjko-Petrič et al. 2008, Son et al. 2008). In those cases in which cats do not show any clinical signs of kidney failure, we can still assess the general status of the animal as well as its renal function by monitoring of haematological and other biochemical parameters – such as urea, creatinine and phosphorus. Clinical sings, such as anorexia, vomiting, and polyuria, manifest when more than 70% of kidneys is no longer functional. For this reason an adequate diagnosis should be done as early as possible, especially in predisposed breeds (Lyons 2010), so to identify cats with PKD in time and prevent them from having offspring and transmit the disease (Criado-Fornelio et al. 2008). The cysts are present from birth, but they are smaller in younger cats (Domanjko-Petrič et al. 2008). Although it is recommended that breeders should not mate cats with PKD, this advice is often not taken. Reasons for non-compliance include: absence of clinical signs, lack of information about the disease, expensive or unavailable diagnostic analysis, and the fact that decisions about breeding are made before the results of the analysis arrive (Lyons 2010).

Seven weeks is considered the earliest time to detect cysts using ultrasound and the sensitivity of this method in cats is 75% at 16 weeks of age, and 91% at 36 weeks of age (Bonazzi et al. 2009). However, when it comes to older cats, ultrasonography is a very practical non-invasive method to diagnose PKD (Bonazzi et al. 2009). High frequency transducers are required (from 7 MHz or higher) to detect cysts on ultrasound, cysts in the cortex are easily detected because the medulla is hypoechoic (Domanjko-Petrič et al. 2008). Cats should be at least 10 months old to claim that they are negative on ultrasound (Barrs et al. 2011).

Lyons and colleagues (Lyons et al. 2004) were the first to describe a molecular tool for detecting the mutation responsible for the development of cysts. Since then, there have been numerous papers aiming at developing more specific and more sensitive molecular methods for the diagnosis of the causes of PKD (Young et al. 2006, Helps et al. 2007, Son et al. 2008, Bonazzi et al. 2009, Lee et al., 2010).

The goals of this study was to analyse the frequency of PKD in group of randomly selected Persian cats from Serbia, and to evaluate the sensitivity and specificity of renal ultrasound and genetic tests in this population. Haematological and biochemical parameters (urea, creatinine, and phosphorus) as indicators of renal function were also examined. To the best of our knowledge, this is the first study in the Republic of Serbia focusing on the occurrence of PKD in Persian cats.

Materials and methods

The study included 70 cats of Persian breed originating from breeders and individual owners (44 females and 26 males). Tested cats were not related and were from different parts of Serbia. The age of the cats ranged from 4 months to 8 years. In addition to haematological and biochemical analyses, kidneys of all cats were examined for the presence of cysts by ultrasound, whilst the presence of mutation on exon 29 was investigated by DNA analysis using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). No urinalysis was performed.

Blood was collected by venepucture of v. cephalica antebraehii dextra in vacutainers with anticoagulant (EDTA) for complete blood count, and in vacutainers without anticoagulant for biochemical analysis. For haematological analysis IDEXX ProCyte Dx was used (IDEXX Laboratories, Westbrook, Maine, USA), and for biochemical analysis (urea, creatinine, phosphorus) IDEXX VetTest 8008 was used (IDEXX Laboratories, Westbrook, Maine, USA).

For ultrasound, animals were examined in dorsal...
position, both kidneys were examined. Aloka ProSound 5000 SSD (Hitachi Aloka Medical, Tokyo, Japan) with 10 MHz linear transducer and 7 MHz convex transducer were used.

For PCR-RFLP detection, DNA was isolated from oral cavity swabs using KAPA Express Extract Kit (Kapa Biosystems, Wilmington, Massachusetts, USA; PN KK7152) following the manufacturer’s protocol. Samples were kept at a temperature of -20°C. The following set of primers was used for the amplification of PKD exon 29: PKD1F (3’ CAGGTAGACGGGATAGACGA 5’) and PKD2R (3’ TTCTTCCGTGTCAACGACTG 5’) by Lyons and colleagues (Lyons et al. 2004). The amplification was conducted in 25 µl reaction volume containing 12.5 µl of KAPA2G Robust HotStart ReadyMix (Kapa Biosystems, Wilmington, Massachusetts, USA; PN KK7152) and 1.25 µl of each primer from PKD1F / PKD2R primer set and 10 µl DNA sample. Thermal protocol recommended for KAPA2G Robust HotStart ReadyMix was used: 3 minutes of initial denaturation at 94°C, followed by 35 cycles of denaturation (30 seconds at 94°C), primer annealing (30 seconds at 55°C), extension (1 minute at 72°C) and a final extension step at 72°C, which lasted 1 minute. Ten microliters of PCR product was digested with 10U of SchI (MlyI) (New England Biolabs, Ipswich, Massachusetts, USA) in a 30 µl reaction containing 1x Buffer Tango. Digestion performed at 37° for 12 hours was followed by inactivation of the enzyme at 65°C for 20 minutes.

