PREVALENCE OF POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN AND AROUND MUMBAI

T H E S I S

Submitted in partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

BY

GAIKWAD ABHISHEK ANIL

Enrolment No: V/16/093

Mumbai Veterinary College, Mumbai

MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY, NAGPUR – 440 001

(INDIA)

2024

Appendix - B

DECLARATION OF THE STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled "**PREVALENCE OF POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN AND AROUND MUMBAI"** or part thereof has not been submitted for any of the other degree or diploma of any university, nor the data have been derived from any thesis or publications of any university or scientific organization. The sources of material used and all assistance received during the course of investigation have been duly acknowledged.

Date:

Place: Mumbai

GAIKWAD ABHISHEK ANIL
Enrolment No. V/16/093
Reg. No. 1873

Dr. J.U. PATIL
Chairman, Advisory committee,
Assistant Professor,
Dept. of Veterinary Epidemiology
and Preventive Medicine,
Mumbai Veterinary College.

Appendix – C(i)

DECLARATION OF ADVISORY COMMITTEE

MR ABHISHEK ANIL GAIKWAD has satisfactorily prosecuted his course of research work for a period of not less than one semester and that the thesis entitled **" PREVALENCE OF POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN AND AROUND MUMBAI "** submitted by his is the result of research work is sufficient to warrant its presentation to the examination in subject of **Veterinary Epidemiology and Preventive Medicine** for the award of **Master of Veterinary Science (M.V. Sc.)** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by her for a degree of any other university.

Place: Mumbai
Date:

(Dr. J.U. PATIL)
Chairman
Assistant Professor,
Dept. of Veterinary Epidemiology
and Preventive Medicine
Mumbai Veterinary College.

ADVISORY COMMITTEE

Sr. No.	**Name**	**Designation**	**Signature**
I.	Dr. D.A. Pawalkar	Assistant Professor, Dept. of Veterinary Epidemiology and Preventive Medicine	
II.	Dr. R. V. Gaikwad	Professor & Head, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence	
III.	Dr. S.V. Gulwane	Professor & Head, Department of Veterinary Gynaecology & obstetrics	
IV.	Dr. M. P. Sawane	Professor & Head, Department of Veterinary Animal Genetics & Breeding	

Appendix -C (ii)

CERTIFICATE

This is to certify that the thesis entitled, "**PREVALENCE OF POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN AND AROUND MUMBAI"** submitted by Mr. Gaikwad Abhishek Anil to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE (M.V.Sc)** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

Name & Signature of External Examiner	**Signature with Seal Head of Department**	**Name & Signature Adviser &Guide**

ADVISORY COMMITTEE

Sr. No.	Name	Designation	Signature
I)	Dr. D. A. Pawalkar	Assistant Professor, Veterinary Epidemiology and Preventive Medicine	
II)	Dr. R. V. Gaikwad	Professor and Head of the department Department of Clinical Medicine, Ethics and Jurisprudence	
III)	Dr. S. U. Gulvane	Professor and Head of the department of Animal Reproduction, Gynaecology and Obstetrics	
IV)	Dr. M. P. Sawane	Professor & Head, Department of Animal Genetics and Breeding	

Signature with Seal
Dean/ Associate Dean
Mumbai Veterinary College

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Dedicated to my Beloved Family

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Date:

Place:Mumbai *Dr. Abhishek Anil Gaikwad*

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LIST OF ABBREVIATIONS

Sr No.	Abbreviations	Full Form
1.	**%**	Percent
2.	**°F**	Degree Fahrenheit
3.	**PKD**	Polycystic Kidney Disease
4.	**PCR**	Polymerase Chain Reaction
5.	**RFLP**	Restriction Length Fragment Polymorphism
6.	**ALP**	Alkaline Phosphatase
7.	**CBC**	Complete Blood Count
8.	**SE**	Standard Error
9.	**Bp**	Base Pair
10.	**AST**	Aspartate Transaminase
11.	**RK**	Right Kidney
12.	**BUN**	Blood Urea Nitrogen
13.	**BW**	Body Weight
14.	**LK**	Left Kidney
15.	**°C**	Degree Celsius
16.	**OPD**	Out Patient Department
17.	**DLC**	Differential Leucocyte Count
18.	**PCV**	Packed Cell Volume
19.	**KFT**	Kidney Function Test
20.	**RBC**	Red Blood Cell

21.	**WBC**	White Blood Cells
22.	**gm%**	Gram Percent
23.	**Gm/dl**	Gram Per Decilitre
24.	**Hb**	Haemoglobin
25.	**IU/I**	International Unit Per Litre
26.	**SGOT**	Serum Glutamate Oxaloacetate Transaminase
27.	**SGPT**	Serum Glutamate Pyruvate Transaminase
28.	**Pg**	Picogram
29.	**TBE**	Tris/Borate/EDTA
30.	**DNA**	Deoxyribonucleic Acid
31.	**et al**	et alia (and other people)
32.	**Hrs**	Hours
33.	**Ml**	Milligram
34.	**Ng**	Nanogram
35.	**nM**	Nanomolar
36.	**q-PCR**	Quantitative Polymerase Chain Reaction
37.	**Rpm**	Revolution per Minute
38.	**RT-PCR**	Real Time-Polymerase Chain Reaction

Introduction

INTRODUCTION

Polycystic kidney disease (PKD) is a genetic disorder that causes many fluids filled cysts to grow in kidneys. Unlike the usual harmless simple kidney cysts that can form in the kidneys later in life, PKD cysts can change the shape of kidneys, including making them much larger. PKD is an inherited disorder in which clusters of cysts developed primarily within kidneys, causing kidneys to enlarge and loss function overtime, cysts are non-cancerous around sacs containing fluid. The cysts vary in size, and they can grow very large. Having many cyst or large cysts can damage kidneys can cause serious complications, including high blood pressure, and kidney failure Polycystic kidney disease is congenital disorder that has been recognized in several breed of cat but in particularly prevalent in Persian cats (Biller and Dibartola 1995). The disease has a mode of inheritance similar to human autosomal dominant PKD, is progressive and may lead to irreversible renal failure. Occasionally, cysts can be found in liver and pancreas (Biller *et al.* 1990, Lyons *et al.* 2004).

The development and expansion of cysts progress gradually, resulting in the deterioration of kidney tissue and a gradual decline in kidney function, ultimately leading to irreversible kidney failure. Feline polycystic kidney disease (PKD) and autosomal dominant polycystic kidney disease (ADPKD) in humans are hereditary conditions transmitted through autosomal dominant inheritance. ADPKD is among the most prevalent genetic disorders in humans. In cats, particularly in the Persian breed, this disease is highly prevalent and ranks as one of the most common genetic ailments. Imaging examinations appear to be the most dependable means for diagnosing the disease, although ongoing efforts involve the development of additional genetic tests to identify the presence of the responsible mutation (Schirrer *et al*., 2021).

Autosomal-dominant polycystic kidney disease is associated with a C→A transversion occurring in exon 29 of the feline PKD1 gene. This genetic alteration leads to a stop codon at position 3284, resulting in the loss of 25% of the C-terminus of the polycystin-1 protein. This particular form of the protein serves as

a target for molecular detection in carriers of ADPKD. Polycystin-1 plays a crucial role in regulating cellular processes such as proliferation, differentiation, and apoptosis. Reduction in its activity often leads to frequent occurrences of lesions and cellular changes within the renal parenchyma (Scalon, 2014).

In cats with Autosomal Dominant Polycystic Kidney Disease (ADPKD), renal function deteriorates progressively at a rate that varies from cat to cat, but typically leads to end stage renal disease by 3 to 10 years of age. ADPKD cats is inherited in an autosomal dominant manner with variable penetrance and no sex predilection. 5-6 current data on the frequency of ADPKD in Persian cats worldwide, based on systematic ultrasound examination of both kidneys, indicates a prevalence of 38% (Barrs *et al.* 2001).

Since 2000, the prevalence of feline PKD have been investigated in several countries, including the United States, Italy, the United Kingdom, Australia, France, Slovenia and Taiwan. Many reports have identified the breeds at high risk for PKD, including Persians and Persian related breeds. Since 2004, reports on the diagnosis of PKD using genetic testing have increased, and the PKD1 mutation rate is reported to be 15.7% in Persian cats in Taiwan, 33.3% in Persian cats in Slovenia, and 37.1% in Persian cats and Exotic Shorthairs in Italy. In our study, the PKD1 mutation rate in Persian cats was 46% (Reeko SATO *et al.* 2019).

The prevalence of polycystic kidney disease (PKD) has been estimated in the USA, Australia, UK, and Germany, but no data are available to date in France. The medical records from two centers (ENVL and ENVA) pertaining to all healthy cats who were screened for PKD using ultrasonography between December 2000 and April 2002 were examined. When one or more anechoic cavities were discovered in a kidney, a cat was considered positive.39.1% of exotic shorthair cats and 41.8% of Persian cats had PKD. PKD was not found in cats belonging to different breeds. Between Persians and Exotic Shorthairs, between males and females, and between the prevalence of PKD in ENVL and

ENVA, there was no discernible variation in PKD prevalence (Barthez *et al.* 2003).

In a metropolitan city with increased urbanization, the number of households with pets has grown drastically over the last decade specifically with regard to feline companions. The ownership of cats is seen in all sections of society and they have achieved the status of beloved pet to almost an extension of the family.

There is a lack of data of the polycystic kidney disease in Persian cats and its implications on the prognosis, hence the present study is planned with the following objectives.

Objectives:

1) Evaluation of Persian cats for ultrasonographic evidence of polycystic kidneys.
2) Effect of Polycystic kidneys on hematobiochemical parameters in Persian cats.
3) Evaluation of PKD1 gene mutation with PCR with RFLP & Gene sequencing of few cases.

Review of Literature

REVIEW OF LITERATURE

Evaluation of Persian cats for ultrasonographic evidence of polycystic kidneys

Biller *et al*., (1996) observed that Polycystic kidney disease in Persian cats culminates in chronic renal failure after a variable clinical course. An affected 6-year-old Persian cat was used to establish a colony of cats with polycystic kidney disease. In affected cats, cysts could be detected by ultrasonography as early as 7 weeks of age. Absence of cysts on ultrasound examination at 6 months of age was correlated with absence of poly-cystic kidney disease at necropsy. Both males and females were affected and, of progeny from affected x unaffected crosses, 42% were affected and 58% were unaffected. In affected x affected crosses, 73% of progeny were affected and 27% were unaffected. These results are compatible with autosomal dominant inheritance of this trait. Polycystic kidney disease in Persian cats resembles autosomal dominant polycystic kidney disease (ADPKD) in human beings, and represents a valuable animal model of the human disease.

Barrs *et al*., (2001) examined 228 cats comprising 162 Persians, 40 Himalayans, 17 Exotics and 9 Burmillas. In Sydney 92 cats were tested, comprising 68 Persians, 8 Himalayans, 6 Ragdolls, 5 Chinchillas and 5 Burmillas. In total, 320 cats of Persian or related breed, ranging in age from 10 months to 10 years, were tested for ADPKD by renal ultrasound examination A disease prevalence of 43% was found. The prevalence's of ADPKD in cats in Sydney (45%) and in Brisbane (42%) were not significantly different (P = 0.71). Similarly, prevalence of ADPKD in Persian cats screened in Sydney (54%) was not significantly different from that in Persian cats screened in Brisbane (43%) (P=0.15). Also, the prevalence in female cats (43%) was not significantly different from the prevalence in male cats (41%) (P=0.72). Two cats, whose results were considered equivocal on initial ultrasound examination, tested positive on a subsequent examination and are included in the positive group.

Cannon *et al*., (2001) observed prevalence of polycystic kidney disease was assessed in 132 Persian cats, 46 of them referred for the investigation and

treatment of medical or surgical conditions, and 86 apparently healthy cats referred specifically to be screened for the disease. Cats referred for the investigation of renomegaly or renal failure were excluded, and cats under 10 months old were only included if they had been examined postmortem. One hundred and twenty-six of the cats were examined ultrasonographically with a 7-5 MHz sector scanner, and the other six cats were examined postmortem. Forty-nine of the 86 cats referred specifically for screening (57-0 per cent) and 16 of the 46 cats referred for other clinical reasons (34-8 per cent) were affected by the disease, giving an overall prevalence of 49-2 per cent.

Lee *et al*., (2010) examined persian-related and non-Persian-related cats by ultrasonography and/or molecular testing to determine the prevalence of feline polycystic kidney disease (PKD) and the presence of a PKD1 gene mutation. PCR was used to amplify exon 29 of the PKD1 gene using genomic DNA extracted from blood samples, and the PCR products were analysed by direct DNA sequencing. Among the 111 cats included in the study, 54 were examined by both ultrasonography and gene testing for a point mutation in exon 29 of the PKD1 gene. The prevalence of PKD diagnosed by ultrasonography was 25.9 per cent in all the cats and 24.2 per cent in Persian-related cats. The prevalence of the transversion mutation in exon 29 of the PKD1 gene was 13.5 per cent in all cats and 15.7 per cent in Persian-related cats. Three cats that were diagnosed with PKD by ultrasonography did not have the mutation within exon 29. Nucleotide analysis of exon 29 indicated that male cats had a higher point mutation rate than female cats.

Yu *et al*., (2019) performed US examinations using an 8 MHz microconvex transducer on a dedicated ultrasound unit (Logiq 9, GE Healthcare, Wauwatosa, WI, USA). Each kidney was scanned in sagittal and transverse planesby either a first-year veterinary radiology resident (K.L.S.) or a board-certified veterinary radiologist (J.S.M.). The length was measured, using internal digital callipers, as the longest point between the cranial and caudal poles in the sagittal plane. Width and height were measured in the transverse plane.

Beck and Lavelle (2001) Studied two hundred and fifty Persian cats, ranging in age from13 weeks to 10 years, were presented to the University of

Melbourne Veterinary Clinic and Hospital for ultrasound examination of both kidneys. The cats were placed in dorsal and lateral recumbency and alcohol and ultrasonic coupling gel were applied to the skin. The kidneys were examined ultrasonographically in longitudinal, sagittal and transverse planes. Results were recorded for each cat at the time of examination as either negative or positive for PKD. In addition, 14 Exotics (short-haired Persians), 4 Ragdolls and 3 British Short-Hair cats were examined. Forty five percent of Persian cats examined were found to be positive for feline polycystic kidney disease on the basis of presence of anechoic cysts within the renal parenchyma. These cats ranged in age from 13 weeks to 10 years. Fifty per cent of the Exotic cats were positive for polycystic kidney disease whereasall Ragdolls and British Short Hairs were negative for the disease. Only one positivecat was reported to be showing clinical signs of renal disease.

