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STATISTICAL CORRELATION OF GENETIC, ULTRASONOGRAPHIC, AND PARACLINICAL DIAGNOSIS IN POLYCYSTIC KIDNEY DISEASE IN CATS FROM ROMANIA

CORELAȚIA STATISTICĂ A DIAGNOSTICULUI GENETIC, ULTRASONOGRAFIC ȘI PARACLINIC ÎN BOALA POLICHISTICĂ RENALĂ LA PISICI DIN ROMÂNIA

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ABSTRACT | REZUMAT

Polycystic kidney disease (PKD) has been recognised for many years as a hereditary, monogenic, autosomal dominant disorder. Recently, more and more studies have shown that a small percentage of close to 5% of cats diagnosed with renal cysts during an ultrasound examination are, in fact, NN homozygous types. It is considered that other mutations (such as missense mutations and point mutations in the PKD2, DZIP1L, and PKDH1 genes) can also cause polycystic kidney disease. The study was conducted between October 2019 and May 2022 on 83 felines of different breeds, including Persian and other related breeds with known high and medium prevalences for polycystic kidney disease. The cats were divided into three groups and examined separately using a clinical evaluation, ultrasound scans, genetic testing for the PKD1 gene, and a serum biochemistry evaluation. Statistical analysis was used to find correlations between diagnostic results and variables taken into account for the study, using Pearson's correlation coefficient and variables such as age, sex, genetic tests (+/-), diagnostic method, and serum biochemistry evaluation. The results obtained in this study have shown the following statistically relevant aspects: a causal relation between age and the discovery of cysts; effects of genetic variability and selective expression in the individual; a lack of association between sexes and the presence or absence of cysts; and breed as an important factor. Although 100% of the ultrasound scans were confirmed by genetic testing, we consider that genetic testing remains the most accurate diagnostic test, especially for younger individuals (4–10 months).

Keywords: PKD1, polygenic, cat, ultrasonography, statistic

Boala rinichilor polichistici (PKD) a fost cunoscută pentru mulți ani ca fiind o eredopatie monogenică, autozomală, dominantă. Recent, din ce în ce mai multe studii au arătat că un mic procent de aproape 5% dintre pisicile diagnosticate cu chisturi renale în timpul unei examinări cu ultrasunete au fost de fapt NN homozigote, considerându-se că alte mutații (cum ar fi mutațiile missense și mutațiile punctiforme în genele PKD2, DZIP1L și PKDH1) pot provoca, de asemenea boală renală polichistică. Studiul a fost realizat în perioada octombrie 2019 – mai 2022, pe 83 de feline din rase diferite, inclusiv rasa Persană și alte rase înrudite cunoscute pentru prevalența medie și înaltă pentru boala polichistică renală. Pisicile au fost împărțite în trei grupuri și au fost examinate separat, folosindu-se evaluare clinică, ultrasonografia, testarea genetică pentru gena PKD1 și evaluarea biochimică a serului. Analiza statistică a fost utilizată pentru a găsi corelații între rezultatele diagnosticului și variabilele luate în considerare pentru studiu, folosind coeficientul de corelație al lui Pearson pentru variabile precum vârsta, sexul, testele genetice (+/-), metoda de diagnosticare și evaluarea biochimiei serului. Rezultatele obținute în acest studiu au evidențiat următoarele aspecte relevante statistic: o relație cauzală între vârstă și apariția chisturilor; efectele variabilității genetice și ale expresiei selective individuale; o lipsă de asociere între sex și prezența sau absența chisturilor; rasa ca factor predispozant. Deși 100% dintre ecografii au fost confirmate prin testare genetică, considerăm că testarea genetică rămâne cel mai precis test de diagnostic, în special pentru indivizii tineri (4-10 luni).

