The Pharma Innovation Journal 2023; 12(5): 1386-1391

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(5): 1386-1391

www.thepharmajournal.com
Received: 07-02-2023
Accepted: 16-03-2023

Sadiq Khan M
M.V.Sc. Scholar, Department of Veterinary Gynaecology and Obstetrics, Veterinary College KVAFSU, Hebbal, Bengaluru, Karnataka, India

Sahadev A
Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Veterinary College KVAFSU, Hebbal, Bengaluru, Karnataka, India

Mohan HV
Assistant Professor, Department of Veterinary Public Health and Epidemiology, Veterinary College, Hebbal, Bengaluru KVAFSU, Karnataka, India

Renukaradhya GJ
Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Hebbal, Bengaluru, KVAFSU, Karnataka, India

Naveen Kumar S
Professor and Head, Department of Animal Genetics and Breeding, Veterinary College, Hebbal, Bengaluru KVAFSU, Karnataka, India

Veena MP
Assistant Professor, Department of Veterinary Physiology, Veterinary College, Hebbal, Bengaluru KVAFSU, Karnataka, India

Corresponding Author:
Sadiq Khan M
M.V.Sc. Scholar, Department of Veterinary Gynaecology and Obstetrics, Veterinary College KVAFSU, Hebbal, Bengaluru, Karnataka, India

Relative accuracy of diagnostic tests compared to canine prostate specific esterase for benign prostatic hyperplasia

Sadiq Khan M, Sahadev A, Mohan HV, Renukaradhya GJ, Naveen Kumar S and Veena MP

Abstract
Male intact dogs aged above 1 year presented to the college hospital during a period of 8 months were screened for benign prostatic hyperplasia (BPH) based on the presence of any of the clinical sign/s *viz*., constipation, dysuria, hematuria, haemorrhagic preputial discharges and also infertility. Additional 20 intact male dogs without any clinical symptom and having normal prostate on sonographic findings (Non-BPH) were considered as control to evaluate the relative accuracy of diagnostic tests *viz*., history and clinical signs, digital rectal examination (DRE), trans-abdominal ultrasonography (USG) and prostatic wash cytology (PWC) compared with canine prostate specific esterase for BPH. Dogs with one or the other clinical signs and with infertility were subjected for further diagnostic tests. Occurrence of BPH was 0.83% with more in dogs aged > 4-8 years (80%) and in Labrador (35%) and German shepherd (25%). Constipation and tenesmus (50%) were the most frequently recorded symptom followed by dysuria and haematuria (40%). The relative accuracy of diagnostic tests compared with CPSE was 92.50 for USG, 85.00 for PWC, 82.50 for clinical symptoms and 65.00% for DRE, respectively.

Keywords: BPH, CPSE, DRE, PWC, USG

Introduction
Benign prostatic hyperplasia (BPH) is a disease that develops spontaneously in intact male dogs, can start as early as 3 years of age as glandular hyperplasia. It is a symptom of ageing, which is characterised by hyperplasia as well as hypertrophy (Kutzler and Yeager, 2005) [19]. Almost all intact male dogs are likely to develop BPH with more than 95 percent dogs contracting the disease by 9 years of age. BPH is a common cause of infertility in the dogs due to the alteration of the biochemistry of the prostatic fluid whose important action on nutrition of spermatozoa is affected. Prostatitis or Prostatic abscess is likely a consequence of presence of blood in the prostate (Romagnoli and Schlafer, 2006) [31].

Dogs affected with BPH do no exhibit clinical signs until the enlarging prostate causes tenesmus or haematuria. Other clinical signs are urethral discharge or haemorrhagic preputial discharges, hemospermia, or rarely a stilted gait secondary to prostatic pain (Pinheiro *et al*., 2017) [27] resulting in discomfort for the animal. Hence, accurate diagnosis of BPH becomes important to address the pain to ensure comfort of the patient (Paclikova *et al*., 2006) [26] and to plan for the appropriate treatment. A tentative diagnosis could be made by history, physical examination, laboratory findings and imaging of the prostate. Though prostatic biopsy allows a definitive diagnosis but is rarely recommended as it is an invasive method (Smith, 2008) [34].