Bonazzi *et al*., (2009) evaluated the sensitivity and specificity of early ultrasound examination and to compare ultrasound and genetic testing for early diagnosis. Sixty-three Persians and seven Exotic Shorthairs were considered. All underwent ultrasonographic and genetic testing (polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) assay) between 2.5 and 3.5 months of age (10e14 weeks). With ultrasound, 41.4% showed renal cysts, while 37.1% were PKD positive by genetic testing and DNA sequencing. Six cats with at least one renal cyst were negative by genetic testing, while only one cat negative at ultrasound resulted positive at genetic test. DNA sequencing of three polycystic cats, negative by genetic test, revealed they were heterozygous for the mutation. Agreement was described by Cohen's kappa that resulted 0.85, considering genetic test and DNA sequencing. Sensitivity and specificity of ultrasound were 96.2% and 91%, respectively. Sensitivity was higher and specificity lower than reported previously. The higher sensitivity could be due to improved technical capabilities of ultrasound machines and transducers. Other causes of PKD could explain the lower specificity. In conclusion, ultrasound resulted in a reliable diagnostic method for feline AD-PKD1 at early age and it should always be used with genetic testing, in order to reach a complete screening programme and eventually to identify other genetic mutations.

Domanjko-Petric *et al*., (2008) reported polycystic kidney disease (PKD) is an inherited autosomal kidney disease which is most commonly identifiedin Persian and Persian related cats. Positive cats have multiple cysts of various sizesthat occur in the renal cortex and medulla and occasionally in other abdominal organs. PKD often leads to renal failure which occurs from mid to late in life. Renalcysts can be diagnosed ultrasonographically after 7 weeks of age by an experienced ultrasonographer and a high-resolution machine. However, ultrasonography is nowbeing replaced by genetic screening. A total of 340 cats of variable breeds aged from 5 months to 18 years were ultrasonographically examined in the past 7 years at the University Veterinary Small Animal Clinic. Of these, 13.8% were PKD positive with very high prevalence in Persian cats (36%). There was no sex predilection identified. The C > A transversion at position 3284 on exon 29 of PKD1 gene, resulting in a stop mutation has been identified in the heterozygous state in eight affected cats examined (Persian breed). All heterozygous cats were also ultrasonographically positive.

Hege *et al*., (2001) reported case is about a 9-year-old male castrated Persian cat with chronic renal failure. After physical examination and ultrasonography polycystic kidney disease (PKD) was diagnosed. Various aspects of etiology, pathophysiology and diagnosis of PKD are discussed.

Reichle *et al*., (2002) described the ultrasonographic (US) and computed tomographic (CT) appearance of autosomal dominant polycystic kidney disease (ADPKD) in cats; to compare renal volume in cats with ADPKD (n = 5; mean age59 * 10 months)) and normal cats(n = 5; mean age 66 * 10 months) using 2 imagingmodalities, US and CT; and to calculate cyst volume using CT. Glomerularfiltration rate (GFR) was determined by 2 methods: 9Y"'Tc-diethylene- triaminepentaacetic acid (9Yn'T~- DPTA) scintigraphic uptake and 99mTc-DTPA plasma clearance. Sonographically, ADPKD affected kidneys were characterized by multiple anechoic to hypoechoic, round to irregularly shaped structures with variation in size. Affected kidneys had indistinct corticomedullary junctions and foci of

mineralization. Intravenous (IV) contrast medium administration allowed more definitive identification of cysts with CT, and identification of distortion of renal pelves by cysts. A significant difference (Welch ANOVA, P = 0.05) was detected between the US-estimated renal volumes of normal and affected cats. No statistically significant differences were detected in CT volume (between thenormal and affected cats, or between US and CT volume measurements) or the 2 GFR methods. In this group of clinically normal, middle-aged ADPKD cats, renalfunction was within normal limits and not significantly different than normal.

Vucicevic *et al*., (2016) observed polycystic kidney disease (PKD) is an inherited autosomal disorder in cats, mostly diagnosed in Persian cats. Renal cystscan 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 andgenetic 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.

Tavasolian *et al*., (2018) detected ultrasonography is an accurate and accessible method for polycystic kidney disease (PKD), an inherited autosomal dominant disease, and other urinary tract diseases. The present work is a preliminary study of PKD and urinary tract abnormalities using

ultrasonography in Persian and other long hair cats in Iran. This study was conducted on 83 cats including 68 Persian cats and 15 Persian related cats from December 2013 to March 2015. The age of cats ranged 3 to 72 months. Cats were classified as PKD-positive when at least one renal cyst was observed. Other urinary system abnormalities were recorded ultrasonographically. Association of personal and nutritional characteristics with PKD and other urinary tract disease detected by ultrasonography was statistically analyzed. The prevalence of PKD among Persian cats and in the total population was 33.80% and 31.30%, respectively. PKD was more prevalent among male cats compared to those in female cats. PKD occurrence was significantly more among cats fed by commercial dry foods compared to those fed by homemade foods. There was no significant association between PKD and age, hair color, eye color, related clinical signs and other kidney abnormalities in ultrasonographic findings. The prevalence of renal calculi, urine sediments and bladder calculus were 2.40%, 32.80% and 3.60%, respectively. Urine sediments were significantly raised with increasing age. Screening program is essential for on□time diagnosis of PKD and to plan therapeutic management and control of the disease.

Wills *et al*., (2009) observed that polycystic kidney disease (PKD) is the most prevalent inherited genetic disease in cats with Persian and Persian-related breeds predominantly affected. Diagnosis of PKD relied on ultrasound scanning until the recent development of the PKD gene test. However, gene testing has limitations as it will only identify the autosomal dominant form of PKD and not other forms of cystic kidney disease. Ultrasound scanning also has the advantage of being able to assessthe severity and progression of disease in PKD affected cats.The aim of this study was to demonstrate the repeatability of ultrasound scanning in the detection of PKD and to assess progression of the disease over time. This study demonstrated 100% repeatability of ultrasound scanning in the detection of PKD and has also demonstrated progression of disease in 75% of PKD positive cats assessed over a 1-year period.

Effect of Polycystic kidneys on haematobiochemical parameters in Persian cats

Biller *et al*., (1996) reported that olycystic kidney disease affected cats, the kidneys are enlarged and irregular, and renal failure develops after a variable number of years. Azotemia, hyperphosphatemia, isosthenuria, non-regenerative anaemia, and metabolic acidosis are present in affected cats with renal failure. The renal cysts are smooth, round, and anechoic on ultrasonography. At necropsy, there are multiple cysts of varying size in the cortex and medulla of both kidneys, and lymphoplasmacytic inflammation and interstitial fibrosis also may be present.

Guerra *et al*., (2019) obtained CBC within 1 hr of blood collection using a haematology analyzer (BC-2800Vet; Shenzhen Mindray Bio Medical Electronics). Evaluated parameters included red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, concentration, platelet count, and white blood cell count and differential. Air-dried Wright–Giemsa- stained blood films were performed immediately after blood collection. Differential white blood cell counts were performed manually by counting 100 nucleated cells per smear. Selected biochemical analytes in the serum were quantified using an automated spectrophotometer clinical wet chemistry analyzer (Liasys; Analyzer Medical System). The analytes included serum urea nitrogen, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), albumin, total protein (TP), phosphate and calcium. Sodium, potassium and chloride were measured using an ion-specific electrolyte analyzer (AVL-OMNI4- Roche).

Noori *et al*., (2019) studied 28 cats with PKD, 9 cats (32%) showed renal failure signs and all the patients, with the exception of one patient, died three months after admission. A Persian cat with uremia (Urea: 137 mg/dl and Creatinine 3.7 mg/dl), and mild anorexia survived more than two

years. This cat also had a persistent eosinophilia with unknown origin. In the present study, there was not any significant correlation between the presence of PKD and clinical signs.

Schirrer *et al.*, (2021) observed that laboratory findings are not specific, mainly indicating renal failure (azotemia, hyperphosphatemia, non-regenerative anemia, and proteinuria). However, clinical stages can be highly variable, as demonstrated in a recent study where several young animals presented azotemia with a remarkably high creatinine concentration, compared to older animals with less important values.

Phoon *et al.*, (2015) examined 6-year-old intact Persian cat for the primary complaint of inappetence and weight loss. Irregular surface of kidneys was palpated during physical examination. Abdominal radiograph findings were indicative of renomegaly. Ultrasonography revealed multiple anaechoic structures within the renal parenchyma. The cortex, medulla and renal pelvis were unable to be differentiated. Both radiographic and ultrasonographic findings were suggestive of polycystic kidney disease. Blood test revealed normochromic, normocytic anaemia with azotaemia whereas urinalysis findings were hypostenuria and proteinuria, consistent of chronic kidney disease due to polycystic kidney. Ultrasound is a useful antemortem diagnostic tool to diagnose polycystic kidney disease in cats.

Evaluation of PKD1 gene mutation with PCR

Domanjko-Petric *et al.*, (2008) reported positive cats have multiple cysts of various sizes that occur in the renal cortex and medulla and occasionally in other abdominal organs. PKD often leads to renal failure which occurs from mid to late in life. Renal cysts can be diagnosed ultrasonographically after 7 weeks of age by an experienced ultrasonographer and a high-resolution machine. However, ultrasonography is now being replaced by genetic screening. A total of 340 cats of variable breeds aged from 5 months to 18 years were ultrasonographically examined

in the past 7 years at the University Veterinary Small Animal Clinic. Of these, 13.8% were PKD positive with very high prevalence in Persian cats (36%). There was no sex predilection identified. The C > A transversion at position 3284 on exon 29 of PKD1 gene, resulting in a stop mutation has been identified in the heterozygous state in eight affected cats examined (Persian breed).

Gendron *et al*., (2013) studied all cats with renal cysts were homozygous wild-type at the variant causing AD-PKD in Persian cats (PKD1: c.10063C>A). Thus, the ultrasonographical changes seen in the Maine Coons are not caused by the known AD-PKD allele. A pedigree analysis of the cats with renal changes revealed familial clustering indicating a genetic cause. Male and female cats were equally affected suggesting an autosomal mode of inheritance. Three Maine Coons affected with renal cysts from two litters were offspring of non-affected parents, excluding a simple dominant mode of inheritance. \

Sato *et al*., (2019) described feline polycystic kidney disease (PKD), an inherited autosomal dominant disease, has been reported to occur mostly in Persian or Persian related cats, and to be associated with a mutation from C to A at position 10063 in exon 29 of the feline PKD1 gene (PKD1 mutation). Many clinical cases have been recognized in Japan, but the mutation rate in cats has not been reported. The objective of this study was to determine epidemiological characteristics and clinical features in cats with the PKD1 mutation. Referring veterinarians sent blood samples of 377 cats for the PKD1 gene evaluation. The blood samples were from 159 cats with renal cysts confirmed by ultrasonography, 60 cats without renal cysts, and 158 cats that did not undergo ultrasonography. In total, 150 cats carried the PKD1 mutation and the signalment, site and number of renal cysts, and results of blood test were evaluated in cats with the PKD1 mutation. The breeds with the highest rate of the PKD1 mutation were Persian (46%), Scottish Fold (54%) and American Shorthair cats (47%). However, mixed breed cats also showed high rates of the PKD1 mutation. Of cats with the mutation, the incidence of high plasma creatinine (≥1.6 mg/dl) was greater

in cats ≥3 years old, although a few cats ≥9 years of age had low plasma creatinine creatinine (≥1.6 mg/dl).The coincidence of renal and hepatic cysts was 12.6%, with the high prevalence in Persian cats (31%).

Helps *et al*., (2007) observed Autosomal-dominant polycystic kidney disease (AD-PKD) is the most prevalent inherited genetic disease of cats, particularly affecting Persians. Until recently the condition has been diagnosed by renal ultrasound screening. With the identification of the genetic mutation responsible for AD-PKD it is now possible to use advanced molecular techniques to screen for the disease. We have developed a rapid, sensitive and specific real□time PCR genotyping assay that can detect the single nucleotide polymorphism responsible for AD-PKD. Of 72 UK Persian and Exotic Shorthair cats submitted for AD-PKD ultrasound screening, 29 were found to have the disease, 41 were negative and 2 were equivocal. The recently published PCR-RFLP method showed the AD-PKD mutation to be present in all 29 diseased cats and absent in the 41 negative and 2 equivocal cats. Our real-time PCR genotyping assay was in complete agreement with the PCR-RFLP results. Of 600 blood or buccal swabs analysed from April 2005 to January 2006, 165 were found to be AD-PKD positive and 435 were negative, giving a prevalence of 27.5%. All 194 cats with AD-PKD were found to be heterozygous for the mutation.

Scalon *et al*., (2014) described a newly developed touchdown polymerase chain reaction (PCR) to detect this single point mutation, using 2 primers specific for the mutant allele, adapted from an existing multiplex amplification refractory mutation system (ARMS PCR). Furthermore, correlations between the clinical outcomes of tested animals and the results of the genetic test were investigated. A total of 334 cats were tested, 188 from the Veterinary Hospital of Small Animals at the University of Brasilia, and 146 from an anti-rabies vaccine campaign of the Federal District. A total prevalence of 9% was evident among the samples, with 33% of the Persian cats testing positive, and 7% of the Brazilian long- and shorthaired cats testing positive. Prevalence was not correlated with gender or hemogram. Positive animals exhibited hyperglobulinemia (P = 0.02). This research

demonstrated that the mutation does not only occur in Persian and Persian-related cats, and that a touchdown PCR can be used to diagnose ADPKD.

Bilgen *et al*., (2020) screened 16 cats from various breeds exhibiting a renal abnormality by ultrasound examination and genotyped them for the c.10063C>A transversion on exon 29 of the polycystin-1 (PKD1) gene, by PCR–restriction fragment length polymorphism (PCR-RFLP). Among these cats, a Siamese nuclear family of 4 cats with ancestral hereditary renal failure were screened by whole- genome sequencing (WGS) to determine novel variations in genes associated with both AD and autosomal recessive PKD in humans. During the study period, one cat died as a result of renal failure and was forwarded for autopsy. Additionally, we screened 294 cats asymptomatic for renal disease (Angora, Van, Persian, Siamese, Scottish Fold, Exotic Shorthair, British Shorthair, and mixed breeds) to determine the prevalence of the mutation in cats in Turkey. Ten of the symptomatic and 2 of the asymptomatic cats carried the heterozygous C → A transversion, indicating a prevalence of 62.5% and 0.68%, respectively. In the WGS analysis of 4 cats in the Siamese nuclear family, novel variations were determined in the fibrocystin gene (PKHD1), which was not compatible with dominant inheritance of PKD.

Grahn *et al*., (2004) noted PKD1- and PKD2-containing clones were identified from RPCI-86 feline BAC library using overgo probing.5 Sequence of the PKD1 BAC clone (GenBank accession number AC145332.26) was obtained by the University of Oklahoma's Advanced Center for Genome Technology (Norman, OK, USA). Microsatellite primers developed from the sequence are PKD1UCD1F: 5¢-TTAAGCATTTACCCGGCATC-3¢ and PKD1UCD1R: 5¢-CATCCAGTCCAGTCCTTGT-3¢; PKD1UCD2F: 5¢-GCAGGGACTGTGAGGTTG-3¢ and PKD1UCD2R: 5¢-GCAGGGACTGTGAGGTTG-3¢. Sub-clones of the PKD2 clone were screened with a (CA)17 probe. Primer sequences for the PKD2- derived microsatellite (GenBank accession number AY727857) are PKD2UCD1F: 5¢- GGAAATGCCAAAAACCAGTG-3¢ and PKD2UCD1 R: 5¢-

TGGGTCTCTAGTCTGCTGTATGA-3¢. PKD1- UCD2 and PKD2UCD1 have (CA)16 and (CA)19 repeats, respectively, whereas PKD1UCD1 is a (TTG)10 repeat.