Cuvinte cheie: PKD1, poligenic, pisică, ultrasonografie, statistic

Autosomal dominant polycystic kidney disease (AD PKD) has long been known as a monogenic hereditary disorder with incomplete penetration that causes the progressive development of cysts in the kidney and sometimes in other organs like the pancreas and liver. It was first described in the cat in 1967; actual research on the hereditary transmission of the disease had truly begun after 1990; and the first study to pinpoint the genetic particularities of PKD in the cat came much later, in 2004, when researchers from U.C. Davis University, United States, highlighted a higher prevalence (38%) in Persian cats (18). Later, other international studies have identified a high prevalence in

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breeds like Persian, Exotic Shorthair, and Himalayan (5, 26, 15) with a geographic distribution of PKD1 gene mutation in affected felines of 42% in France (1); 41% in Italy (4); 36% in Slovenia (8); 36% in Iran (20, 21); 46% in Japan (24); 49% in Great Britain (6); 50% in Australia; 67% in New Zealand; 39% in USA; 9% in Germany; 21% in the Netherlands; 64% in Spain; and 16% in Taiwan (6, 14, 21, 24). However, other breeds, such as Birman, Siamese, British Shorthair, Scottish Fold, Angora, Ragdoll, Maine Coon, Chartreaux, and their crossbreeds, can be affected by this disease. Because of this genetic mutation's hereditary character and the fact that approximately 80% of actual cat breeds have a certain degree of crossbreeding with Persian cats, the disease could be found in every type of cat breed (25). Tubule degeneration, necrosis, and apoptosis play a key role in the development of cysts, explaining the pathogenetic mechanism of this disease (3, 26, 2, 17), but variable genetic expressivity determines variable expression of traits and diseases in individuals, which means that some cats with ADPKD have a small number of cysts and the disease never progresses towards kidney failure, while other cats have a severe and rapid progression towards kidney failure in just a few years (5, 26).

The most recent data presented by **Online Mendelian Inheritance in Animals** suggests a genetic polymorphism of PKD in the cat. Rodney et al. (2021) describe polycystic kidney disease (PKD) in some cat breeds as being caused by a new frameshift mutation in polycystin-2 (PKD2), capable of perturbing protein function in a Siberian cat diagnosed with PKD through ultrasound examination (23). This is a single base deletion mutation that causes truncated protein synthesis (p.Lys737Asnfs*2). However, screening of other Siberian cats diagnosed with PKD through ultrasound examination did not identify the **c.2211delG** in PKD2, which suggests the presence of a ***de novo*** mutation capable of causing the disease in Siberian cats. Other studies support a multiple mutation theory because a small percentage of approximately 5% of individuals diagnosed with kidney cysts through ultrasound or microscopic pathology examination have tested negative for the disease, with all individuals presenting a NN homozygous genotype, which indicates the absence of the PKD1 gene mutation (7, 9,10, 12, 15, 19).

Recent studies have focused on the identification of new molecular markers to help develop new complementary genetic tests for feline ADPKD. These used the study model of the disease in humans and mice, where mutations in the PKD1 (85%) and PKD2 (15%) genes have been identified as being responsible for autosomal dominant and recessive polycystic kidney disease in humans; a mutation of the PKDH1 gene being responsible for polycystic disease of the kidneys and liver; and a mutation of the Nek88 gene being responsible for PKD in mice and zebra fish (10, 13, 16, 18, 22, 27). Due to the growing popularity of the polygenic hypothesis of this disease in cats, some medical research in the field of genetics is centred around the identification and localization of mutated genes from the genetic map of Persian cats. Results have shown a significant nonrecombinant link between the PKD disease locus and the FCA476 marker situated on the E3 feline chromosome. This data suggests that the PKD1 gene or some other gene from this region could cause feline PKD (27). Therefore, PKD can be considered a polygenic genetic disorder, with mutations in the FCA476 microsatellite of the PKD1 gene from the E3 feline chromosome being the principal cause of this disease. A small but significant percentage of mutations are represented by changes in the PKD2 gene from the B1 chromosome, situated between the FCA6 12 and FCA097 microsatellites, as well as by mutations in the DAZ interacting zinc finger protein 1 like (DZIP1L) and PKDH1 genes. These new mutations target the ciliary structures and can lead to different severities of clinical manifestations of PKD, which suggests a possible epistatic interaction (11).