During physical examination enlarged prostate can be detected by rectal palpation (Lopate, 2013) [21]. Prostatic cytology and bacterial culture may be beneficial to rule out or to confirm the co-existence of multiple prostatic disorders but do not provide definitive diagnosis of BPH. Radiography allows evaluating prostatic size and positioning but it over estimates the prostate size (Feeney *et al*., 1987) [10]. Ultrasonographically confirmed prostatomegaly may be useful to confirm BPH with findings of above methods (Romagnoli and Schlafer, 2006) [31]. However, differential diagnosis of BPH from other prostatic diseases like prostatitis, squamous metaplasia, prostatic cyst and abscess and prostatic neoplasia is difficult due to the resemblance of the clinical and ultrasonographic findings. Under these limitations, (Levy *et al*., 2014) [20] suggested prostatic cytology or biopsy and estimation of serum biomarkers *viz*., canine prostate-specific esterase (CPSE) and dihydrotestosterone (DHT) for the BPH diagnosis.

The current study was carried out to record the incidence of BPH in intact male dogs (≥1 year) and to evaluate the relative accuracy of certain diagnostic methods considering the serum CPSE as standard for BPH.

Materials and Methods

Design of the experiment

Permission from Institutional Animal Ethics Committee was accorded to carry out this study in dogs (VCH/IAEC/2022/30; Dtd 10.08.2022). A total of 2404 male intact dogs aged ≥ 1 year presented to the college hospital during a period of 8 months were screened for BPH irrespective of age and breed that had showed any of the clinical signs *viz*., constipation, dysuria, haematuria, haemorrhagic/purulent preputial discharges, hemospermia, rectal tenesmus, dyschezia, dysuria, stranguria, caudal abdominal pain, gait abnormalities and with complaint of infertility. Among them, after excluding certain specific conditions with similar symptoms, 16 with clinical symptoms and 04 dogs with complaint of infertility were suspected for BPH and further they were subjected to digital rectal examination (DRE), ultrasonography of prostate (USG), prostatic wash cytology (PWC) and serum CPSE and DHT estimation. Based on history and preliminary examination, apparently healthy 20 intact male dogs with normal sonographic findings of the prostate (Non-BPH group) were taken for comparison. Accuracy parameters such as sensitivity, specificity, positive and negative predictive value of various routine diagnostic methods were evaluated comparing with the serum CPSE.

History and clinical signs: Detailed history such as the breed, age, gonadal status, history and duration of infertility (if any), clinical signs *viz*., general body condition, gait abnormalities, constipation/flattened stools, haemorrhagic/purulent preputial discharges (if present), normal colour/bloody/pus mixed urine, presence of pain on palpation of caudal abdomen and duration of illness presented to clinics were recorded. Twenty BPH suspected male dogs and 20 healthy intact male dogs without any such signs were subjected to further diagnostic tests.

Digital rectal examination (DRE): Following clinico-andrological examination the prostate of each dog including 20 healthy intact dogs was palpated perrectally using a lubricated gloved index finger and the information recorded was

a. Location of the prostate.
b. Consistency.
c. Symmetry.
d. Mobility.
e. Presence or absence of pain.

Trans-abdominal ultrasonography (USG): The dog was positioned in dorsal oblique recumbency, the hair in the supra-pubic area was clipped, coupling gel was applied liberally to enhance the contact and USG was carried out using a real time sector trans-abdominal probe (Aloka Prosound α6). The probe was placed against the ventral abdominal wall cranial to the pubis to image the gland. Urinary bladder was used as a landmark and the gland was located posterior to the trigone of the bladder, around the pelvic urethra (De Souza *et al*., 2017) [7]. After visualization, the capsule of the gland was evaluated for echogenicity, thickness and regularity and the prostatic parenchyma was examined for the echogenicity, presence or absence of cysts/abscesses, if any. Actual volume of the prostate gland was obtained by measuring height, length and width with the help of electronic calliper using double B mode in the ultrasound machine.

Volume ratio (V-ratio) of the prostate was computed using the formula, V-ratio = Actual prostatic volume/Expected prostatic volume (Alonge *et al*., 2018) [1]. The expected prostatic volume (cm^3) was estimated using the formula, Expected prostatic volume = 0.33 X body weight (kg) +3.28 (Sannamwong *et al*., 2012) [33]. V-ratio of ≥ 1.5 was taken as suggestive of BPH as recommended by Alonge *et al*. (2018) [1].