Bonazzi *et al*., (2009) evaluated the sensitivity and specificity of early ultrasound examination and to compare ultrasound and genetic testing for early diagnosis. Sixty- Persians andseven Exotic Shorthairs were considered. All underwent ultrasonographic and genetic testing (polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) assay) between 2.5 and 3.5 months of age (10e14 weeks). With ultrasound, 41.4% showed renal cysts, while 37.1% were PKD positive by genetic testing and DNA sequencing. Six cats with at least one renal cyst were negative by genetic testing, while only one cat negative at ultrasound resulted positive at genetic test. DNA sequencing of three polycystic cats, negative by genetic test, revealed they were heterozygous for the mutation. Agreement was described by Cohen's kappa that resulted 0.85, considering genetic test and DNA sequencing. Sensitivity and specificity of ultrasound were 96.2% and 91%, respectively. Sensitivity was higher and specificity lower than reported previously. The higher sensitivity could be due to improved technical capabilities of ultrasound machines and transducers. Other causes of PKD could explain the lower specificity. In conclusion, ultrasound resulted in a reliable diagnostic method for feline AD-PKD1 at early age and it should always be used with genetic testing, in order to reach a complete screening programme and eventually to identify other genetic mutations.

Kappe *et al*., (2005) reported a sample of the German Persian cat population and seven Exotic Shorthair cats were scanned for the prevalence of this presumptive PKD causative mutation. 116 Persian cats including four families and seven Exotic Shorthair cats were examined for polycystic kidney disease using ultrasonography. DNA samples of all cats were used for further investigations. The identified mutation causes a second restriction site. So PCR products of mutated and normal alleles can be differentiated by RFLP analysis. None of the 58 unaffected cats displayed

the mutation. 95% of the affected Persian cats and all three affected Exotic Shorthair cats showed the mutation. In all these cases, the mutation was found in the heterozygous state. The ultrasonographic examination and genetic testing are concordant in 97.4%. These data support the assumption that the stop mutation causes most cases of feline PKD. The homozygous mutation seems to be a lethal factor. Not all PKD affected cats have the investigated mutation. In these cases, the disease might be caused by another mutation.

Materials and Methods

Materials and Method

The present study was conducted on Persian cats referred to the Department of Veterinary Epidemiology and Preventive Medicine, Mumbai Veterinary College, Parel and the Cats presented at the medical ward of Bai Sakarabai Dinshaw Petit Hospital for Animals (BSDPHA) affiliated to Mumbai Veterinary College, Parel, Mumbai- 400012 from March 2023 to September 2024.

3.1 Statutory permission to undertake the research work

The present study was undertaken after the approval from the Board of Studies in Veterinary Epidemiology and Preventive Medicine, Resolution no:VEPM/00/2023 and Subject no: VEPM /02/2023. Dated: 20/07/2023.

3.2 Selection of animals

3.2.1 Inclusion Criteria

Persian cats of age > 6 months of any sex will be included.

3.2.2 Exclusion Criteria

Persian cats of age < 6 months will be excluded from the study.

3.3 Prevalence

The prevalence of Polycystic Kidney Disease was calculated on the basis of cysts in kidneys during study period i.e. from September 2023 to February 2024.

The prevalence Polycystic Kidney Disease of was calculated on the basis of following formula

$$\text{Prevalence (\%)} = \frac{\text{Total number of persian cats positive for Polycystic Kidney Disease during study period}}{\text{Total number of persian cats presented during study period}} \times 100$$

The prevalence study was done on the basis of age, sex, cysts present in kidneys in persian cats.

3.4 Parameters understudy

The following parameters were undertaken: (Appendix I, case record sheet)

1. Patient details (age, sex, breed etc.)
2. Complete blood count (CBC).
3. Liver function and kidney function tests (LFT and KFT).

3.5 Collection of Blood

On the day of presentation, 2ml blood sample was collected in EDTA tube (EDTA K3 Tube) for hematological analysis, 2ml blood in clot activator tube for biochemical analysis and serological test.

Following tests were conducted on collected blood samples:

3.5.1 Haematological Examination

2 ml blood collected in EDTA K3 tubes was analyzed for complete blood count by a fully automated Mindray BC-3000 haemoanalyzer. Parameters studied were:

1. Haemoglobin (Hb, gm %)
2. Packed Cell Volume (PCV, %)
3. Total Erythrocytic count (TEC, $\times 10^6$/cmm)
4. Mean Corpuscular Volume (MCV, fl)
5. Mean Corpuscular Hemoglobin (MCH, pg)
6. Mean Corpuscular Hemoglobin Concentration (MCHC, gm/dl)
7. Total Leucocyte Count (TLC, $\times 10^3$/cmm)
8. Differential Leucocytic Count (DLC):

a) Neutrophils (N %),
b) Basophils (B, %),
c) Eosinophils (E, %),
d) Monocyte (M, %),
e) Lymphocyte (L, %)

9. Platelets (PLT, lacs/cmm)

10. Reticulocyte count (%) (Using brilliant cresyl blue staining technique)

3.5.2 Biochemical examination

Blood samples collected in a plain vial were analyzed for biochemical parameters on semi-automated and fully automated analyzer ARX-100 & FA200 using Erba Manheim kits.

I. Liver function tests

i. Total Bilirubin (TB, mg/dl) (Diazo method, end point)
ii. Indirect bilirubin (DB, mg/dl), (Diazo method, end point)
iii. Direct bilirubin (IB, mg/dl), (Diazo method, end point)
iv. SGOT (IU/L), (IFCC recommended methodology)
v. SGPT (IU/L), (IFCC recommended methodology)
vi. ALP (IU/L), (DGKC-SCE recommended procedure)
vii. Total protein (TP, gm/dl), (Direct Biuret method)
viii. Albumin (gm/dl), Globulin (gm/dl), (Bromocresol green methodology)
ix. Albumin:Globulin (A/G)

II. Kidney Function Tests

i. Serum Blood Urea Nitrogen (BUN, mg/dl), (Urease/GLDH methodology)
ii. Serum Creatinine (mg/dl). (Modified Jaffe's method)

3.6 Confirmative Diagnosis

3.6.1 Ultrasonographic Examination

Persian cats were subjected to ultrasonographic evaluation and following ultrasonographic parameters were studied:

a) No of cysts present in both kidneys

3.6.2 Restraining and positioning of the animals

All cats were examined in the conscious state without the use of any medication or sedative. Cats were placed on both recumbencies i.e., right and left recumbency for evaluation of left and right kidney respectively as well sometimes dorsal recumbency particularly for examination of the right kidney as per necessity.

3.6.3 Instrumentation

a) The animals were scanned using an ultrasonographic machine named SONOSCAPE S2V with the help of a convex transducer (curvilinear transducer) of frequency 5-8 MHz mostly, however, if required convex transducer (curvilinear transducer) of frequency 7-10 MHz was also used for a clear view of the image. i.e change in frequency, gain, depth, and section width was done as per requirement.

b) Ultrasound gel was applied liberally over the entire abdominal skin to ensure intimate contact of the transducer head with the body surface.The images on the monitor were frozen, different measurements were recorded and selected prints were taken on ultrasonographic paper: The images were interpreted and various morphometric measurements were analyzed to find out interferences.

3.6.3 Sonographic Protocol

a) Kidney

In all animals, regardless of their age, sex, or size, the left kidney was scanned first, followed by the right kidney, the abdominal aorta, caudal vena cava, and urinary bladder. For renal sonography, the animals were restrained on dorsal recumbency or left/right lateral recumbency. Ultrasound gel was applied liberally on the skin. Grayscale, B-mode, real-time scanner 5-8 MHz convex transducer was used mostly sometimes 7-10 MHz for increasing the resolution for scanning of kidneys. The left kidney was examined with the transducer in contact with the ventral abdominal wall or flank caudal to the last ribs. The spleen was used as an acoustic window, to scan the left kidney. The transducer was directed caudolaterally. The right kidney was scanned in dorsal

recumbency by placing the transducer caudal to the right costo spinal angle. It was also scanned on left lateral recumbency, by placing the transducer in the middle of the last intercostals space the scan beam directing caudally in necessity. Movement of the transducer at these locations assisted in targeting the impulse from the transducer on the kidneys which appeared on the B-mode monitor. A clear image of the kidney on the monitor was frozen, and distance measurement mode was activated to measure the renal dimensions in centimeter (cm) for both right and left kidneys, A clear image of the kidney on the monitor was frozen, and distance measurement mode was activated to measure the renal dimensions in centimeter (cm) for both right and left kidneys. The entire kidney was scanned on sagittal /longitudinal planes and its echo texture, anatomical location, and size were recorded. The following parameters were recorded:

a) Kidney length: a minimum of three measurements of kidney length (both left and right) in the sagittal plane were recorded to calculate the mean for further ata analysis.

b) Kidney width: minimum of three measurements of kidney width (both left and right) sagittal plane were recorded to calculate mean for further data analysis.

c) Cortical thickness: cortical thickness was measured at a minimum of three different locations in an image in the sagittal plane and recordings were used for further data analysis in each kidney.

d) Medullary thickness: medullary thickness was measured at three different locations in an image in the sagittal plane and recordings were used for further data analysis in each kidney.

3.6 POLYMERASE CHAIN REACTION

- Collection of blood samples

About 2 ml of venous blood sample was collected through

cephalic or sephanous vein puncture from each persian cat in EDTA vacutainer tube. The blood samples were immediately transported to the laboratory at 4 °C and genomic DNA was isolated within 24 hrs.

The plasticware used was acquired from M/s Tarsons (Kolkata, India), Genaxy Scientific Pvt. Ltd. All the instruments used were either procured pre-sterilized or sterilized before use by wrapping appropriately and autoclaving at 121˚C, 15lbs for 45 mins. Neutral glasswares from M/s Borosil (India) were used after being treated with 1% HCl overnight and then rinsed thoroughly with distilled water.

All reagents and equipment used for detection of PKD1 gene mutation in polycystic kidney disease by are listed in Appendix II.

3.6.1 DNA EXTRACTION

Qiagen Kit Method

Protocol: DNA Purification from Blood or Body Fluids (Spin Protocol) This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes, and body fluids using a microcentrifuge. Equilibrate samples to room temperature (15–25°C). Heat a water bath ors heating block to 56°C for use in step 4. Equilibrate Buffer AE or distilled water to room temperature for elution in step 11. Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on page 20. If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

Procedure

1) Pipet 20 µL QIAGEN Protease (or proteinase K) into the bottom of a 1.5 mL microcentrifuge tube.

2) Add 200 μL sample to the microcentrifuge tube. Use up to 200 μL whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 106 lymphocytes in 200 μL PBS. If the sample volume is less than 200 μL, add the appropriate volume of PBS. QIAamp Mini spin columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 μL of an RNase A stock solution (100 mg/mL) should be added to the sample before addition of Buffer AL. Note: It is possible to add QIAGEN Protease (or proteinase K) to samples that have already been dispensed into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.
3) Add 200 μL Buffer AL to the sample. Mix by pulse-vortexing for 15 s. To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. If the sample volume is larger than 200 μL, increase the amount of QIAGEN Protease (or proteinase K) and Buffer AL proportionally; for example, a 400 μL sample will require 40 μL QIAGEN Protease (or proteinase K) and 400 μL Buffer AL. If sample volumes larger than 400 μL are required, use of QIAamp DNA Blood Midi or Maxi Kits is recommended; these can process up to 2 mL or up to 10 mL of sample, respectively. Note: Do not add QIAGEN Protease or proteinase K directly to Buffer AL.
4) Incubate at 56°C for 10 min. DNA yield reaches a maximum after lysis for 10 min at 56°C. Longer incubation times have no effect on yield or quality of the purified DNA.
5) Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
6) Add 200 μL ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid. If the sample volume is greater than 200 μL, increase the amount of ethanol proportionally; for example, a 400 μL sample will require 400 μL of ethanol.

7) Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2 mL collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 mL collection tube (provided), and discard the tube containing the filtrate. Close each spin column to avoid aerosol formation during centrifugation. Centrifugation is performed at 6000 x g (8000 rpm) to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Mini spin column is empty. Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.
8) Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 mL collection tube (provided), and discard the collection tube containing the filtrate.It is not necessary to increase the volume of Buffer AW1 if the original sample volume is larger than 200 µL. Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach.
9) Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
10) Recommended: Place the QIAamp Mini spin column in a new 2 mL collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. This step helps to eliminate the chance of possible Buffer AW2 carryover.
11) Place the QIAamp Mini spin column in a clean 1.5 mL microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µL Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min. Incubating the QIAamp Mini spin column loaded with Buffer AE or water for 5 min at room

temperature before centrifugation generally increases DNA yield. A second elution step with a further 200 µL Buffer AE will increase yields by up to 15%. Volumes of more than 200 µL should not be eluted into a 1.5 mL microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation. Elution with volumes of less than 200 µL increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. For samples containing less than 1 µg of DNA, elution in 50 µL Buffer AE or water is recommended. Eluting with 2 x 100 µL instead of 1 x 200 µL does not increase elution efficiency. For long-term storage of DNA, eluting in Buffer AE and storing at –30 to –15°C is recommended, since DNA stored in water is subject to acid hydrolysis. A 200 µL sample of whole human blood (approximately 5 x 106 leukocytes/mL) typically yields 6 µg of DNA in 200 µL water (30 ng/µL) with an A260/A280 ratio of 1.7–1.9.

i. The provided protocol outlines the steps for DNA extraction using the QIAamp Mini Kit from QIAGEN. Here's a summarized version of the protocol:

Materials Required:

- QIAamp Mini Kit
- QIAGEN Protease (or proteinase K)
- Buffer AL
- Ethanol (96–100%)
- Buffer AW1
- Buffer AW2
- Buffer AE or distilled water
- Microcentrifuge tubes (1.5 mL)
- Pipettes and tips
- PBS (if needed)
- RNase A stock solution (if RNA-free genomic DNA is required)

Protocol:

1. Pipet 20 µL QIAGEN Protease (or proteinase K) into a 1.5 mL microcentrifuge tube.
2. Add 200 µL sample (whole blood, plasma, serum, buffy coat, body fluids, or lymphocytes in PBS) to the tube. Adjust volume with PBS if necessary.
3. Add 200 µL Buffer AL to the sample and mix thoroughly by pulse-vortexing for 15 s.
4. Incubate at 56°C for 10 min.
5. Briefly centrifuge to remove drops from the inside of the lid.
6. Add 200 µL ethanol (96–100%) to the sample, mix by pulse-vortexing for 15 s, and centrifuge briefly.
7. Apply the mixture to the QIAamp Mini spin column and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the filtrate tube.
8. Add 500 µL Buffer AW1 to the column, centrifuge at 6000 x g (8000 rpm) for 1 min, and discard the filtrate tube.
9. Add 500 µL Buffer AW2 to the column, centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
10. Place the column in a new tube and centrifuge at full speed for 1 min.
11. Place the column in a clean microcentrifuge tube, add 200 µL Buffer AE or distilled water, incubate, and centrifuge at 6000 x g (8000 rpm) for 1 min.