MATERIALS AND METHODS

The study was done from October 2019 to May 2022 on a group of 83 felines from different breeds. The cats were given a thorough clinical examination, underwent an ultrasound examination, and blood was collected for genetic and paraclinical tests. Individuals diagnosed through ultrasound examination were divided into three groups:

A) **29 individuals** (different breeds, ages, and sexes) with a positive PKD diagnosis, divided into 4 age groups: < 1 year, 1–3 years, 3–7 years, and > 7 years, from different types of breeds: Persian, British Shorthair, Scottish Fold, Birman, European, and their crossbreeds.

B) **56 individuals** with a negative PKD diagnosis, divided into groups according to breeds and their crossbreeds, age groups, and sex. These cats were screened through an ultrasound examination and evaluated based on epidemiological criteria related to their breed-related genetic predisposition.

C) For the correlation of the feline polycystic kidney disease 1 (PKD1) gene mutation with ADPKD cases diagnosed through ultrasound imaging, and in order to establish a statistical correlation between the method of diagnosis and the presence or absence of a mutation in the PKD1, out of the 83 evaluated subjects included in the study, we have selected a number of 15 cases for the **control group**. These individuals were selected according to the following two criteria:

1. individuals diagnosed through ultrasound, both positive and negative for ADPKD;

2. individuals belonging to the Persian breed, known for having the highest prevalence, and other breeds like British Longhair, British Shorthair, and their crossbreeds classified as high-risk for mutations of the PKD1 gene.

Sample characteristics and protocol

Ultrasound examination of the patients was done using various medical devices, including the Samsung HS 30, SonoScape portable, Esaote MyLab60, GE LogiQ F6, and Mindray DC-40. The transducers used were both microconvex and linear, including the 6C2 microconvex transducer and the 7L4B linear transducer, offering sufficient permeation of ultrasound waves for a visualisation of the whole kidney and a good resolution for a detailed evaluation of the renal structure. Clinical biochemistry tests were performed using the ARKAY SPOTCHEM EZ-SP-4430 dry biochemistry analyser. Blood samples were collected and centrifuged for 5 minutes at a speed of 4.000 rotations per minute. The resulting serum was tested for the following parameters: urea, creatinine, liver enzymes (GPT/ALT, GOT/AST, and GGT), and total bilirubin for patients with hepatic cysts.

Genetic testing has been done for the mutation in the PKD1 gene (c.10063C>A mutation in exon 29 PKD1). For this test, 1 mL of peripheral venous blood was collected in EDTA-coated vials. Mutations in the PKD gene were evaluated using the TaqMan SNP test. This method of genotyping uses the nuclease activity of the 5th Taq polymerase (an enzyme used for in vitro DNA synthesis through the PCR technique) in order to generate a fluorescent signal during the chain polymerization reaction (PCR). For every single nucleotide polymorphism (SNP), the test utilises two TaqMan samples with different sequences in the SNP locus, with sample complementary to the wild-type allele and the other to the variant allele.

Statistical analysis

The statistical evaluation of clinical biochemistry test results was centred around changes in the creatinine parameter and was done in order to form a correlation between the results and the variables taken into consideration during the study, using Pearson's correlation coefficient and variables such as age, sex, genetic tests (+/-), diagnostic method, and serum biochemistry parameters. Although Pearson's linear correlation coefficient (r) does not have causal significance outside of experimental conditions, it can reveal links, associations, or variance between values, according to utilised variables. The interpretation of the determination coefficient (R2) was done through multiple linear regression in order to create a predictable model (breed and symptomatology; age and number of cysts ultrasound examination and positive genetic tests, etc). In order to extrapolate the genetic testing results of the control group to the total number of cats included in the study, and according to breed types, the mutation rate (%) of PKD1 was calculated using the formula: [(number of cats with a positive mutation / total number of cats) x 100].

RESULTS AND DISCUSSIONS

Group A, with 29 cases confirmed with ADPKD through ultrasound examination, representing 35% of the total 83 cases from the study, was gathered from different clinics and small practices in different cities in Romania. The cats diagnosed with PKD belong to many of the internationally recognised high-risk breeds for this type of disease (Fig. 1).