Prostatic wash cytology: Prostatic wash was collected from the dogs in the study for the cytological evaluation as per the procedures described by Johnston *et al*. (2001) [17]. The aspirate was centrifuged at 1500 rpm for 10 minutes and the cytosmears were prepared from sediment. The smears were air dried, fixed with methanol for 30-60 seconds and stained with giemsa for 20 minutes. The cytosmears after air drying were examined under high power (40X) for uniform sheets of prostatic epithelial cells with round nucleus and basophilic cytoplasm to conclude as BPH; neutrophils and bacteria to rule out prostatitis (Plate 1, 2 & 3). Based on the findings of prostatic wash cytology, the diagnosis was made as BPH or BPH with concurrent prostatitis.

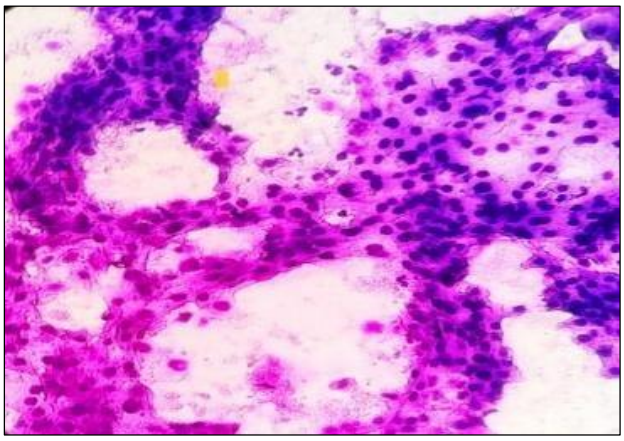

Plate 1: Photomicrograph of sheets of prostatic epithelial cells (Giemsa stain; 200 magnification)

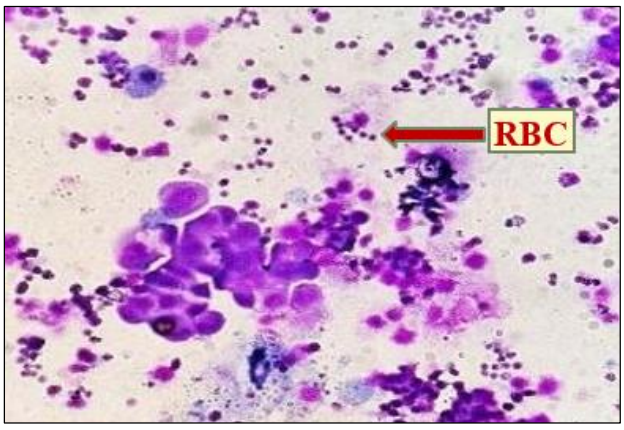

Plate 2: Photomicrograph of prostatic wash cytology showing cluster of prostatic epithelial cells with RBC (Giemsa stain, 400 magnification)

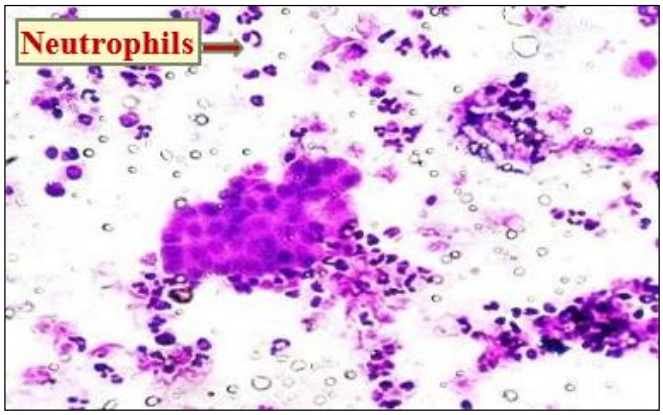

Plate 3: Photomicrograph of Prostatic wash cytology showing cluster of prostatic epithelial cells with neutrophils (Giemsa stain 400 magnification)

Estimation of serum biomarker: The study dogs after being subjected for DRE, USG and prostatic wash cytology, 2 mL of blood was collected in clot activated vacutainers and centrifuged at 2000-3000 rpm for 20 minutes. The serum was separated and stored at -20 ℃ until estimation of CPSE and DHT. Serum CPSE and DHT were assayed by standard sandwich ELISA method using kits (Canine prostate specific antigen, PSA ELISA kits and DHT ELISA kits, Chongquing Biospes Co. Ltd., Jiulongpo, China). The procedure of the estimation of CPSE and DHT was as per the instructions given by the manufacturer. Dogs were grouped as BPH and Non-BPH (Healthy intact) after confirmation with serum CPSE.