Additional Notes:

1) Proper mixing and centrifugation are crucial for efficient DNA extraction.
2) Adjust volumes proportionally for larger sample sizes.
3) Follow recommendations for elution volume based on desired DNA concentration and storage conditions.

4) Ensure proper disposal of waste materials to prevent contamination.
5) Maintain appropriate storage conditions for extracted DNA to preserve integrity.

3.6.2 Quantification of DNA

DNA concentration was determined by both quantitative analysis on Nano drop and qualitative analysis on Agarose gel.

3.6.3 PCR Test Reactions

Primer gene	
Forward Primer Sequence (5’-3’)	**CAGGTAGACGGGATAGACGA**
Reverse Primer Sequence	**TTCTTCCTGGTCAACGACTG**

(Guerra *et al* 2019)

A 559 base pair (bp) PCR fragment containing exon 29 was amplified using the same pair of primers.

The chemicals required for PCR reactions test were thawed on ice and added in PCR vials in following order.

DNA Template	1 µl
Forward Primer	1 µl
Reverse Primer	1 µl
PCR Master Mix	12.5 µl
Nuclease Free Water	9.5 µl

PCR reaction cycle were set as follows

1) Initial Denaturation at 94^0 C for 3 min.

2) Denaturation at 94^0 C for 1 min.
3) Primer Annealing at 58^0 C for 1 min.
4) Extension at 72^0 C for 1 min.
5) Steps 2 to 4 were repeated 35 times and then Final extension at 72^0 C for 1 min.

In the experimental procedure, an amplification product of 559 base pairs corresponding to exon 29 was obtained.

A mutation was identified which alters a restriction enzyme site for MLY1, resulting in the production of two fragments, one of 316 base pairs and the other of 243 base pairs upon digestion.

Approximately 5 microliters of the amplification product were subjected to digestion using 10 units of MLY1 enzyme in a 10 microliter reaction containing 1X TAE Buffer.

The digestion was carried out at 37°C for 3 hours followed by enzyme inactivation at 65°C for 10 minutes. The entirety of the digestion reaction was then analyzed on 1.8 to 2% agarose gels.

3.6.3 Visualization of PCR products by Agarose gel electrophoresis:

PCR amplicons were electrophoresed on agarose gel (two per cent) and the size of electrophoresed amplicons was determined by comparing with 100 bp DNA ladder. The procedure for agarose gel electrophoresis is as mentioned here under:

1. The required amount of agarose (0.6 grams) was dissolved in 1X TBE buffer (30 ml) in glass conical flask and subjected to low energy microwave heat.Care was taken not to overheat or to avoid vaporization of buffer, so as to obtain a final concentration of two per cent gel.
2. The agarose solution was allowed to cool down below 60 °Cand ethidium bromide (0.5 μg/ml)was added and mixed thoroughly, poured into

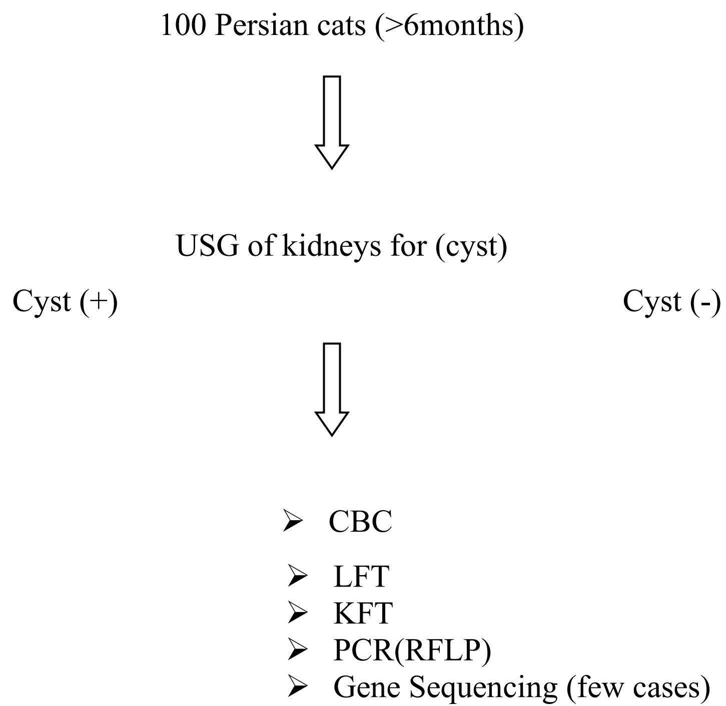
asealed gel tray and the appropriate sized comb was inserted for formation of wells.

3. Gel was allowed to set at room temperature for period of 30-45 min; later both comb and sealing tape were removed carefully from the tray. The gel tray was submerged in a submarine electrophoresis tank and adequate amount of freshly prepared TBE buffer was added.
4. Five micro liter of PCR product was mixed with 2 µl of loading dye and electrophoresed at 100 V for 30 min in 1X TBE buffer.
5. DNA ladder(100 bp) was also loaded and ran alongside the PCR products.
6. After half an hour, the power supply was turned off and the lid was removed from the gel tank. The gel was visualized under UV trans-illuminator, and presence of a single compact band of expected sized PCR amplicons were ascertained and photographed using Gel Documentation System (Bio rad Molecular imager Gel Doc XR+, USA)

3.7 Analysis of Data

The data gathered in the research plan was subjected to statistical analysis to determine whether the results were of significance or not, by methods illustrated by Snedecor and Cochran (2014) by using WASP 2.0 software. Microsoft Excel was used to determine significance in recorded data via Two Sample T-test.

FLOW CHART OF WORK



Results and Discussion

RESULT AND DISCUSSION

The current study entitled " PREVALENCE OF POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN AND AROUND MUMBAI" involved 100 Persian cats who were chosen based on the inclusion and exclusion criteria outlined in the materials and methods section. The blood samples were collected and subjected for hematological, biochemical parameters.The ultrasonography was carried out at the Ultrasonography Unit of Animal Reproduction, Gynaecology, and Obstetrics, Mumbai Veterinary College, Parel, Mumbai. Detection of PKD gene mutation by PCR was carried out at department of Animal Genetics & Breeding.The study covered cases reported to the outpatient department (OPD), Mumbai Veterinary College, and inpatient cases admitted to Bai Sakarabai DiNShaw Petit Hospital for Animals (BSDPHA), Parel, Mumbai. The findings of these investigatioNS have been documented, discussed thoroughly and are shown here:

4.1 ULTRASONOGRAPHY

In feline polycystic kidney disease (PKD) diagnosis, imaging tests are pivotal. For advanced cases with multiple large cysts, ultrasound emerges as the most successful examination method.

Among the 100 cats that underwent ultrasonographic evaluation, it was found that renal cysts were present in 9 Persian cats. The distribution of cysts observed in cats affected by Polycystic Kidney Disease (PKD) can be compared with the findings from Guerra (2019), which established that the optimal threshold for discriminating between ADPKD and non-ADPKD animals was three cysts in one or both kidneys. This comparison may provide iNSights into the diagnostic accuracy and relevance of the observed cystic burden in the context of PKD in feline populatioNS.Ultrasound shows cysts present in the kidneys Plate 1.

The number of cysts observed in cats affected by Polycystic Kidney Disease (PKD) is illustrated in Table no.4.1& Fig 1

Table 4.1 Cysts in kidneys on USG (n=9)

PKD cats	Cyst in kidneys		TOTAL
	RK	LK	
1	1	3	4
2	2	2	4
3	3	1	4
4	2	1	3
5	2	1	3
6	1	1	2
7	2	2	4
8	2	2	4
9	1	3	4
	MEAN±SE		3.55±1.19

Correlating this data with Guerra's findings (2019) is iNSightful. Guerra determined that a threshold value of three cysts present in either one or both kidneys could effectively distinguish between individuals with Autosomal Dominant Polycystic Kidney Disease (ADPKD) and those without, achieving optimal seNSitivity and specificity. In the context of the presented dataset, the mean cyst count of 3.55 suggests that, on average, the cats exhibit a cyst burden coNSistent with PKD. Moreover, the standard deviation of 1.19 highlights the variability in cyst counts among the PKD-affected feline cohort, underscoring the heterogeneity of PKD presentation.

According to Guerra et al. (2019), ultrasound is a non-invasive, widely accessible, safe, cost-effective modality adept at identifying kidney cysts. These cysts typically manifest as hypo- to anechoic spherical cavities, occasionally exhibiting contrast enhancement, and varying in size from one to over twenty millimeters.

Lee et al. (2010) suggests that supplementing renal ultrasounds with liver ultrasounds can improve diagnostic accuracy.

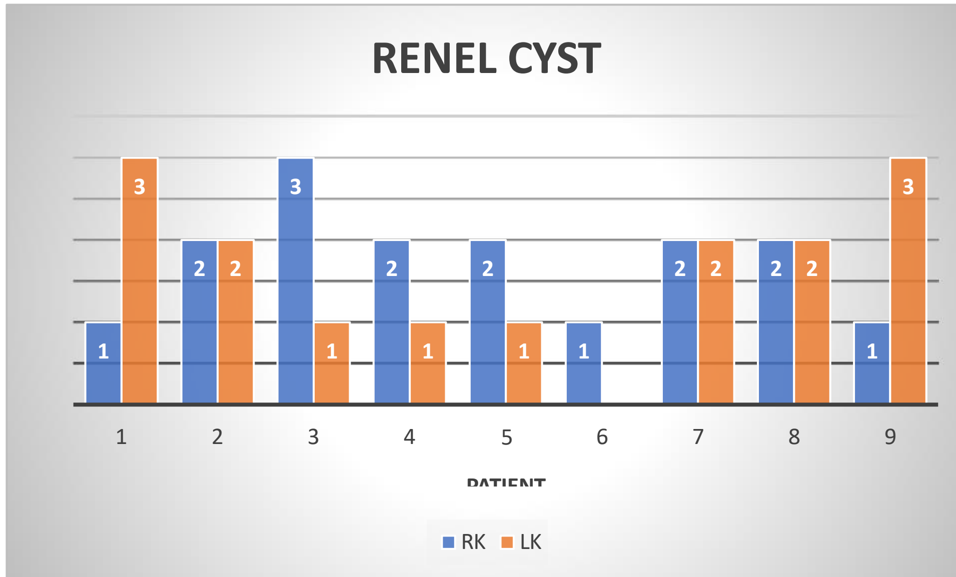


Fig 1 cyst wise distribution of PKD affected cats

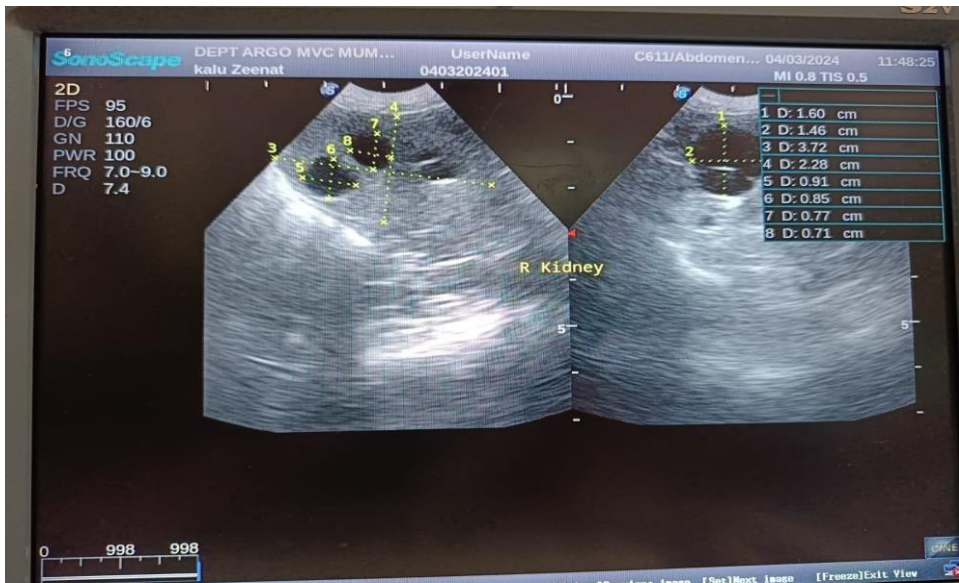


Plate 1.USG image of Cysts in kidney

Ultrasound demoNStrates seNSitivity, specificity, and repeatability levels ranging around 91–96.2%, 91–100%, and 100%, respectively, as reported by Bonazzi (2007) and Barr (2001).

Clinical recommendatioNS advocate for ultrasound examinatioNS to be conducted by specialist veterinariaNS utilizing high-resolution ultrasound machines equipped with a 7.5–12 MHz multifrequency linear traNSducer, as highlighted by Cooper *et al.,* (2000), Bonazzi *et al.,* (2007, 2009), and Noori *et al.,*(2019).

Ultrasound emerges as a valuable diagnostic modality for detecting polycystic kidney disease (PKD) in cats, as emphasized by Phoon (2005) and Beck and levelle *et al.,*(2001), who diagnosed feline PKD using ultrasonography in Persian cats, reporting a prevalence of 45% in their study.

In a study by Lee *et al.,* (2010), only a subset of cats underwent both ultrasonographic and genetic examinatioNS, with limited overlap between the two groups. Specifically, out of 15 cats diagnosed with the PKD1 gene mutation, only 11 underwent ultrasonography.

Cannon *et al.,* (2001) highlighted the utility of high-definition ultrasound for diagnosing PKD, noting its ability to detect fluid-filled cysts within the renal parenchyma.

A comparative study by Bonazzi (2009) evaluated ultrasound and genetic testing for early PKD diagnosis in cats, including 63 PersiaNS and 7 Exotic Shorthairs.

The study revealed that ultrasound identified renal cysts in 41.4% of cats, while genetic testing and DNA sequencing identified PKD in 37.1% of cats. Ultrasound demoNStrated a seNSitivity of 96.2% and specificity of 91%.

Bonazzi *et al.,* (2009) recommended using ultrasound in conjunction with genetic testing for compreheNSive screening of PKD in cats.

4.2 Haematology

The mean hematological values of Persian cats affected by polycystic kidney disease (PKD) and PKD -ve counterparts are summarized in Table 4.2.

There were no significant differences ($P < 0.05$) observed in the mean values of hemoglobin (10.79±1.58 vs 11.37±0.35 g/dl), packed cell volume (32.12±3.99 vs 34.45±1.04 %), and total erythrocyte count (7.36±0.88 vs 7.32±0.23 $\times 10^6$/ µl) between PKDaffected and PKD -ve Persian cats.

However, no significant reduction ($P < 0.05$) in mean corpuscular volume (MCV) was noted in affected cats compared to PKD -ve ones (43.54±2 vs 47.39±1 fl). Conversely, no significant differences ($P < 0.05$) were observed in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values between PKD-affected and PKD -ve Persian cats.

Biller et al. (1996) proposed the presence of non-regenerative anemia, azotemia, hyperphosphatemia, isosthenuria, and metabolic acidosis in affected cats with renal failure, aligning with the hematological findings observed in this study.

Regarding the leukogram, no significant changes ($P < 0.01$) were noted in total leukocyte count (20.5±3.52 vs 17.33±1.26 x 10^3/ µl) and neutrophil count (74.44±6.11 vs 69.67±1.64 %) between PKD-affected and PKD -ve Persian cats.