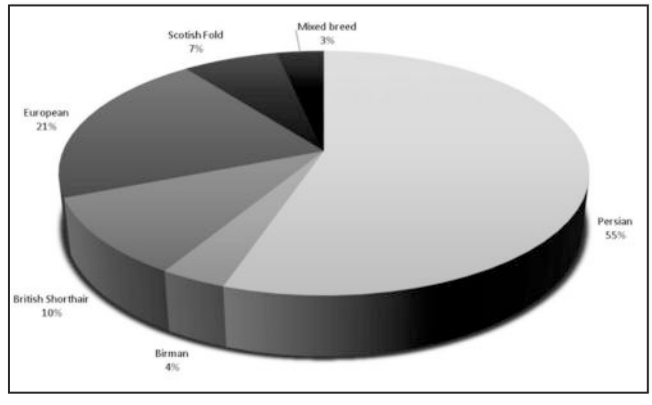

Fig. 1. Incidence of ADPKD according to patient breed

Out of the total 29 cases diagnosed with ADPKD, 15 individuals presented PKD symptomatology (52%), and 14 individuals (48%) did not have clinical signs of kidney disease. Statistical processing in a $P<0.05$ confidence interval indicates a significant positive correlation ($R2 = 0.96$) between age, the presence of cysts, and renal symptomatology (Fig. 2).

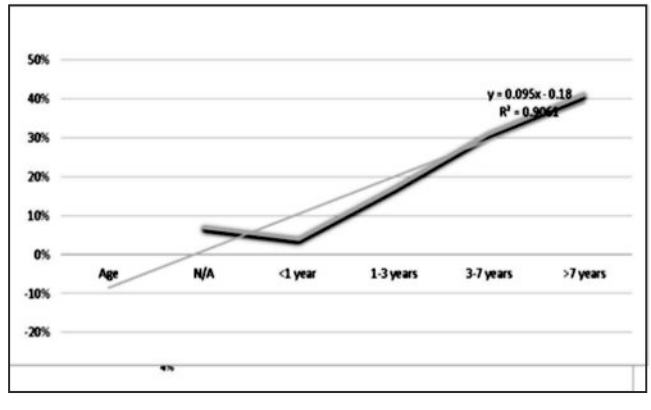

Fig. 2. Statistical evaluation through determination coefficient R2 according to subject age

The correlation between patient age and the number of cysts has shown that from a total number of 29 cases diagnosed through ultrasound examination, 8 individuals had unilateral cysts (28%), and 21 individuals had bilateral cysts (72%; Fig. 3).

Study results have demonstrated that the number of cysts was proportional with patient age: individuals between 3 and 7 years had 1-3 cysts, and individuals over 7 years had over 3 renal cysts. Furthermore, cyst size was also proportional with age; the incidence of large cysts between 0.5 and 1 cm and over 1 cm was greater in individuals aged 3 to 7 years and older. With a value of R2 = 0.84 we estimated that there is a significant association between the number of cysts and a cat's age (Fig. 4), similar to other research data in this field (11).

The negative value of the determination coefficient indicates a lack of association between sex and ADPKD (Fig. 5).

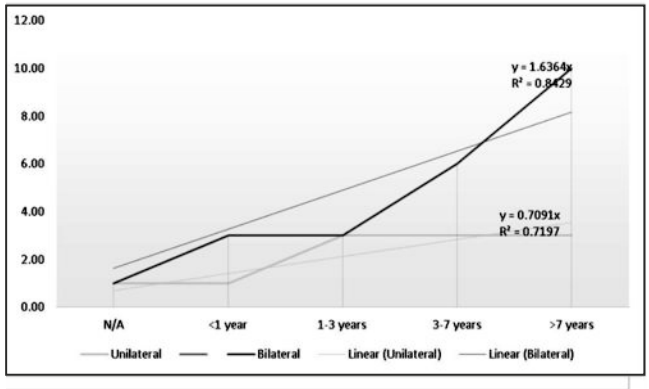

Fig. 3. Statistical evaluation through the R2 coefficient according to patient age and the number of cysts