Statistical analysis: The serum CPSE, serum DHT levels and V-ratio of Prostatic volume of BPH and Non BPH dogs were analysed by unpaired t-test as per Steel and Torrie (1980) [35] using GraphPad prism version 5.0. The accuracy, sensitivity, specificity, positive and negative predictive values of each diagnostic test was obtained (Glaros and Kline, 1988) [13].

Outcome of the diagnostic test	Positive (BPH)	Negative (Non-BPH)
Positive	True positive (A)	False positive (B)
Negative	False negative (C)	True negative (D

Number of BPH dogs = A+C Number of Non-BPH dogs = B+D
Sensitivity = A/(A+C) x 100 Specificity = D/(B+D) x 100
Positive predictive value = A/(A+B) x 100 Negative predictive value = D/(C+D) x 100
Overall diagnostic accuracy = A+D/(A+B+C+D) x 100

Results and Discussion

Occurrence

Occurrence of BPH was 0.83 percent (20 out of 2404) in the present study confirmed by serum CPSE and similar incidence of 0.65 percent was reported by Dwivedi *et al.* (2021) [9] based on DRE and ultrasonography of the prostate. Age-wise occurrence of BPH in dogs in the present study was 20 percent (04) in dogs aged 1-4 years, 40 percent (08) each in dogs aged ≥ 4-8 and ≥ 8-12 years. Dhivya *et al.* (2012) [8] reported the incidence of 42 percent in dogs more than 8 years of age, 39 percent in 6-8 years and 19 percent in 4-6 years of age which is in agreement with the present study. In the present study breed-wise occurrence of BPH was found to be 35 percent (07) in Labrador retriever, 20 percent (04) in German shepherds, 10 percent (02) each in Shih Tzu and non-descript followed by 5 percent (01) each in Beagle, Boxer, Pug, Rottweiler and Cocker spaniel. Kraweic and Heflin (1992) [18] opined that Doberman pinscher and German shepherd were more frequently affected than other breeds. Gautam *et al.* (2019) [12] recorded 31.5 percent occurrence of BPH in Labrador retriever followed by German shepherd and Indian spitz, with 15.7 percent each which is almost similar with the present study. Nonetheless, the occurrence depends on sample size, region-based management practices and popularity of the breeds, duration of illness and diagnostic methods used for confirmation of BPH (Dhivya *et al*., 2012; Dwivedi *et al*., 2021) [8, 9].

Serum CPSE

In the present study the mean value of serum CPSE level in Non-BPH dogs was 57.69±1.55 ng/mL and ranged from 44.24-64.84 ng/mL, whereas the CPSE level in BPH dogs was 101.0 ±8.19 ng/mL with a range of 80.04-245.24 ng/mL and difference between Non-BPH and BPH dogs was significant (p<0.05). Similarly, Bell *et al*. (1995) [2] reported that the mean serum CPSE concentrations of BPH dogs were significantly higher (189.7 ng/mL) than in normal intact dogs (41.8 ng/mL). Paclikova *et al*. (2006) [26], Lévy *et al*. (2014) [20] and Wolf *et al.* (2012) [36] also reported that serum CPSE values to be significantly higher in BPH than in non-BPH dogs. A clinical threshold of 61ng/mL (range 54 ng/mL-67 ng/mL) of serum CPSE using ELISA kit (Odelis® CPSE, Virbac, France) for the diagnosis of BPH has been considered by several researchers (Lévy *et al*., 2014; Pinheiro *et al*., 2017; Cavalca Cardoso, 2019) [3, 20, 27] and 57.69 ± 1.55 ng/mL with a range of 44.24-64.84 ng/mL was considered as the clinical threshold in the present study using research kits.