Furthermore, there were no significant differences observed in platelet counts (2.29±0.27x 10^3/ µl vs 2.37±0.09 x10^3/ µl) between PKD-affected cats and PKD -ve Persian cats.

These hematological findings provide valuable iNSights into the physiological changes associated with PKD in Persian cats and may aid in the diagnosis and management of the disease.

Table 4.2 Mean haematological values in PKD -ve and Diseased persian cats

SR. NO	Parameters	MEAN±SE (PKD -ve)	MEAN±SE (Diseased)	T value	Table T (p≤0.05)	Table T (P≤0.01)
1.	Hb (gm%)	11.37±0.35	10.79±1.58	0.474 ^{NS}		
2.	TEC ($\times10^6$ /µl)	7.32±0.23	7.36±0.88	0.049 ^{NS}		
3.	PCV (%)	34.45±1.04	32.12±3.99	0.658 ^{NS}		
4.	RDW	20.03±0.67	22.74±3.4	0.785 ^{NS}		
5.	MCV (fl)	47.39±1	43.54±2	1.185 ^{NS}		
6.	MCH (pg)	16.53±0.56	14.37±0.54	1.198 ^{NS}		
7.	MCHC (g/dl)	32.72±0.34	33.29±1.52	0.486 ^{NS}	1.984	2.627
8.	TLC ($\times10^3$/µl)	17.33±1.26	20.5±3.52	0.76 ^{NS}		
9.	Neutrophils (%)	69.67±1.64	74.44±6.11	0.861 ^{NS}		
10.	Eosinophils (%)	3.47±0.75	0.67±0.37	1.168 ^{NS}		
11.	Lymphocytes (%)	25.88±1.62	23.11±5.95	O.506 ^{NS}		
12.	Monocytes (%)	1.59±0.11	1.78±0.28	0.529 ^{NS}		
13.	Basophils (%)	0±0	0±0	0	0	0
14.	Platelets ($\times10^3$/µl)	2.37±0.09	2.29±0.27	0.233 ^{NS}	1.984	2.627

^{NS} – *Non significant* * - *Significant (P<0.05)* ** - *Highly significant (P < 0.01)*

Table 4.3 Mean biochemical values in PKD -ve and Diseased Persian cats

SR. NO	Parameters	MEAN±SE (PKD -ve)	MEAN±SE (Diseased)	T value	Table T ($p \leq 0.05$)	Table T ($P \leq 0.01$)
1.	Bilirubin (Total) (mg/dl)	0.86±0.22	0.66±0.15	0.284 ^{NS}	1.984	2.627
2.	Bilirubin (Direct) (mg/dl)	0.47±0.14	0.25±0.08	0.508 ^{NS}		
3.	Bilirubin (Indirect) (mg/dl)	0.39±0.08	0.4±0.07	0.062 ^{NS}		
4.	SGOT (IU/L)	80±11.53	41.92±6.01	1.033 ^{NS}		
5.	SGPT (IU/L)	106.7±16.43	41.38±4.61	1.244 ^{NS}		
6.	ALP (IU/L)	93.75±21.76	35.39±8.31	0.839 ^{NS}		
7.	Total Protein (g/dl)	6.76±0.10	6.97±0.52	0.395 ^{NS}	2.294	3.328
8.	Albumin (g/dl)	2.77±0.05	2.59±0.16	0.978 ^{NS}	1.984	2.627
9.	Globulin (g/dl)	4±0.10	4.38±0.51	0.739 ^{NS}	2.293	3.325
10	A/G Ratio	0.74±0.03	0.64±0.06	1.051 ^{NS}	1.984	2.627
11	BUN (mg/dl)	26.2±1.13	86.1±24.44	2.445*	2.305	3.352
12	Creatinine (mg/dl)	1.13±0.04	4.57±0.93	3.687**	2.305	3.354

^{NS} *– Non significant* * *- Significant ($P<0.05$)* ** *- Highly significant ($P < 0.01$)*

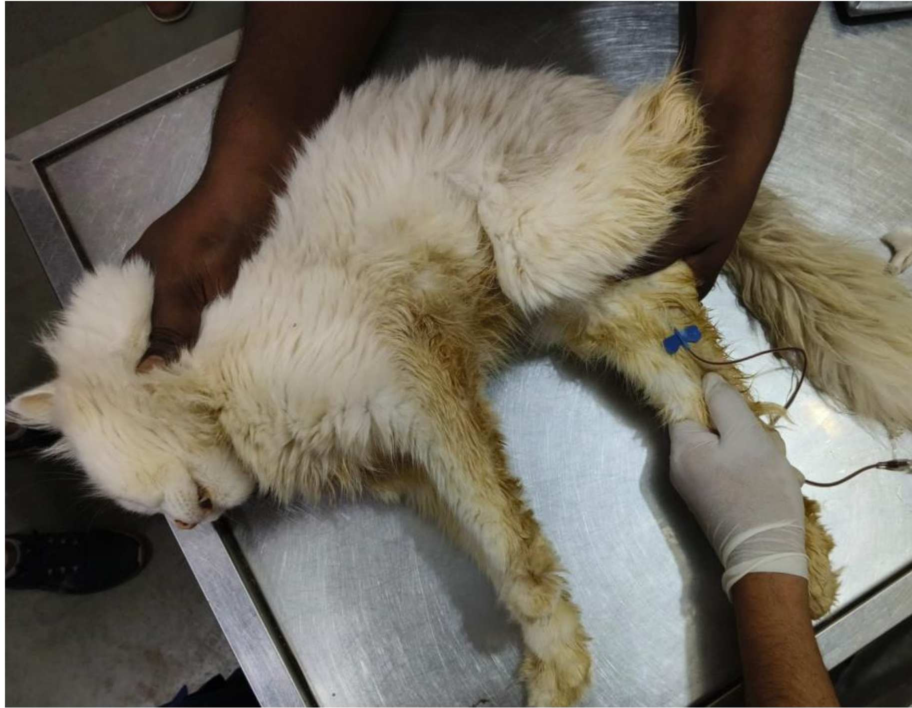


Plate 2. Blood Collection in Persian Cats

4.3 Biochemistry

Average biochemical parameters observed in Persian cats diagnosed with Polycystic Kidney Disease (PKD) alongside their PKD-negative counterparts presented in Table 4.3

Plasma urea nitrogen values in persian cats suffering from polycystic kidney disease revealed significant ($P < 0.05$) increase (86.1±24.44 mg/dl vs 26.2±1.13 mg/dl) with PKD as compared to PKD -ve cats.

Polycystic kidney disease affected persian cats revealed highly significant ($P < 0.01$) increase in plasma creatinine (4.57±0.93 mg/dl vs 1.13±0.04 mg/dl) values compared to normal PKD -ve cats.

In Persian cats affected by polycystic kidney disease (PKD), significant increases in SSerum urea nitrogen (86.1±24.44 mg/dl vs 26.2±1.13 mg/dl) and Serum creatinine (4.57±0.93 mg/dl vs 1.13±0.04 mg/dl) were observed compared to PKD -ve cats, as indicated by Schirrer *et al.,* (2021), Phoon *et al.,* (2015), and Noori *et al.,* (2019).

Specifically, Schirrer et al. (2021) noted higher creatinine concentratioNS in younger animals, while Reeko Sato *et al.,* (2019) observed a greater incidence of high plasma creatinine levels (≥1.6 mg/dl) in cats aged ≥3 years with the PKD mutation.

Mean values of SGPT (41.38±4.61 vs 106.7±16.43 IU/L), SGOT (41.92±6.01 vs 80±11.53 IU/L) and ALP (35.39±8.31vs 93.75±21.76 IU/L) revealed non-significant ($P < 0.05$) in polycystic kidney disease affected and PKD -ve persian cats.

Regarding liver enzymes, there were no significant differences in mean values of SGPT, SGOT, and ALP between PKD-affected and PKD -ve Persian cats, as reported by Guerra et al. (2019). Similarly, Guerra et al. (2019) found no distinctioNS in ALT, AST, and ALP levels between the two groups.

Laboratory findings typically indicate renal dysfunction in PKD-affected cats, as suggested by Biller et al. (1996), while Vucicevic et al. (2016) noted that all hematological and biochemical parameters remained within normal range values in examined cats.

It's worth noting that disease progression in PKD can vary, with some cats exhibiting extrarenal manifestatioNS like liver cysts, although less frequently observed compared to humaNS with PKD. The pathogenesis of PKD involves mutated polycystin-1 affecting cell proliferation and differentiation, leading to apoptosis and renal fibrosis, thereby contributing to cyst formation and eventual renal failure. Despite advancements, various mutatioNS and diseases can mimic PKD, necessitating thorough coNSideration in diagnosis and a compreheNSive understanding of disease mechanisms.

However, the precise etiology of PKD remaiNS elusive and is subject to ongoing research in both human and veterinary medicine.

4.4 Epidemiology

A total of 100 clinical cases of Persian cats admitted Department of Veterinary Epidemiology and Preventive Medicine, Mumbai Veterinary College, Parel and the Cats presented at the medical ward of Bai Sakarabai DiNShaw Petit Hospital for Animals (BSDPHA) affiliated to Mumbai Veterinary College, Parel, Mumbai- 400012.

4.4.1 Age- wise prevalence

Age - wise prevalence of polycystic kidney disease in persian cats has been shown in Table 4.4 and fig 2.

This study examined the age distribution of 100 Persian cats, focusing on the prevalence of cases within different age categories. The analysis revealed that the majority of cases (96%) were concentrated within the age range of 0.5 to 5 years. In contrast, only four cases were observed in cats older than 5 years (Fig 4).

In this study, the prevalence of polycystic kidney disease (PKD) among Persian cats was investigated across different age groups. The overall prevalence was found to be 9%. Among cats aged 0.5 to 5 years, the prevalence was 8.33%, whereas it was 1% among cats older than 5 years. Remarkably, the highest incidence of PKD was observed in cats younger than 5 years of age.

Age-wise prevalence analysis of polycystic kidney disease (PKD) in Persian cats revealed the highest occurrence in the above 5-year age group.

Correlating with previous findings, the prevalence of PKD tends to increase with age in felines, with 57.1% of cases observed in cats aged seven years or older (Lee, 2010). Interestingly, comparable prevalence rates were noted between cats younger than nine months and those aged nine months or older (Bonazzi, 2007).

Additionally, the oNSet of autosomal dominant PKD in cats can lead to end-stage renal failure as early as three years of age, although clinical sigNS typically manifest later in life (Barrs, 2001). However, no iNStances of PKD were documented in cats below 0.5 years of age.

Hege *et al.,* (2001) reported a PKD case in a 9-year-old male castrated Persian cat suffering from chronic renal failure, with age not statistically influencing PKD prevalence in Persian cats.

4.4.2 Sex-wise prevalence

Sex - wise prevalence of polycystic kidney disease in persian cats has been illustrated in Table 4.5 and fig 3.

This investigation examined the sex distribution of 100 Persian cats, focusing on the prevalence of cases among males and females. The analysis revealed that 57% of the cases were male, while 43% were female, as detailed in Table 4.4 and visually represented in Figure 5.

Sex wise overall Percent prevalence of polycystic kidney disease in present study is about 16.35% in which male is 14.03 % and female is 2.32 %.

Barrs *et al.,* (2001) observed prevalence in female cats (43%) was not significantly different from the prevalence in male cats (41%) (P=0.72).

Barthez *et al.,* (2003) found that the prevalence of polycystic kidney disease (PKD) was comparable between male (41.8%) and female (39.1%) Persian and Exotic Shorthair cats in France.

Bear *et al.,* (1992) suggested that males with autosomal dominant polycystic kidney disease (ADPKD) might undergo a faster progression of kidney failure in comparison to females.

Sato *et al.,* (2019) observed that male cats exhibit a higher incidence of concurrent renal and hepatic cysts in feline polycystic kidney disease. The effect of sex on prevalence rate was found to be non-significant.

4.4. Overall prevalence

Out of 100 clinical cases of Persian cats 9 were found positive for polycystic kidney disease, indicating an overall prevalence of 9 % in and around Mumbai.

Various studies have coNSistently observed high prevalence rates of polycystic kidney disease (PKD) in Persian cats. Cannon *et al*., (2001) and Barrs *et al*., (2001) reported rates of 49.2% and 45.0% respectively. Barrs et al. (2001) specifically noted an overall prevalence of 42.0% in Persian cats from Brisbane,

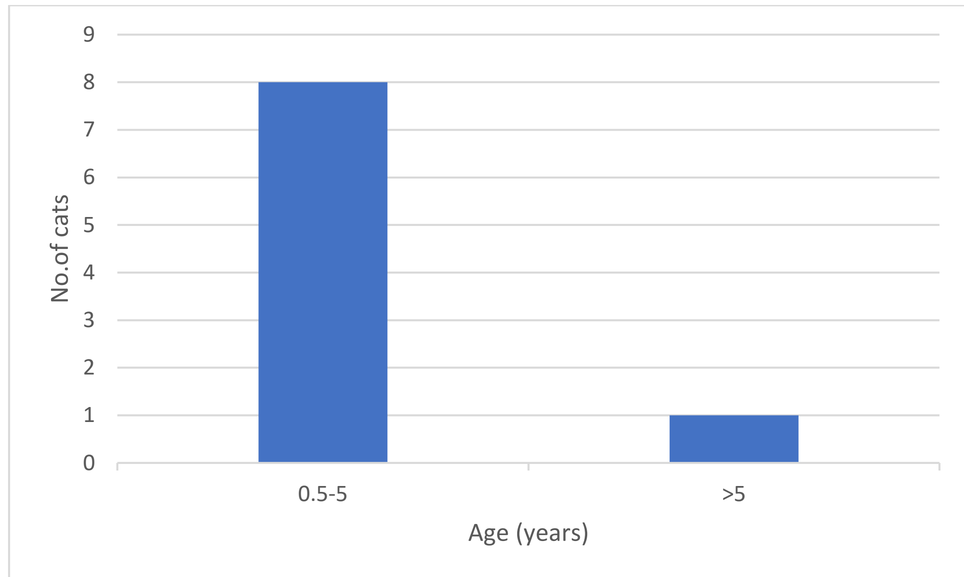


Fig 2 Age wise prevalence of PKD in persian cats (n=9)