The PCR products were visualised with UV light after staining the 2% agarose gel with ethidium bromide using commercial O’RangeRuler™ 100 bp DNA Ladder (Fermentas, Waltham, Massachusetts, USA).

Formalin fixed paraffin embedded (FFPE) liver sample obtained from a PKD positive cat was used as a positive control for PCR. DNA isolated from a buccal swab obtained from a 10-year old PKD negative domestic cat was used as a negative control.

Results

Haematological and biochemical parameters

Haematological parameters in all examined cats were within the normal range. Biochemical parameters (urea, creatinine, and phosphorus) in all cats were also within reference intervals (normal ranges of analyzer were 5.7-12.9 mmol/l for urea, 71-212 µmol/l for creatinine and 1-2.42 mmol/l for phosphorus). The mean values of urea in PKD positive cats were 7.5 mmol/l, creatinine 103 µmol/l and phosphorus 2.05 mmol/l.

Ultrasound

The cysts in the kidneys were spherical, anechoic or hypoechoic cavities (Figure 1). Cysts were detected in the renal parenchyma of 13 of 70 cats (18.6%; 10 females and 3 males). The largest number of cysts was located in the cortex of the kidneys. The youngest cat with PKD was 3.5 years old.

PCR - RFLP

Polymerase chain restriction-fragment length polymorphism resulted in only 1 fragment of 559 bp on agarose gel in samples from cats without the PKD mutation, while samples from cats with the PKD mutation produced 2 more fragments of 316 bp and 243 bp (Figure 2). The results revealed 34 PKD affected individuals (23 females and 11 males) representing 48.6% of considered cats (Table I). Genetic analysis confirmed the presence of mutation causing the disease for all 13 cats resulted positive for PKD on ultrasound. The age of these animals ranged from 4 months to 8 years. It is important to stress that the 21 cats which ranged from 4 months to 3.5 years, were found to be PKD positive by PCR-RFLP analysis, although no signs of PKD were detected on ultrasound.

Discussion

Polycystic kidney disease is one of the most common genetic diseases in cats, which may lead to death (Egenvall et al. 2009). Mutation causing PKD is very common in Persian cats and related breeds, reaching the frequency of 40%. There is no therapy for this disease, so prevention is crucial. Prevention could be achieved by exclusion of PKD positive individuals from reproduction and this requires reliable diagnostic tools such as PCR-RFLP able to detect PKD in early stages of life (Criado-Fornelio et al. 2008).
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were within reference ranges in all PKD positive cats. Haematology results indicated good health, and the number of erythrocytes, leukocytes, and thrombocytes was normal in all animals. Mean values of urea (7.5 mmol/l), creatinine (103 µmol/l) and phosphorus (2.05 mmol/l) were in accordance with reference values and literature data (Bosje et al. 1998). That could be explained by the fact that clinical signs, symptoms and health issues occur in older individuals (Barrs et al. 2011), and most of cats in our study were younger than 5 years. Domanjko-Petrič and colleagues (Domanjko-Petrič et al. 2008) found higher levels of creatinine in 10 cats with the disease, but their average age was 12 years. These findings suggest that haematological and biochemical parameters may be within reference interval in PKD positive individuals, especially in young ones.