Clinical symptoms

Out of 20 dogs confirmed for BPH using serum CPSE, 10 dogs (50%) exhibited constipation and tenesmus followed by 08 dogs each (40%) with dysuria and haematuria, haemorrhagic preputial discharge in 04 (20%), 02 (10%) each were with flattened stools, caudal abdominal pain and gait abnormality. However, 04 dogs (20%) did not exhibit any of the symptoms based on the history provided by the owner. Similar to the present study, common symptoms in dogs with BPH have also been reported by Kutzler and Yeager (2005) [19] and Holt (2007) [15]. Krawiec and Heflin (1992) [18] recorded constipation together with tenesmus as one of the important clinical signs of prostatic disease in aged dogs, due to compression of the colon by the enlarged prostate gland flattened stools (Das *et al*., 2017) [5]. In BPH dogs, dysuria or/and haematuria reported by some authors is due to centrifugal growth of the gland, dilated prostatic veins' inward growth result in compressing the urethra, sanguineous discharge from or ruptured blood vessels of highly perfused hyperplastic prostatic tissue (Reihmann and Bruskewitz, 1993; Read and Bryden, 1995; Smith, 2008) [29, 30, 34]. However, some dogs are asymptomatic initially and also without overt clinical signs during dog's life time (Pinheiro *et al*., 2017) [27] despite evidence of BPH based on CPSE. The

variations in recording the signs might be attributed to the time of presentation, failure to notice the symptoms and to differentiate from other conditions (Dwivedi *et al*., 2021) [9].

Digital rectal examination

In the present study, out of 20 dogs the location of the gland was intra-pelvic in 30 (06), partly intra-pelvic in 50 (10) and intra-abdominal in 20% (04) dogs. Consistency of the prostate was smooth and firm (spongy) in 60 (12) and hard in 40% (08) of dogs. Asymmetry of the prostate was found in 60 (12) and remaining 40% (08) had symmetrical prostate lobes. Prostate was movable in 75 (15) and fixed in 25% (05) of 20 dogs. Pain on palpation was observed in 90 (18) dogs and was absent in 10% (02) dogs.

Gadelha *et al.* (2009) [11] found mainly pelvic location in 1-3 years old dogs and 50% abdominal in intact dogs older than seven years by rectal palpation. In severe and advanced cases of BPH, the cranial portion of the prostate may be difficult to palpate by DRE suggestive of change in location of the prostate in disease process (Memon *et al*., 2007; Christensen, 2018) [4, 23]. Soft consistency of prostate was reported in 94.7 percent in dogs with BPH (Das *et al*., 2017) [5] and 18-46.2 percent of dogs with prostatic asymmetry was also reported by DRE (Mukaratirwa and Chitura, 2007; Ruetten *et al*., 2021) [25, 32]. Kutzler and Yeager (2005) [19] suggested that the lack of mobility of the prostate should be considered as one of the signs of prostatic disease including BPH. Pain on palpation was found in 90 percent and absent in 10 percent of dogs with BPH in the present study, which is almost similar with report of Das *et al.* (2017) [5]. Variations in findings and the proportion by different authors might be due to high subjective nature of the test and also, it is difficult to diagnose slight prostatomegaly when the prostate gland remains in the pelvic canal (Levy *et al*., 2014) [20].

Ultrasonographic findings of dogs with BPH and Non-BPH dogs

In the present study, the mean V-ratio in healthy dogs (1.11± 0.06) was significantly lesser (p<0.05) compared to that of the dogs with BPH (2.60 ± 0.20). Similar mean V-ratio was reported in BPH dogs by Holst *et al*. (2017) [14] where they considered a V-ratio of more than 2.5 for diagnosis of BPH with clinical signs and a CPSE threshold of 90 ng/mL. Alonge *et al*. (2018) [1], reported that the V-ratio > 1.5 is suggestive of BPH in asymptomatic dogs which is in agreement with the present study. Out of 20 BPH dogs, the prostatic capsule was normal and echogenic (55%) followed by thickened capsule (30%) and irregular capsule (15%) (Table 1). Prostatic parenchyma was cystic in 70% and normal and uniformly iso-echogenic in 30% of dogs. Prostatic capsule and parenchyma were normal and uniformly isoechogenic with a mean V-ratio of 1.11± 0.06 in all 20 Non-BPH dogs. Similar observations were also reported in many of the prostatic diseases (Johnston *et al.*, 2000; Davidson, 2003) [6, 16]. Irregular prostatic capsule has been considered as a diagnostic evaluation for BPH in dogs (Pinheiro *et al.*, 2017; Alonge *et al.*, 2018) [1, 27]. Menon (2008) [24] recorded 73.3 percent and Mantziaras *et al*. (2017) [22] also reported 74.3 percent of the BPH affected dogs with multiple anechoic/hypoechoic areas (cysts) in the prostatic parenchyma which concords with the present study.