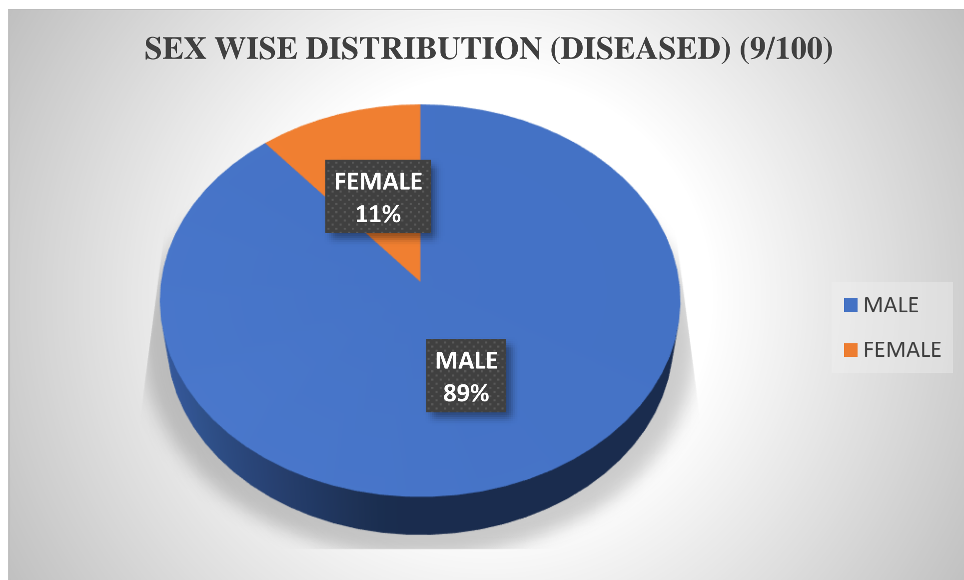


Fig 4.2 Sex wise prevalence of PKD in Persian cats

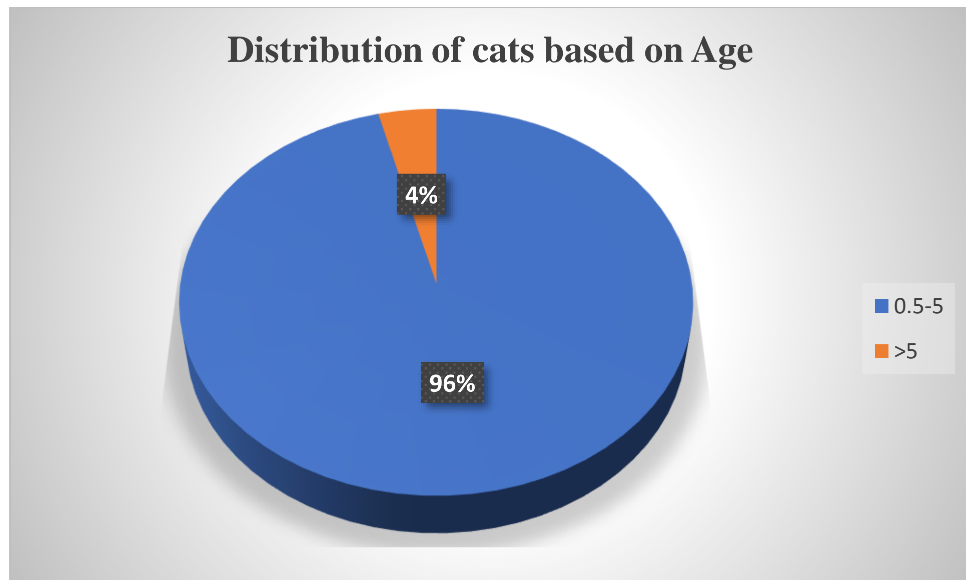


Fig 4.3 Distribution of cats based on Age

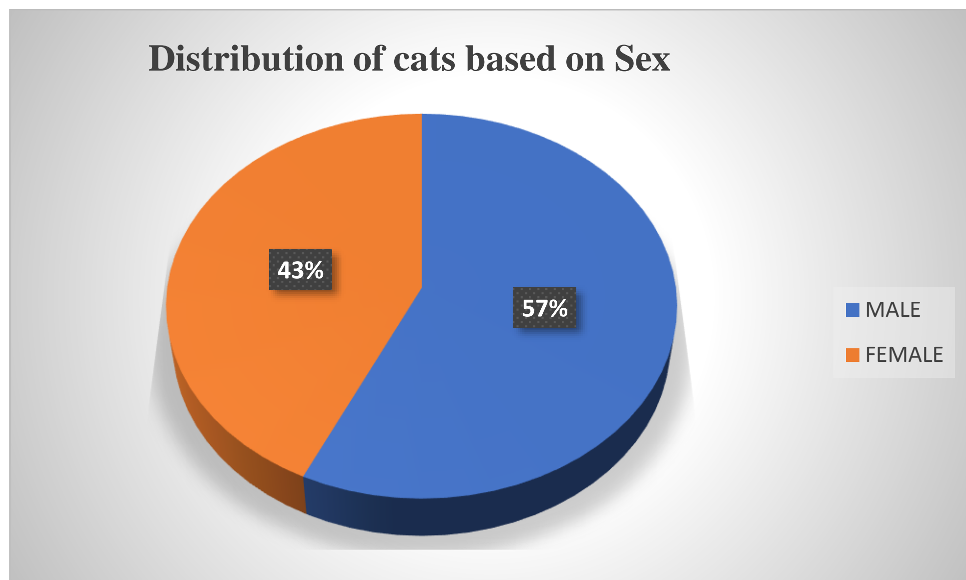


Fig 4.4 Distribution of cats based on Sex

while Barthez et al (2003) reported a prevalence of 41.8% in Persian cats from France.

Domanjko-. Petric *et al,*(2007) found a prevalence of 13.8% PKD positive cases, with a particularly high prevalence of 36% in Persian cats. Reeko Sato et al. (2019) observed a 12.6% coincidence of renal and hepatic cysts, with a high prevalence of 31% in Persian cats.

Lee *et al., (*2010) found a prevalence rate of 24.2% in Persian-related cats. These findings collectively highlight the substantial prevalence of PKD in Persian cats across various regioNS.

The variation in prevalence rates may be due to difference of geographical area, management and environmental factors.

Table 4.4 : Age wise prevalence of PKD in Persian cats (n=9)

Sr. No	Age (Years)	No. Screened	No. affected	Percent cases	Percent Prevalence
1	0.5 – 5	96	8	0.08	8.33
2	>5 yrs	4	1	0.01	25
Total		100	9	0.09	

NS – Non significant $p < 0.05$

Table 4.5 : Sex wise prevalence of PKD in Persian cats (n=9)

Sr. No	**Sex**	**No. Screened**	**No. affected**	**Percent cases**	**Percent Prevalence**
1	Male	57	8	0.08	14.03
2	Female	43	1	0.01	2.32
Total		100	09	0.09	

NS – Non significant $p < 0.05$

4.4 Polymerase Chain Reaction (PCR)

The elimination of PKD within the Persian breed and similar breeds requires early diagnosis for breeding management. Molecular testing correlates well with

ultrasound findings but is limited by cost and access. Genetic testing is preferred for confirming the causal mutation, especially in younger cats, while ultrasound remaiNS the primary method for diagnosing and monitoring PKD progression. Both tests are recommended synergistically for compreheNSive medical diagnosis, particularly in breeding programs aimed at detecting feline PKD.

4.4.1 Isolation of genomic DNA

The isolation of genomic DNA from blood samples obtained from Persian cats was carried out by following Qiagen kit method. Each of 2 ml blood samples processed yielded about 100 µg of genomic DNA.

4.4.2 Estimation of the quality and the quantity of genomic DNA

The ratio of the optical deNSity of DNA at 260 and 280 nm (A260/280) was used to assess the purity of nucleic acids. Spectrophotometer readings at 260 and 280 nm for the DNA samples from present study were in the range of 1.72 to 2.0 and the mean OD value was 1.835 ± 0.006, which was an indicative of good quality genomic DNA. Further, the quality of isolated DNA was confirmed through agarose gel (2 per cent) electrophoresis. Majority of the genomic DNA samples showed clear and distinct bands under UV-traNSilluminator, which was indicative of good quality DNA(Plate 3).

4.4.3 Polymerase Chain Reaction (PCR)

The presence or absence of the C→A traNSversion at position 3284 of exon 29 in the feline PKD1 gene was investigated in all cats included in this study using a PCR-RFLP assay.

4.4.4. Selection and designing of PCR Primers

A newly designed and custom synthesized primer pair was successful in amplification of an expected length of 559 bp fragment from PKD

gene sequence. PCR amplified fragment of PKD gene sequence has been deposited in Gen Bank (Accession No. AY612847)

4.4.5 Composition of PCR mixture and optimization of PCR protocol

Prior to successful PCR amplification of the desired sequence of PKD gene from the studied sample of genomic DNA, various steps of PCR optimization procedure were carried out. ~1 pmol of each forward and reverse primer, 1.25 mM dNTP, 1.75 mM MgCl2,1 × PCR buffer, and 0.375U of Amplitaq (Applied Biosystems) polymerase in 10 µl reaction volume resulted successful amplification of expected PCR amplicoNS of PKD gene. The details of optimized PCR conditioNS and number of PCR cycles are presented in (material method) The PCR products obtained were subjected to direct mutation analysis through restriction enzyme digestion using MLY1 (NEB), following previously established protocols.

Enzymatic digestion was carried out at 37°C for 45 minutes, followed by enzyme deactivation at 67°C for 20 minutes. Subsequently, the digested products were visualized on 2% agarose electrophoresis gels stained with a dye.(Plate 3)

4.4.6 Electrophoretic analysis of PCR amplicoNS

The amplified products were electrophoresed on a two per cent agarose gel. The amplified fragments obtained from studied persian cat population were of 559 bp in size (Plate 4).

4.4.6 Electrophoretic analysis of PCR amplicoNS

The amplified products were electrophoresed on a two per cent agarose gel. The amplified fragments obtained from studied persian cat population were of 559 bp in size (Plate 4).

4.4.7 Analysis of Restriction Fragment Length Polymorphism (RFLP)

The PCR RFLP technique was employed to verify the polymorphism in PKD gene persian cat.The representative PCR samples were submitted for sequencing from a private firm (CHROMOXPERT RESEARCH &DIAGNOSTICS Pvt. Ltd).

Nine of the 100 cats with cystic kidneys were found heterozygous for the PKD mutation by PCR RFLP, with DNA sequencing confirming a C→A traNSversion. After the presence of the mutation had been confirmed, owners were informed of their cat's disease status. To limit spread of the mutant allele in the cat population, sterilization was suggested.

Scalon *et al.,* (2014) reported a prevalence of autosomal dominant polycystic kidney disease (ADPKD) in cats as 9%, with a notably higher incidence of 33% observed in Persian cats. The utilization of Touchdown PCR has been highlighted as an effective method for diagnosing the ADPKD mutation in various cat populatioNS. Positive cats exhibited hyperglobulinemia, indicating the presence of the disease.

Genetic testing through PCR techniques can identify carriers of the ADPKD mutation, thereby aiding in the prevention of disease traNSmission. Furthermore, ADPKD prevalence varies among different cat breeds. The use of PCR, including the touchdown PCR technique, has been developed for the detection of ADPKD in cats.

Helps *et al.,* (2007) demoNStrated the successful use of PCR in detecting the ADPKD mutation in cats with renal cysts, particularly affecting Persian breeds.

Additionally, Bonazzi *et al.,* (2009) utilized PCR/RFLP assays for genetic testing of polycystic kidney disease in cats, although PCR alone was not specifically mentioned.

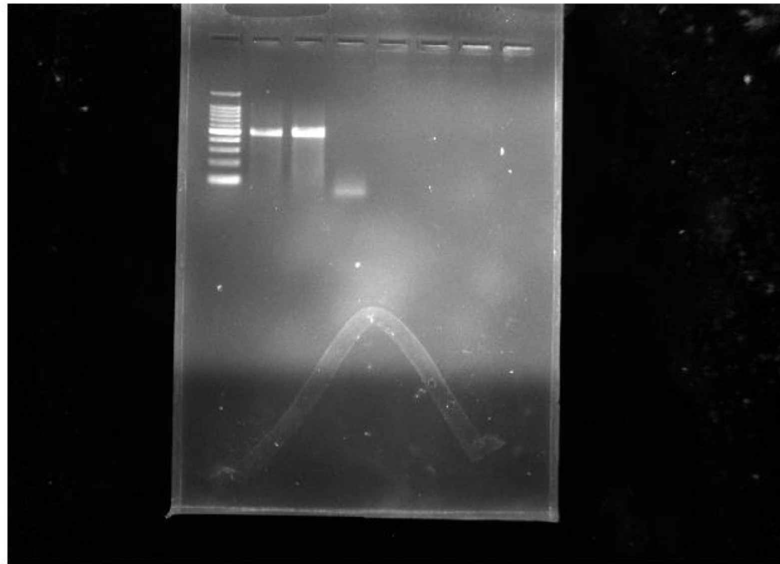


Plate 4.2: Agarose gel (2%) electrophoresis showing (A) 100 bp ladder and (B) amplified DNA (316 bp) amplicon size positive for PKD GENE.

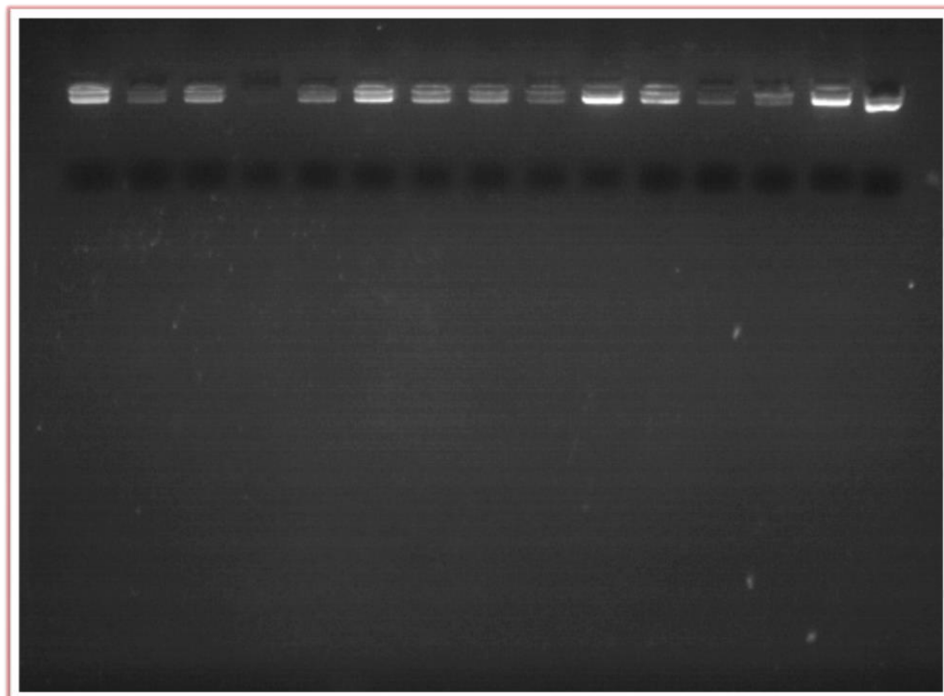


Plate 4.3 Assessments of DNA after DNA extraction by gel electrophoresis.