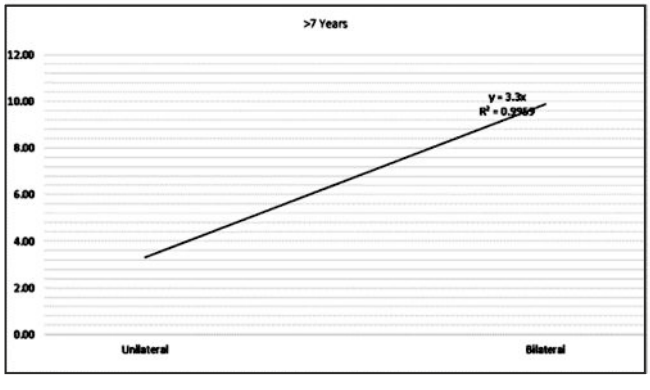

Fig. 4. Statistical evaluation through the R2 determination coefficient for the > 7-years-age segment

An evaluation of serum creatinine levels has shown that out of a total of 29 cases of cats diagnosed with ADPKD included in the study, 9 individuals had normal creatinine values (31%), and 20 individuals (69%) presented elevated creatinine values. Statistical evaluation of R2 = 0.56 has shown a moderate association between age and serum creatinine values, which may suggest that PKD evolves with age but may also evolve differently in individuals (Fig. 6).

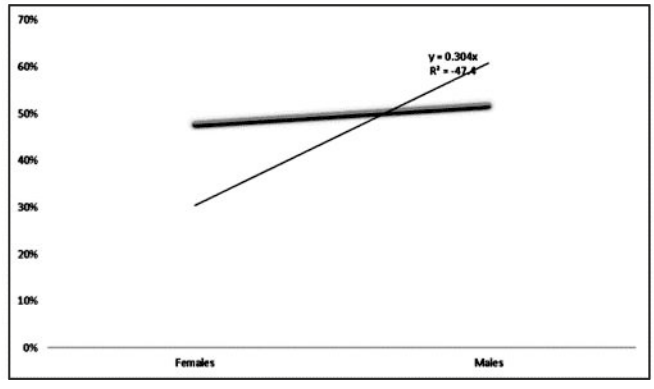

Fig. 5. Statistical evaluation through the R2 determination coefficient according to sex

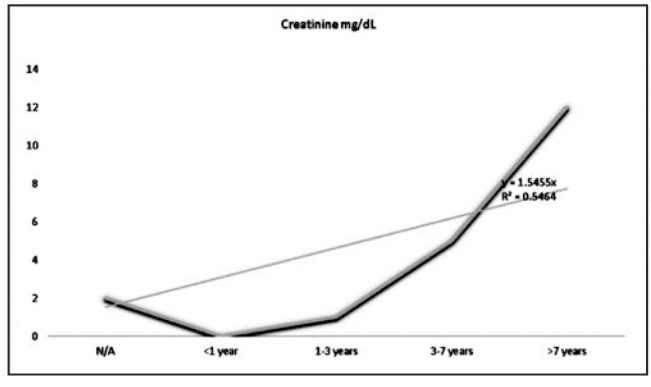

Fig. 6. Statistical evaluation of creatinine values (mg/dL) in relation to cat age through the R2 determination coefficient

Group B was formed out of individuals selected according to breed genetic predisposition for the PKD1 gene mutation without any type of renal symptomatology but screened through ultrasound examination.

Individuals were divided into groups according to breed, age, and sex, in a similar manner to the previous group. Incidence according to breed was for British shorthair (35%); Persian (24%); Scotish fold (13%); European (11%); British longhair (8%); Birman (7%); and crossbreed cats (2%).

Group C was made in order to confirm the ultrasound diagnostic by means of genetic testing and also correlate the results obtained through these two different diagnostic methods.

Out of the total 83 cases evaluated through ultrasound investigation (+/- diagnosis) for PKD, a control group was formed from 15 individuals (18%) of breeds that are predisposed to this genetic disorder and underwent genetic testing (Table 1).