Table 1: Ultrasonographic evaluation of prostate in Non-BPH and BPH dogs

Parameters	Non-BPH dogs (n=20)	BPH dogs (n=20)
V-ratio of the prostate (Mean ±SE)	1.11± 0.06 (0.69-1.95)	2.60 ± 0.20 * (1.42-4.69)
Prostatic capsule		
Normal and echogenic	20 (100%)	11 (55%)
Thickened	0	6 (30%)
Irregular	0	3 (15%)
Prostatic parenchyma		
Normal	20 (100%)	6 (30%)
Cyst/cysts	0	14 (70%)

Note: (Values in the parenthesis indicate the range/percentage)
*Significant at p<0.05.

Prostatic wash cytology

Microscopic examination of the cytosmear prepared using the sediment of prostatic wash revealed uniform sheets (clumps) of prostatic epithelial cells (Plate 1) with few RBCs (Plate 2) in 11(55%) dogs and 3 (15%) dogs out of 20 dogs with BPH revealed uniform sheets of epithelial cells along with RBC and WBC (Plate 3). Prostatic wash cytology without uniform sheets of epithelial cells was recorded in 6 (30%) dogs with BPH and 20 healthy (Non-BPH) dogs. Pinheiro *et al*. (2017)[27] conclusively diagnosed 100 percent of BPH cases by prostatic cytology by US-FNA in which they observed uniform clusters of prostatic epithelial cells. Further, they found 27.5 percent of dogs with BPH showed concomitant prostatitis (presence of a large number of neutrophils and associated macrophages and uniform clusters of prostatic cells. Powe *et al.* (2004) [28] have also reported similar results by US-FNA with prostatic histopathology.

Serum dihydrotestosterone (DHT)

Serum DHT levels in dogs with and without BPH were 5260 ± 390 pg/mL (2210-8860) and 4300 ± 310 pg/mL (1400-6290), respectively. The serum DHT level of dogs with BPH and without BPH did not vary significantly (Table 2). Wolf *et al.* (2012) [37] reported no differences in blood serum concentrations of DHT between the normal and BPH dogs but recorded highest prostatic fluid concentrations of DHT in dogs of >4 years of age (412.2 ± 181.1 pg/mL), ≤ 2 years (291.7 ± 166.0 pg/mL) and >2-4 years (313.8 ± 162.7 pg/mL). Recently, Yoon *et al.* (2020) [37] investigated the variations in serum sex hormone concentrations, DHT levels, prostatic volume and reported no significant difference in the serum sex hormone concentrations and prostatic blood volume of normal dogs and they also found four times higher intra-prostatic DHT level in dogs with BPH which was ascertained by western blot analysis. Further, they suggested that the rise in DHT levels in dogs with BPH despite the normal serum testosterone/DHT concentrations was due to blood retention within the prostate. Based on the literature available, it was evident that the accuracy of estimation of DHT in diagnosis of canine BPH is inconclusive.

The relative accuracy, sensitivity and specificity of diagnostic tests compared with CPSE was 92.50, 95.00 and 85.00 for USG, 85.00, 70.00 and 100 for PWC, 82.50, 80.00 and 85.00 for clinical symptoms and 65.00, 55.00 and 75.00% for DRE, respectively as mentioned in Table 2.

Table 2: Relative accuracy of various diagnostic methods for canine BPH in comparison with CPSE (n=20)

Diagnostic methods	USG	Prostatic Wash Cytology	Clinical symptoms	DRE
Sensitivity (%)	95.00	70.00	80.00	55.00
Specificity (%)	85.00	100	85.00	75.00
Positi Positive Predictive Value (PPV) (%)	90.00	100	84.21	69.00
Negative Predictive Value (NPV) (%)	94.73	86.96	81.00	62.50
Accuracy (%)	92.50	85.00	82.50	65.00

Conflict of interest
There is no competing interest among the authors.

Acknowledgement
The first author is highly thankful to the Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bengaluru for the facilities provided to carry out the work.