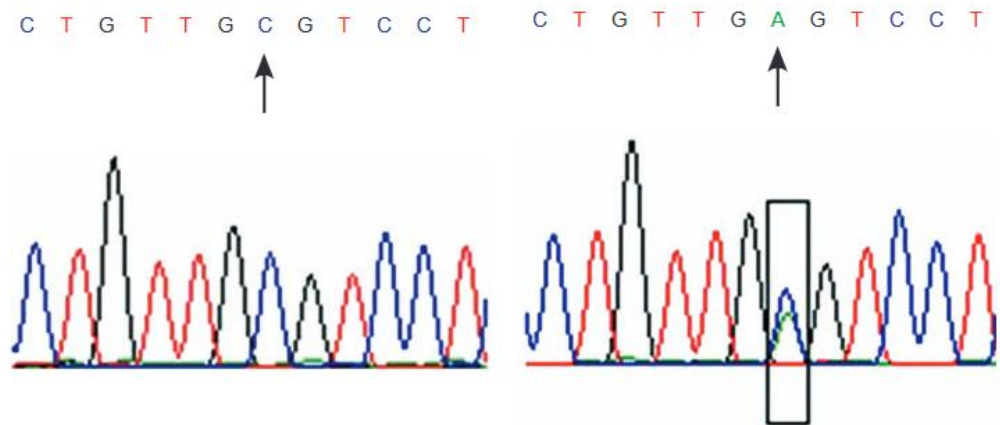


Fig 6: Nucleotide sequences of the (a) wild-type and (b) mutated feline PKD gene. A single nucleotide mutation (C to A transversion [arrow]) was identified in exon 29; this point mutation was heterozygous in all samples, as indicated by the coexistence of cytosine and adenine (shown in the box)

Bilgen *et al.,* (2020) detected polycystic kidney disease in cats using PCR-RFLP, with a prevalence of 62.5% in symptomatic and 0.68% in asymptomatic cases.

Schirrer *et al.,* (2023) affirmed that PCR methods, such as RFLP-PCR and realtime PCR, are capable of diagnosing polycystic kidney disease by identifying the respoNSible mutation.

Domanjko-Petric *et al.,* (2008) indicated the use of PCR for identifying cats with the PKD mutation, facilitating the determination of whether cats will develop polycystic kidney disease in the future.

4.4.8 Gene Sequencing of PKD1 gene

To obtain positive controls, PCR products from DNA cats were sequenced in the forward and reverse direction.

The sequences were initially analysed using the NCBI/BLAST/blastn suite (https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome).

Summary and Conclusions

SUMMARY AND CONCLUSION

The current study entitled "PREVALENCE OF POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN AND AROUND MUMBAI" was conducted on 100 feline patients presented at the Department of Veterinary Epidemiology and Preventive Medicine, Mumbai Veterinary College, Parel, Mumbai 400012. All the Persian cats more than 6 months of age included in the study, haemato-biochemical analysis and ultrasonography of the kidneys for evaluation of the cysts present in kidneys. Detection of PKD gene mutation by PCR was carried out for polycystic Persian cats.

Among the 100 Persian cats,57% were male (n=57/100) and 43% (n=43/100) were female cats. In the present study, age-wise prevalence analysis of polycystic kidney disease (PKD) in Persian cats revealed a prevalence of 8% occurring in cats aged 0.5-5 years and 1% in cats aged above 5 years. Notably, the highest occurrence was observed in cats aged below 5 years.

The MEAN±SE of total cysts present in kidneys of polycystic kidney patients were 3.55±1.19. the Mean cyst counts of 3.55 suggests that, on average, the cats exhibit a cyst burden consistent with PKD. Moreover, the standard deviation of 1.19 highlights the variability in cyst counts among the PKD-affected feline cohort, underscoring the heterogeneity of PKD presentation.

The MEAN±SE of hemoglobin (10.79±1.58 vs 11.37±0.35 g/dl), packed cell volume (32.12±3.99 vs 34.45±1.04 %), and total erythrocyte count (7.36±0.88 vs 7.32±0.23 $\times 10^6$/ µl) between PKD-affected and PKD -ve Persian cats. There were no significant differences ($P < 0.05$) observed in the Mean values of hemoglobin.

The MEAN±SE of in total leukocyte count (20.5±3.52 vs 17.33±1.26 x 10^3/ µl) and neutrophil count (74.44±6.11 vs 69.67±1.64 %) between PKD-affected and PKD -ve Persian cats. Regarding the leukogram, no significant changes ($P < 0.01$) were noted in total leukocyte count.

The MEAN±SE of platelet counts (2.29±0.27x 10^3/ µl vs 2.37±0.09 x10^3/

µl) between PKD-affected cats and PKD -ve Persian cats. there were no significant differences observed in platelet counts.

The MEAN±SE of SGPT (41.38±4.61 vs 106.7±16.43 IU/L), SGOT (41.92±6.01 vs 80±11.53 IU/L) and ALP (35.39±8.31vs 93.75±21.76 IU/L) between PKD-affected cats and PKD -ve Persian cats. Regarding liver enzymes, there were no significant differences in Mean values of SGPT, SGOT, and ALP between PKD-affected and PKD -ve Persian cats

The MEAN±SE of Serum urea nitrogen (86.1±24.44 mg/dl vs 26.2±1.13 mg/dl) and plasma creatinine (4.57±0.93 mg/dl vs 1.13±0.04 mg/dl). Serum urea nitrogen values in Persian cats suffering from polycystic kidney disease revealed significant ($P < 0.05$) increase. Polycystic kidney disease affected persian cats revealed highly significant ($P < 0.01$) increase in plasma creatinine.

The current investigation aimed to explore gene mutations associated with PKD in Persian cats using the PCR-RFLP technique. Genomic DNA was extracted from blood samples obtained from a representative cohort of 100 Persian cats utilizing the Qiagen kit method. The quality and quantity of the DNA were assessed through spectrophotometry and agarose gel electrophoresis. Specifically designed primers were employed to amplify exon 29 of the PKD gene. The amplification process included an initial denaturation at 94°C for 3 minutes, followed by denaturation at 94°C for 1 minute, primer annealing at 58°C for 1 minute, extension at 72°C for 1 minute, repeated denaturation and extension steps for 35 cycles, and a final extension at 72°C for 1 minute.

Following the optimized PCR protocol, a 559bp fragment corresponding to the PKD1 gene was successfully obtained. The amplified PCR products underwent digestion with the MLY1 restriction enzyme to generate distinctive RFLP patterns. The enzymatic digestion of the PCR product occurred at 37°C with 10 units of the restriction enzyme, followed by a 12-hour incubation period. Subsequently, the restriction fragments were separated on a two percent agarose gel via electrophoresis. Among the 100 Persian cats, nine were identified as heterozygous for the PKD mutation using PCR RFLP analysis, withsubsequent DNA sequencing confirming a C→A transversion.

Conclusion:

1. This research emphasizes that PKD affects 9 out of every 100 Persian cats in and around Mumbai.
2. Ultrasound is the preferred imaging method for diagnosing advanced feline polycystic kidney disease characterized by multiple large cysts.
3. Hematological parameters showed limited variation between PKD-affected and PKD-negative cats, except for significant elevations in plasma urea nitrogen and creatinine levels in affected cats.
4. Genetic analysis revealed a notable mutation in the PKD1 gene, shedding light on the genetic basis of PKD in Persian cats. These findings contribute to our understanding of PKD in felines and may inform future diagnostic and therapeutic approaches.

Bibliography

BIBLIOGRAPHY

Barrs, V. R., Gunew, M., Foster, S. F., Beatty, J. A., and Malik, R. (2001). Prevalence of autosomal dominant polycystic kidney disease in Persian cats and related-breeds in Sydney and Brisbane. *Australian veterinary journal*, *79*(4), 257–259. https://doi.org/10.1111/j.1751-0813.2001.tb11977.x

Barthez, P. Y., Rivier, P., and Begon, D. (2003). Prevalence of polycystic kidney disease in Persian and Persian related cats in France. *Journal of feline medicine and surgery*, *5*(6), 345–347. https://doi.org/10.1016/S1098-612X(03)00052-4

Beck, C., and Lavelle, R. B. (2001). Feline polycystic kidney disease in Persian andother cats: a prospective study using ultrasonography. *Australian veterinary journal*, *79*(3), 181–184. https://doi.org/10.1111/j.1751-0813.2001.tb14573.x

Bilgen, N., Bişkin Türkmen, M., Çınar Kul, B., Isparta, S., Şen, Y., Akkurt, M. Y., Çıldır, Ö. Ş., and Bars, Z. (2020). Prevalence of *PKD1* gene mutation in cats in Turkey and pathogenesis of feline polycystic kidney disease. *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, *32*(4), 549–555. https://doi.org/10.1177/1040638720935433

Biller, D. S., Chew, D. J., and DiBartola, S. P. (1990). Polycystic kidney disease in a family of Persian cats. *Journal of the American Veterinary Medical Association*, *196*(8), 1288–1290.

Biller, D. S., DiBartola, S. P., Eaton, K. A., Pflueger, S., Wellman, M. L., and Radin, M. J. (1996). Inheritance of polycystic kidney disease in Persian cats. *The*

Journal of heredity, *87*(1), 1–5. https://doi.org/10.1093/oxfordjournals.jhered.a022945

Bonazzi, M., Volta, A., Gnudi, G., Bottarelli, E., Gazzola, M., and Bertoni, G. (2007). Prevalence of the polycystic kidney disease and renal and urinary bladder ultrasonographic abnormalities in Persian and Exotic Shorthair cats in Italy. *Journal of feline medicine and surgery*, *9*(5), 387–391. https://doi.org/10.1016/j.jfms.2007.03.004

Bonazzi, M., Volta, A., Gnudi, G., Cozzi, M. C., Strillacci, M. G., Polli, M., Longeri, M., Manfredi, S., and Bertoni, G. (2009). Comparison between ultrasound and genetic testing for the early diagnosis of polycystic kidney disease in Persian and Exotic Shorthair cats. *Journal of feline medicine and surgery*, *11*(6), 430–434. https://doi.org/10.1016/j.jfms.2008.10.003

Cannon, M. J., MacKay, A. D., Barr, F. J., Rudorf, H., Bradley, K. J., and Gruffydd-Jones, T. J. (2001). Prevalence of polycystic kidney disease in Persian cats in the United Kingdom. *The Veterinary record*, *149*(14), 409–411. https://doi.org/10.1136/vr.149.14.409

Cooper B: Autosomal dominant polycystic kidney disease in Persian cats. Feline Prac 28: 20-21, 2000

Domanjko-Petric, A., Cernec, D., and Cotman, M. (2008). Polycystic kidney disease: a review and occurrence in Slovenia with comparison between ultrasound and genetic testing. *Journal of feline medicine and surgery*, *10*(2), 115–119. https://doi.org/10.1016/j.jfms.2007.07.004

Gendron, K., Owczarek-Lipska, M., Lang, J., and Leeb, T. (2013). Maine Coon renal screening: ultrasonographical characterisation and preliminary genetic analysis for common genes in cats with renal cysts. *Journal of feline medicine and surgery*, *15*(12), 1079–1085. https://doi.org/10.1177/1098612X13492164

Grahn, R. A., Biller, D. S., Young, A. E., Roe, B. A., Qin, B., and Lyons, L. A. (2004). Genetic testing for feline polycystic kidney disease. *Animal genetics*, *35*(6), 503–504. https://doi.org/10.1111/j.1365-2052.2004.01213.x

Guerra, J. M., Cardoso, N. C., Daniel, A. G. T., Onuchic, L. F., and Cogliati, B. (2021). Prevalence of autosomal dominant polycystic kidney disease in Persian and Persian-related cats in Brazil. *Brazilian journal of biology = Revista brasleira de biologia*, *81*(2), 392–397. https://doi.org/10.1590/1519-6984.227131

Guerra, J. M., Freitas, M. F., Daniel, A. G., Pellegrino, A., Cardoso, N. C., de Castro, I., Onuchic, L. F., and Cogliati, B. (2019). Age-based ultrasonographic criteria for diagnosis of autosomal dominant polycystic kidney disease in Persian cats. *Journal of feline medicine and surgery*, *21*(2), 156–164. https://doi.org/10.1177/1098612X18764591

Hege, R., Zimmer, C., and Reusch, C. (2001). Polyzystische Nierenerkrankungbei einer Perserkatze [Polycystic kidney disease in a Persian cat]. *Schweizer Archiv fur Tierheilkunde*, *143*(4), 203–207.

Helps, C. R., Tasker, S., Barr, F. J., Wills, S. J., and Gruffydd-Jones, T. J. (2007). Detection of the single nucleotide polymorphism causing feline autosomal-dominant polycystic kidney disease in Persians from the UK using a novel real-time PCR assay. *Molecular and cellular probes*, *21*(1), 31–34. https://doi.org/10.1016/j.mcp.2006.07.003

Helps, C., Tasker, S., and Harley, R. (2007). Correlation of the feline PKD1 genetic mutation with cases of PKD diagnosed by pathological

examination. *Experimental and molecular pathology*, *83*(2), 264–268. https://doi.org/10.1016/j.yexmp.2007.04.002

Kappe, E. C., Hecht, W., Gerwing, M., Michele, U., and Reinacher, M. (2005). Polycystic kidney disease in the German population of Persian cats. A comparative study of ultrasonographical examination and genetic testing.

Lee, Y. J., Chen, H. Y., Hsu, W. L., Ou, C. M., and Wong, M. L. (2010). Diagnosis of feline polycystic kidney disease by a combination of ultrasonographic examination and PKD1 gene analysis. *The Veterinaryrecord*, *167*(16), 614–618. https://doi.org/10.1136/vr.c4605

Noori, Z., Moosavian, H. R., Esmaeilzadeh, H., Vali, Y., and Fazli, M. (2019). Prevalence of polycystic kidney disease in Persian and Persian related-cats referred to Small Animal Hospital, University of Tehran, Iran. *Iranian journal of veterinary research*, *20*(2), 151–154.

Phoon, M. H. E., Khor, K. H., Lau, S. F., and Saw, M. H. (2015). Polycystic kidney disease in a Persian cat. *J Vet Malaysia*, *27*(1), 20-23.

Reichle, J. K., DiBartola, S. P., and Léveillé, R. (2002). Renal ultrasonographic and computed tomographic appearance, volume, and function of cats with autosomal dominant polycystic kidney disease. *Veterinary radiology and ultrasound : the official journal of the American College of Veterinary Radiology and the International Veterinary Radiology Association*, *43*(4), 368–373. https://doi.org/10.1111/j.1740-8261.2002.tb01020.x

Sato, R., Uchida, N., Kawana, Y., Tozuka, M., Kobayashi, S., Hanyu, N., Konno, Y., Iguchi, A., Yamasaki, Y., Kuramochi, K., and Yamasaki, M. (2019). Epidemiological evaluation of cats associated with feline polycystic

kidney disease caused by the feline PKD1 genetic mutation in Japan. *The Journal of veterinary medical science*, *81*(7), 1006–1011. https://doi.org/10.1292/jvms.18-0309

Scalon, M. C., da Silva, T. F., Aquino, L. C., Carneiro, F. T., Lima, M. G., Lemos, M. D., and Paludo, G. R. (2014). Touchdown polymerase chain reaction detection of polycystic kidney disease and laboratory findings in different cat populations. *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, *26*(4), 542–546. https://doi.org/10.1177/1040638714536561

Schirrer, L., Marín-García, P. J., and Llobat, L. (2021). Feline Polycystic Kidney Disease: An Update. *Veterinary sciences*, *8*(11), 269. https://doi.org/10.3390/vetsci8110269

Vucicevic, M., Slijepcevic, D., Davitkov, D., Avdalovic, V., Aleksic-Kovacevic, S., Stevanovic, J., and Stanimirovic, Z. (2016). First report of Polycystic kidney disease occurrence in Persian cats in Serbia. *Veterinaria italiana*, *52*(1), 51–56. https://doi.org/10.12834/VetIt.599.2885.2

Wills, S. J., Barrett, E. L., Barr, F. J., Bradley, K. J., Helps, C. R., Cannon, M. J., and Gruffydd-Jones, T. J. (2009). Evaluation of the repeatability of ultrasound scanning for detection of feline polycystic kidney disease. *Journal of feline medicine and surgery*, *11*(12), 993-996.