Table 1

A control group of cats was selected to confirm the ultrasound diagnosis by means of genetic testing

No.	Breed	Age	Sex	Results of genetic testing (NP.NA–PKD1)	Hormonal status
1.	Angora-British Shorthair crossbreed	4	M	Positive (NP heterozygous genotype)	Neutered
2.	British Shorthair	0.5	M	Positive (NP heterozygous genotype)	Intact
3.	Persian	2	M	Positive (NP heterozygous genotype)	Intact
4.	Persian	6	F	Positive (NP heterozygous genotype)	Spayed
5.	British Shorthair	3	F	Negative (NN homozygous genotype)	Intact
6.	British Shorthair	2	F	Negative (NN homozygous genotype)	Intact
7.	British Shorthair	5	M	Negative (NN homozygous genotype)	Intact
8.	British Shorthair	3	M	Negative (NN homozygous genotype)	Intact
9.	British Shorthair	4	F	Negative (NN homozygous genotype)	Intact
10.	British Shorthair	4	F	Negative (NN homozygous genotype)	Intact
11.	British Longhair	2	F	Negative (NN homozygous genotype)	Intact
12.	British Longhair	4	F	Negative (NN homozygous genotype)	Intact
13.	British Longhair	2	F	Negative (NN homozygous genotype)	Intact
14.	British Longhair	4	M	Negative (NN homozygous genotype)	Intact
15.	Persian crossbreed	3	M	Negative (NN homozygous genotype)	Neutered

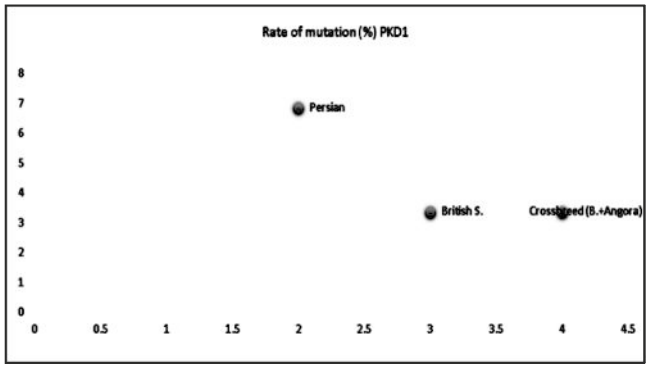

Fig. 7. The rate of mutation (%) for PKD1

In order to extrapolate the results obtained through genetic testing of the control group to the total number of cats evaluated in this study, depending on breed predisposition, the rate of mutation (%) for PKD1 was calculated using the formula: [(number of cats positive for the mutation / total number of cats) x 100] (Fig. 7).

2/29 x 100 = 6.89% Persian

1/29 x 100 = 3.4% British Shorthair

1/29 x 100 = 3.4% Angora-British Shorthair crossbreed

Group A

Statistical evaluation of individuals in group A using association methods has highlighted the relationship between patient age, sex, the size and number of renal cysts, the presence or absence of renal symptomatology, and certain serum biochemical parameters with symptomatology.Furthermore, kidney cysts were smaller in size in younger cats and larger in older individuals, in correlation to the degenerative nature and the constant clinical progression of this genetic disorder. The highest incidence was found in Persian cats (55%), which makes this breed the perfect 'candidate' for this genetic disorder, alongside other diseases such as diabetes and feline lower urinary tract disease (18, 14). Statistical processing of data has shown that the presence of the PKD1 gene mutation was significantly correlated with age, which indicates that the probability of detecting cysts through ultrasound examination increases with patient age. The study did not identify statistical differences between males and females, which suggests that the genetic inheritance of PKD is not sex-related.

Serum creatinine was used as an indicator for kidney function in patients with ADPKD and as a correla-

tion factor for renal deterioration with patient age and the number and size of renal cysts. Creatinine values began to rise around the age of three and reached their highest value at the age of 7. Statistical processing of the data indicates that individuals over the age of 7 suffered from a greater deterioration of kidney function, a probable correlation to the increased incidence of larger cysts for this age category. On the other hand, the age group between 3 and 7 years presented either moderate increases in creatinine levels or physiological serum creatinine values. These findings are not surprising, and there are other studies that have identified normal serum creatinine values (<1.6 mg/Dl) even in individuals with a positive PKD1 gene mutation and over 9 years old. These results suggest that PKD evolves differently in the individual as a result of variable genetic expressivity. Selective and variable expressivity in individuals determined by genetic variability has led led some cats with a positive diagnosis of the PKD1 gene mutation to never develop clinical manifestations of kidney disease. For these reasons, we cannot formulate any predictions over the phenotypical evolution of the disease for any of the 29 cases that tested positive, which underlines the necessity of regular re-evaluation of patients through ultrasound and clinical biochemistry tests.