Conclusion
It was concluded that the incidence of BPH in dogs was 0.83 percent with more occurrence in the dogs aged >4 years in the present study. Considering the Serum CPSE as standard to diagnose BPH in male dogs the accuracy of USG was 92.50% followed by prostatic wash cytology (85.00%), clinical symptoms (82.50%) and DRE (65.00%). However, further studies with larger sample size using canine specific kits for CPSE estimation and DHT is required for diagnostic confirmation of BPH.

References
1. Alonge S, Melandri M, Leoci R, Lacalandra GM, Aiudi G. Canine prostate specific esterase (CPSE) as a useful biomarker in preventive screening programme of canine prostate: CPSE threshold value assessment and its correlation with ultrasonographic prostatic abnormalities in asymptomatic dogs. Reproduction Domestic Animals. 2018;53(2):359-364.
2. Bell FW, Klausner JS, Hayden DW, Lund EM, Liebenstein BB, Feeney DA, *et al*. Evaluation of serum and seminal plasma markers in the diagnosis of canine prostatic disorders. Journal of Veterinary Internal Medicine. 1995;9(3):149-153.
3. Cavalca Cardoso N. Development of Synchrotron Based Imaging Tools for Benign Prostatic Hyperplasia Using an Induced Canine Model. PG Thesis, University of Saskatchewan Saskatoon, Saskatchewan, Canada, 2019.
4. Christensen BW. Canine Prostate Disease. Veterinary Clinics of North America: Small Animal Practice. 2018;48(4):701-719.
5. Das MR, Patra RC, Das RK, Rath PK, Mishra BP. Haemato-biochemical alterations and urinalysis in dogs suffering from benign prostatic hyperplasia. Veterinary World. 2017;10(3):331-335.
6. Davidson EB. How I treat prostatic cysts and abscesses. In: Proc. North American Veterinary Conference, Orlando, Florida, USA; c2003. p. 1034-1035.
7. De Souza MB, Da Silva LD, M., Moxon R, Russo M, England GC. Ultrasonography of the prostate gland and testes in dogs. In Practice. 2017;39(1):21-32.
8. Dhivya TR, Sridevi P, Kulasekar K, Kumarasamy P. Incidence of benign prostatic hyperplasia in dogs. Indian Journal of Animal Reproduction. 2012;33(2):54-55.
9. Dwivedi PK, Shahi A, Mishra A, Jawre S, Singh R, Das B. Incidence of benign prostatic hyperplasia in dogs. Journal of Pharmaceutical Innovation. 2021;10(11):892-894.
10. Feeney D, Johnston G, Klausner J, Perman V, Leininger V, Tomlinson JR. Report of reproductive studies: canine prostatic disease-comparsion of radiographic appearance with morphologic and microbiologic findings: 30 cases (1981-1985). Journal of the American Veterinary Medical Association. 1987;190:1018-1026.
11. Gadelha CRF, Vicente WRR, Ribeiro APC, Apparício MF, Covizzi GJ, Machado LDS. Age-related ultrasonography, cytology and microbiologic exam of canine prostate. Brazilian Journal of Veterinary Research and Animal Science. 2009;61:1261-1267.
12. Gautam A, Sharma AK, Saxena AC, Kumar N, Gautam D, Verma N. Prostate affections in dogs: incidence and clinical analysis. Journal of Entomology and Zoology Studies. 2019;7:413-417.
13. Glaros AG, Kline RB. Understanding the accuracy of tests with cutting scores: The sensitivity, specificity and predictive value model. Journal of Clinical Psychology. 1988;44(6):1013-1023.
14. Holst BS, Holmroos E, Friling L, Hanas S, Langborg LM, Franko MA. The association between the serum concentration of canine prostate specific esterase (CPSE) and the size of the canine prostate. Theriogenology. 2017;93:33-39.
15. Holt PE. Prostate disease, In: SEVC (Southern European Veterinary Conference) Proceedings, International Veterinary Information Service, Ithaca, New York; c2007.
16. Johnston SD, Kamoplatana K, Root-Kustritz MV, Johnston GR. Prostatic disorders in the dog. Animal Reproduction Science. 2000;60:405-415.
17. Johnston SD, Kustritz MVR, Olson PNS. Disorders of the canine prostate, In: Canine and feline theriogenology, Edn. 1, W.B. Saunders, Phildelphia; c2001. p. 337-355.
18. Krawiec DR, Heflin D. Reports of retrospective studies: Study of prostatic disease in dogs: 177 cases (1981-1986). Journal of the American Veterinary Medical Association. 1992;200:1119-1122.
19. Kutzler M, Yeager A. Prostatic diseases. In: Textbook of veterinary internal medicine, Edt. Ettinger. Edn. 6, W.B. Saunders, Elsevier; c2005. p. 1809-1819.
20. Levy X, Nizanski W, Von Heimendahl A, Mimouni P. Diagnosis of common prostatic conditions in dogs: an update. Reproduction Domestic Animals. 2014;49(2):50-57.
21. Lopate C. Clinical approach to male pathologies. In: Reproduction and neonatology of dogs and cats, Edn. 2, veterinary editions, Torino; c2013. p. 202-206.
22. Mantziaras G, Alonge S, Faustini M, Luvoni GC. Assessment of the age for a preventive ultrasonographic examination of the prostate in the dog. Theriogenology. 2017;100:114-119.
23. Memon MA. Common causes of male dog infertility. Theriogenology. 2007;68(3):322-328.
24. Menon K. A clinical study on prostatic diseases of dogs. M.V.Sc. thesis, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, India; c2008.
25. Mukaratirwa S, Chitura T. Canine subclinical prostatic