Yu, Y., Shumway, K. L., Matheson, J. S., Edwards, M. E., Kline, T. L., and Lyons, L. A. (2019). Kidney and cystic volume imaging for disease presentation and progression in the cat autosomal dominant polycystic kidney disease large animal model. *BMC nephrology*, *20*(1), 259. https://doi.org/10.1186/s12882-019-1448-1

Appendices

APPENDIX -1

Case paper- (Patient ID:)

Case no:		Patient name		Owner's name		Ph no:	
Age:		Breed:		Sex: M/F			

Name	Right Kidney	Left Kidney

Abdominal Palpation:

Cyst Present in Kidneys:

Haemato-Biochemistry:

Parameters	Values	Units
Haemoglobin		gm%
PCV		%
TEC		10^6/cmm
TLC		10^3/cmm
Neutrophils		%
Eosinophils		%
Lymphocytes		%
Monocytes		%
Basophils		%
Platelets		Lakh/cmm

Total Bilirubin		mg/dl
Direct Bilirubin		mg/dl
Indirect Bilirubin		mg/dl
Alkaline Phosphatase		IU/L
Aspartate Transaminase		IU/L
Alanine Transaminase		IU/L
Total Protein		gm/dl
Albumin		gm/dl
Globulin		gm/dl
A: G Ratio		NIL
BUN		mg/dl
Creatinine		mg/dl

APPENDIX - II

List of Equipment used for PCR

Sr. No.	Name	Manufacturer
1.	Micropipettes	Eppendorf
2.	Vortex	Spinix
3.	Mini centrifuge	GeNei
4.	Nanodrop Spectrophotometer	Thermo Scientific, USA
5.	Ice Maker	Sanyo SIM-F40, Japan
6.	Thermal Cycler	Veriti Thermal Cycler, Applied Biosystems, USA
7.	Cooling Centrifuge	Remi CM -12 plus, mLabs
8.	Hot Water Bath	Neolab Circulator
9.	Deep Freezer	Voltas
10.	Submarine gel electrophoresis apparatus	Scie-plas, UK
11.	Electrophoresis Power Supply	Tarsons
12.	Ohaus Weighing Balance	Pioneer
13.	Equitron Autoclave Pad	Medica instrument Mfg, Co.
14.	Gel doc EZ Gel Documentation System	Bio-Rad

Contents of QIAamp Mini Kit:

- QIAGEN Protease (or proteinase K)
- Buffer AL
- Ethanol (96–100%)
- Buffer AW1
- Buffer AW2
- Buffer AE or distilled water
- Microcentrifuge tubes (1.5 mL)
- Pipettes and tips
- PBS (if needed)
- RNase A stock solution (if RNA-free genomic DNA is required.

Appendix iii:Gene sequencing of PKD1 GENE

0224-185_001_PCR_50_KS_Bgcox3_F_A06.ab1

0224-185_001_PCR_50_KS_Bgcox3_F

KB 1.4.2.6 KB.bcp

KB_3730_POP7_BDTv3.mob

Signal: G:53 A:75 T:81 C:62 AvgSig: 67

C#:48 W:A6 Plate Name:08022024A

TS:47 CRL:563 QV20+:562

TAATTATCTTCTACTCTATTTGATCCTCTATTTTCATGATAAAATATGGATGTTAATACTAATACAGCACCTATACAT

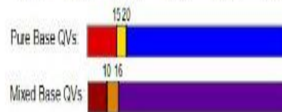
ACATGTGATGAATGTAAACCGGTGATTATTAAAAATAAAGTTGTAAACCAACTGTGATTAACAGCTGATTGTAATAATGAATA

CTCATCACCTTGATAGGATATGAAAGCTACAGCTACAAATGTTAACACAGCAACTAGTTTTTCAGTCTCAGAATTTTCTCCTG

AGATATTTTTATGAAGCATAAATGATACTATAACACTACCAATATTTAATGTACTAGTGATATTTAAAAATGGTTCAATATTA

Inst Model/Name:3730xl/Eurofins1-15104-028

Sequence Scanner Software 2 v2.0



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Electropherogram Data Page 1 of 5

Appendix iv: Haematological values of PKD cats

Cat Name	Age Yrs	Sex	Hb	TEC	PCV	RDW	MCV	MCH	MCHC	TLC	Neutrophil	Eosino	Lymphocyte	Monocyte	Platlet (Lacs/cmm)
LEO	5	M	7.7	5.48	31.9	47.6	58.2	14.1	24.2	21.9	75	0	23	2	3.5
CHINU	4	M	7.3	5.59	23.1	18.5	41.32	13.06	31.6	35.2	89	2	8	1	2.41
TIGER	6	M	12	8.05	35.6	16.6	44.22	14.91	33.71	1.4	34	3	62	1	0.61
ZOE	3.5	F	14.3	9.94	42.4	19.1	42.66	14.39	33.73	26.7	56	1	42	1	1.72
KALU	3.5	M	11	7.66	32.2	18.2	42.2	14.4	34.2	23.33	80	0	17	3	2.1
WHITY	4	M	18.2	10.94	48.7	16.1	44.5	16.6	37.4	16.98	90	0	9	1	2.8
EMIR	5	M	5.5	3.64	14.2	27.7	39	15.2	39	30.03	78	0	20	2	2.5
SIMBA	5	M	16.1	10.22	43.7	15.8	42.8	15.7	36.8	21.17	80	0	17	3	2.8
GORU	4	M	5	4.72	17.3	25.1	37	11	29	7.8	88	0	10	2	2.2

Appendix v: Biochemical values of PKD cats

Bilirubin T	Bili Direct	Bili Indirect	SGOT	SGPT	ALP	Total Protein	Albumin	Globulin	A/G	BUN	Creat
0.69	0.05	0.64	36.48	58.31	16.61	6.98	2.62	4.36	0.6	29.29	2.19
0.2	0.1	0.1	26	29	35	8.3	2.4	5.9	0.41	53.1	2.5
1.4	0.5	0.8	64	52	11	6.8	2.8	4	0.7	48.1	2.7
0.3	0.1	0.2	32	64	76	6.7	3.1	3.6	0.8	89.1	3.9
0.49	0.16	0.33	79.01	29.07	17.9	6.28	2.21	4.07	0.54	115.8	3.6
0.42	0.13	0.29	42	44	70	7.7	3.5	4.2	0.83	9.54	5.77
1.37	0.81	0.56	29.04	29.52	15.5	9.89	2.29	7.6	0.3	255.32	9.4
0.61	0.21	0.4	40.46	37.34	53.5	5.53	2.56	2.97	0.86	56.78	2.28
0.48	0.16	0.32	28.25	29.17	23	4.56	1.86	2.7	0.69	117.91	8.79

Abstract

Appendix-G

THESIS ABSTRACT

1.	Title of the thesis (in Capital letters)	:	**PREVALENCE OF POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN AND AROUND MUMBAI**
2.	Full name of student	:	Gaikwad Abhishek Anil
3.	Name and address of Major Advisor	:	**Dr. J.U. Patil** Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, Mumbai Veterinary College, Parel, Mumbai-400012
4.	Degree to be awarded	:	M. V. Sc.
5.	Year of award of degree	:	2024
6.	Major subject	:	Veterinary Epidemiology and Preventive Medicine
7.	Total number of pages in the thesis	:	
8.	Number of words in the abstract	:	
9.	Signature of Student	:	
10.	Signature, Name and address of forwarding authority (HOD/ SH)	:	
11.	Signature of the Associate Dean	:	

ABSTRACT

The current study entitled "PREVALENCE OF POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN AND AROUND MUMBAI" was conducted on 100 feline patients presented at the Department of Veterinary Epidemiology and Preventive Medicine, Mumbai Veterinary College, Parel, Mumbai 400012. All the Persian cats more than 6 months of age included in the study, haemato-biochemical analysis and ultrasonography of the kidneys for evaluation of the cysts present in kidneys. Detection of PKD1 gene mutation by PCR was carried out for polycystic Persian cats.

Out of hundred clinical cases involving Persian cats, Nine were diagnosed with polycystic kidney disease, resulting in an overall prevalence rate of 9% in the Mumbai area. When considering gender, the prevalence of the disease was found to be 16.35%, with males at 14.03% and females at 2.32%. Analysing by age, the prevalence of polycystic kidney disease in Persian cats was 33.33%, with 8.33% occurring in cats aged 0.5-5 years and 25% in cats aged above 5 years. Notably, all cases in cats aged above 5 years were diagnosed with the disease.

Plasma urea nitrogen values in Persian cats suffering from polycystic kidney disease revealed significant ($P < 0.05$) increase. Polycystic kidney disease affected persian cats revealed highly significant ($P < 0.01$) increase in plasma creatinine.

Upon ultrasonographic examination, cysts were detected in 9 Persian cats, subsequently followed by PCR RFLP analysis. Further investigation involving gene sequencing confirmed the presence of polycystic kidney disease.

These discoveries enhance our comprehension of polycystic kidney disease in felines and could potentially guide the development of future diagnostic and treatment strategies.

Appendix – G

थीसिस गोषवारा

अ)	प्रबंधाचे शीर्षक	:	**मुंबई आणि आसपासच्या पर्शियन मांजरींमध्ये पॉलीसिस्टिक किडनी रोगाचा प्रसार**
ब)	विद्यार्थ्याचे पूर्ण नाव	:	गायकवाड अभिषेक अनिल
क)	प्रमुख सल्लागाराचे नाव आणि पत्ता	:	**डॉ. ज. उ. पाटील,** असिस्टंट प्रोफेसर, मुंबई पशुवैद्यकीय महाविद्यालय, मुंबई
ड)	पदवी प्रदान केली जाणार आहे	:	M. V. Sc.
इ)	पदवीचे वर्ष	:	२०२४
f)	प्रमुख विषय	:	पशुवैद्यकीय साथरोग विज्ञान व रोगप्रतिबंधक औषधशास्त्र विभाग
g)	प्रबंधातील एकूण पृष्ठांची संख्या	:	
h)	गोषवारामधील शब्दांची संख्या	:	
i)	विद्यार्थ्याची स्वाक्षरी	:	
j)	स्वाक्षरी, अग्रेषण प्राधिकरणाचे नाव आणि पत्ता (HOD/SH)	:	
	असोसिएट डीनची स्वाक्षरी		

गोषवारा

"मुंबईमध्ये आणि आसपासच्या पर्शियन मांजरींमध्ये पॉलीसिस्टिक किडनी रोगाचा प्रादुर्भाव" या शीर्षकाचा सध्याचा अभ्यास पशुवैद्यकीय साथीच्या रोगविज्ञान आणि प्रतिबंधात्मक औषध विभाग, मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई 400012 मध्ये 100 रुग्णांवर केला गेला. अभ्यासामध्ये 6 महिन्यांपेक्षा जास्त वयाचा समावेश आहे, मूत्रपिंडामध्ये उपस्थित असलेल्या सिस्टचे मूल्यांकन करण्यासाठी हेमेटो-बायोकेमिकल विश्लेषण आणि मूत्रपिंडाचे अल्ट्रासोनोग्राफी, पॉलीसिस्टिक पर्शियन मांजरींसाठी PCR द्वारे PKD1 जनुक उत्परिवर्तनाचा शोध घेण्यात आला.

पर्शियन मांजरींचा समावेश असलेल्या शंभर क्लिनिकल प्रकरणांपैकी, नऊ जणांना पॉलीसिस्टिक किडनी रोगाचे निदान झाले, परिणामी मुंबई परिसरात एकूण प्रादुर्भाव दर 9% आहे. लिंग विचारात घेता, रोगाचा प्रसार 16.35% आढळून आला, पुरुषांमध्ये 14.03% आणि महिलांमध्ये 2.32%. वयानुसार विश्लेषण करताना, पर्शियन मांजरींमध्ये पॉलीसिस्टिक किडनी रोगाचा प्रसार 33.33% होता, 0.5-5 वर्षे वयोगटातील मांजरींमध्ये 8.33% आणि 5 वर्षांपेक्षा जास्त वयाच्या मांजरींमध्ये 25% आढळतो. उल्लेखनीय म्हणजे, 5 वर्षांपेक्षा जास्त वयाच्या मांजरींमधील सर्व प्रकरणांमध्ये या रोगाचे निदान झाले.

पॉलीसिस्टिक किडनीच्या आजाराने ग्रस्त असलेल्या पर्शियन मांजरींमध्ये प्लाझ्मा युरिया नायट्रोजन मूल्यांमध्ये लक्षणीय (पी <0.05) वाढ दिसून आली. पॉलीसिस्टिक किडनी रोगाने प्रभावित पर्शियन मांजरींमुळे प्लाझ्मा क्रिएटिनिनमध्ये अत्यंत लक्षणीय (पी <0.01) वाढ दिसून आली.

अल्ट्रासोनोग्राफिक तपासणीनंतर, 9 पर्शियन मांजरींमध्ये सिस्ट आढळले, त्यानंतर PCR RFLP विश्लेषण केले गेले. जनुकांच्या क्रमवारीचा समावेश असलेल्या पुढील तपासणीने पॉलीसिस्टिक किडनी रोगाच्या उपस्थितीची पुष्टी केली. या शोधांमुळे मांजरींमधील पॉलीसिस्टिक किडनीच्या आजाराची आमची समज वाढते आणि भविष्यातील निदान आणि उपचार धोरणांच्या विकासासाठी संभाव्य मार्गदर्शन होऊ शकते.

Vita

VITA

The author of this dissertation, Dr Gaikwad Abhishek Anil was born on 12 th July 1998 at Ramling Mudgad, Latur, Maharashtra. He finished his schooling from Ramling Vidyalaya, Ramling Mudgad and also passed his Secondary School Certificate Examination in the year 2013 with distinction and Higher Secondary School Certificate Examination from Mahatma Basweshwar college, Latur in the year 2015 with first class with distinction. With the interest of taking care of animals, he joined the Mumbai Veterinary College, Parel, Mumbai in the year 2016 and completed his graduation obtaining the degree B.V. Sc &A.H. with first class in the year 2022. Further being interested in the field of small animal practice, he joined the Department of Veterinary Epidemiology and Preventive Medicine, Mumbai Veterinary College, Parel, Mumbai for pursuing his Post graduation education, which he completed successfully.

He was an active student all throughout the school and college days and had actively participated in extracurricular activities like cultural events, NSS camps, sports (MAFSU volleyball team captain), tournaments etc. During graduation days, in student council, he was a head of Public Relation committee.

He has successfully done his masters credit seminar on the topic of "RABIES GLOBAL SCENARIO: AN APPROACH OF EPIDEMIOLOGIST". He fulfilled the course work effectively and submitted thesis on "PREVALENCE OF POLYCYSTIC KIDNEY DISEASE OF PERSIAN CAT IN AND AROUND MUMBAI". He is presently a member of Maharashtra state veterinary council. The author can be contacted at abhishekgaikwad827@gmail.com