Group B

For the 54 subjects of different ages and sexes included in group B, the ultrasound diagnosis for PKD was negative. These individuals being chosen for their breed's high prevalence of PKD. From this group, 11 cats (included in group C) tested positive for the PCR real-time genotyping of the PKD1 gene mutation and confirmed 100% of the ultrasound diagnosis results, all individuals being (-) NN homozygous types. The aim of forming this group has its foundation in the practical advantages of using the ultrasonography as a successful diagnostic method that is both fast and accurate. Ultrasonography remains the sole method of appreciating the severity and progression of the disease and was, for many years, the sole method of diagnosis for polycystic kidney disease in cats. Furthermore, an ultrasound investigation is relatively affordable and widely available in veterinary clinics, with the advantages of being a non-invasive test that is both safe for the patient and efficient for the detection of renal cysts.

Group C

The third group was used as a control, in order to highlight the causal mutation in the PKD1 feline gene as an etiologic factor of this autosomal dominant genetic disease, and in order to establish a correlation between the diagnostic method and the presence or absence of a mutation in the PKD1 gene. The 15 cases included were selected as follows: 4 cases were randomly selected from group A [individuals with a **positive ultrasound diagnosis** for ADPKD] that tested positive for genetic testing (NP heterozygous genotype); 11 cases were randomly selected from group B [individuals with a **negative ultrasound diagnostic** for ADPKD] that tested negative for the NN homozygous genotype, with two normal copies of the allele. Individuals with this genotype lack the mutation in the PKD1 gene. Thereby, we can appreciate that genetic testing has highlighted the presence of the mutation in individuals that tested positive during the ultrasound investigation and the absence of the mutation in individuals that tested negative during the ultrasound investigation, even though there was a breed-related predisposition. In order to extrapolate the incidence of the disease according to the total number of cases (n = 83), we have used the mutation rate (%) of the PKD1 gene. Results have shown that the Persian breed had the highest mutation rate of the PKD1 gene (6.8%), followed by British Shorthair and their crossbreeds, both being predisposed for this mutation.

CONCLUSIONS

Results obtained in this study have shown the following statistical aspects: causal relations between age and cyst presence, with cats beginning to develop clinical signs of kidney function decline starting from the age of 3, with a variable clinical evolution; a moderately significant association between serum creatinine levels and patient age was indicative of the effects of genetic variability and selective expressivity between individuals; a lack of association between sex and the presence or absence cysts; breed as an important correlation factor for the different phenotypes of ADPKD in the cat; all the cases that were diagnosed negative for ADPKD during the ultrasound examination were also confirmed, having also tested negative for the genetic evaluation, being identified as NN homozygous genotypes with two normal copies of the allele, without a PKD1 mutation. A complete diagnosis for ADP KD should always include genetic testing, especially in the current context of the polygenic character of the disease. However, there are some very important aspects that for objective reasons (besides the economical aspect, owner consent, etc.), limit the large-scale utilisation of genetic testing. In some cases, a negative test does not necessarily mean that a patient will not develop ADPKD, because genetic testing is 100% accurate only for certain breeds for which the specific test has been approved (excluding errors during sample collection, transportation, processing etc.). This means that the clinician should choose a genetic test according to breed predisposition, use monogenic tests or tests that highlight new candidate genes for

the development of ADPKD, permanently evaluate de novo mutations that can change the phenotypic aspects of the disease (the presence of hepatic or pancreatic cysts, etc.), interpret the results in a clinical context alongside existing pathology, and issue predictions about the evolution of the disease.

Although genetic testing has confirmed 100% of ultrasound results, we consider that *genetic* testing remains the only *sure* diagnostic method, especially in animals younger than 4–10 months, and the only confirmation for causal mutations, which is useful in covering all the candidate genes that can suffer mutations and lead to the onset of polycystic renal disease in the cat. *Ultrasonography* remains the *primary* diagnostic method for polycystic renal disease and especially useful for monitoring disease progression in the cat and consequently establishing supporting therapy management because early diagnosis of the disease is not always accompanied by chronic kidney disease symptomatology.

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