disease: histological prevalence and validity of digital rectal examination as a screening test. Journal of South African Veterinary Assocociation. 2007;78:66-68.
26. Paclikova K, Kohout P, Vlasin M. Diagnostic possibilities in the management of canine prostatic disorders. Veterinarni Medicina. 2006;51(1):1-13.
27. Pinheiro D, Machado J, Viegas C, Baptista C, Bastos E, Magalhães J, *et al*. Evaluation of biomarker canine-prostate specific arginine esterase (CPSE) for the diagnosis of benign prostatic hyperplasia. BMC. Veterinary Research. 2017;13(1):1-7.
28. Powe JR, Canfield PJ, Martin PA. Evaluation of the cytologic diagnosis of canine prostatic disorders. Veterinary Clinical Pathology. 2004;33:150-154.
29. Read RA, Bryden S. Urethral bleeding as a presenting sign of benign prostatic hyperplasia in the dog: a retrospective study (1979-1993). Journal of American Anmal Hospital Association. 1995;31:261-267.
30. Reihmann H, Bruskewitz RC. Prostatic Diseases. In: Benign prostatic hyperplasia: manifestations and intervention. Edt. Lepor H and Lawson RK, WB Saunders Co. Philadelphia, USA; c1993. p. 80-108.
31. Romagnoli S, Schlafer DH. Disorders of sexual differentiation in puppies and kittens: a diagnostic and clinical approach. Veterinary Clinics of North America-Small Animal Practice. 2006;36(3):573-606.
32. Ruetten H, Wehber M, Murphy M, Cole C, Sandhu S, Oakes S, *et al*. A retrospective review of canine benign prostatic hyperplasia with and without prostatitis. Clinical Theriogenology. 2021;13(4):360-366.
33. Sannamwong N, Saengklub N, Sriphuttathachot P, Ponglowhapan S. Formula derived prostate volume determination of normal healthy intact dogs in comparison to dogs with clinical BPH. In: 7th International Symposium on Canine and Feline Reproduction, Whistler; c2012 July. p. 226.
34. Smith J. Canine prostatic disease: A review of anatomy, pathology, diagnosis and treatment. Theriogenology. 2008;70:375-383.
35. Steel RGD, Torrie JH. Principles and procedures of statistics, a biometrical approach, Edn. 2, McGraw-Hill Kogakusha, New York; c1980.
36. Wolf K, Kayacelebi H, Urhausen C, Piechotta M, Mischke R, Kramer S, *et al*. Testicular steroids, prolactin, relaxin and prostate gland markers in peripheral blood and seminal plasma of normal dogs and dogs with prostatic hyperplasia. Reproduction in Domestic Animals. 2012;47:243-246.
37. Yoon S, Alfajaro MM, Cho KO, Choi US, Je H, Jung J, *et al*. Perfusion change in benign prostatic hyperplasia before and after castration in a canine model: Contrast enhanced ultrasonography and CT perfusion study. Theriogenology. 2020;156:97-106.