For every patient involved in this study PKD was diagnosed by ultrasound, the findings were then confirmed by DNA analysis showing the underlying mutation. However, in 21 animals (the youngest was 4 months and the oldest was 1.5 year old) the presence of mutation was found by the molecular genetic method, while the presence of cysts was not observed on ultrasound. The cause of this may be that cysts in younger cats are so small that they cannot be visualized by ultrasound. Therefore, it is reasonable to recommend molecular genetic methods for PKD detection if a cat is younger than 3 months (Domanjko-Petrič et al. 2008). Some authors suggest that DNA analysis is the only method for PKD diagnostic in cats younger than 10 months (Barrs et al. 2011), as with DNA testing it is possible to detect the mutations at a very early age – right after birth. In contrast to that, PKD diagnostic by ultrasound require constant monitoring of appearance and progression of cysts. Nonetheless, ultrasound allows for assessing the size, shape, and location of the kidneys and may also be used to assess renal blood flow (Platt 1997). Besides, if compared to the preparation for ultrasound, sampling (swab) for DNA analyses is much easier and the discomfort of individuals is considerably smaller.

Cysts are present right after birth, but clinical symptoms usually manifest in older animals because the growth of cysts increases pressure on renal parenchyma, leading to renal insufficiency (Domanjko-Petrič et al. 2008). The moment of manifestation of signs and symptoms varies over a period ranging from 3 to 10 years (Barrs et al. 2011).

In this study, DNA analysis using PCR-RFLP method revealed the mutation causing PKD in 34 animals (48.6%). In 21 cats (30%), the disease was diagnosed only using PCR-RFLP (cysts were not found by ultrasound). Cysts were found by ultrasound only in 13 cats (18.6%). The PKD frequency found in this study is higher than the one reported in USA (where 20% of cats resulted positive) (Lyons et al. 2004), Slovenia (36%) (Domanjko-Petrič et al. 2008), Italy (41.1%) (Bonazzi et al. 2009), France (41.8%) (Barthez et al. 2003), and results estimated on the global level also (37%) (Di Bartola 2000). However, these results are similar to those in Great Britain (49.2%) (Cannon et al. 2001) and Australia (45%) (Barrs et al. 2011). Estimates assess that about 6% of cats in USA have PKD, which makes it the most common inherited disorder in cats (Lyons et al. 2004).

Among the cats we analysed, females were more affected by PKD than males (52.3% vs. 42.3%). Yet the small number of samples limits us from asserting whether this disease is more frequent in females; still our results are consistent with data from the literature (Barrs et al. 2011, Domanjko-Petrič et al. 2008).

In this study, haematology and selected biochemical parameters (urea, creatinine, and phosphorus) were within reference ranges in all PKD positive cats. Haematology results indicated good health, and the number of erythrocytes, leukocytes, and thrombocytes was normal in all animals. Mean values of urea (7.5 mmol/l), creatinine (103 µmol/l) and phosphorus (2.05 mmol/l) were in accordance with reference values and literature data (Bosje et al. 1998). That could be explained by the fact that clinical signs, symptoms and health issues occur in older individuals (Barrs et al. 2011), and most of cats in our study were younger than 5 years. Domanjko-Petrič and colleagues (Domanjko-Petrič et al. 2008) found higher levels of creatinine in 10 cats with the disease, but their average age was 12 years. These findings suggest that haematological and biochemical parameters may be within reference interval in PKD positive individuals, especially in young ones.

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Table I. Ultrasound and molecular detection using PCR of PKD in Persian cats of different sexes.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of samples</th>
<th>Prevalence N(%)</th>
<th>Ultrasonography</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>26</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>10</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>13 (18.6)</td>
<td>34 (48.6)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Ethidium bromide stained agarose gel showing amplification products of exon 29 after digestion. PKD negative cats will produce just one fragment (559 bp) of the wild-type allele and PKD positive cats will produce two more bands (316 bp and 243 bp). M = Ladder; 1 = Positive control (PKD positive cat); 2-13 = Sampled cats; 14 = Negative control (PKD negative cat).
In conclusion, molecular genetic methods are the most efficient and reliable for timely diagnosis of PKD and can be used from the first day of life. Also, sampling for genetic tests involves significantly lower stress levels for the animal than ultrasound. Furthermore, genetic tests take considerably less time compared to procedures required for haematological and biochemical analyses or ultrasound. DNA tests for the mutation causing PKD should be done on all Persian cats, before owners and breeders decide to include them in reproduction (Young et al. 2004). Finally, as breeders usually make breeding strategy even before tests are taken, it is important to offer them continuing education, aimed at preventing the disease spreading and providing longer, more comfortable lives to cats.

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