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CANINE PROSTATE SPECIFIC ESTERASE (CPSE) AS AN USEFUL BIOMARKER IN PREVENTIVE SCREENING PROGRAM OF CANINE PROSTATE: CPSE THRESHOLD VALUE ASSESSMENT AND ITS CORRELATION WITH ULTRASONOGRAPHIC PROSTATIC ABNORMALITIES IN ASYMPTOMATIC DOGS

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STRUCTURED SUMMARY

Due to the increased attention that pet-owners devote to their animals and to the improved veterinary care, investigations regarding methods to early detect prostatic disorders that might affect canine life quality have been performed. Canine Prostatic Serum Esterase concentration (CPSE) was reported to be higher in dogs suffering from prostatic diseases. This study aimed to estimate the canine-prostate specific arginine esterase (CPSE) threshold as biomarker to early identify prostatic diseases in asymptomatic dogs. The ultrasonographic exam of the prostate was performed in 19 dogs (6-40 kg; 1-5 years) with no symptoms of prostatic diseases. Dogs were grouped according to the presence (group A) or absence (group B) of prostatic disorders at ultrasound (altered appearance, presence of cysts or irregular borders). For each dog, a venous blood sample was collected to measure serum CPSE and the ratio between calculated and normal expected prostatic volume was assessed for each dog. The CPSE data were statistically analyzed (t-Test, $p<0.05$) and the CPSE threshold in blood serum between groups was calculated by ROC. In 11 dogs, ultrasonography showed signs of prostatic abnormalities (group A, 2-5 years) while no signs were detected in 8 dogs (group B, 1-3 years). The calculated/estimated volume ratio resulted greater than 1.5 in group A dogs. The CPSE was statistically different between groups ($p<0.0001$): higher in group A (mean=184.9, SD=126 ng/ml) than in group B (38.9±22.1 ng/ml). The cut-off CPSE threshold was 52.3 ng/ml (ROC, AUC=0.974, SE 95.6%, SP 89.2%). This study suggests that CPSE serum concentration higher than 50 ng/ml in asymptomatic dogs is associated with ultrasonographic alterations and increased prostatic size (volume by 1.5 times greater than the normal size). Since the onset of prostatic disorders often remains asymptomatic, the rapid assessment of CPSE could be suitable for selecting preventively those animals that would require further accurate evaluation.

Running head: CPSE a biomarker for the canine prostatic health screening.

Keywords: CPSE, dog, ultrasonography, prostatic disease.

INTRODUCTION

Due to the increased attention that pet-owners devote to their animals and to the improved veterinary care, investigations regarding methods to early detect prostatic disorders that might affect the life quality of the dogs have been performed (Mukaratirwa and Chitura, 2007; Levy et al., 2014; Mantziaras et al., 2017). The most relevant clinical diseases of the gland are benign prostatic hyperplasia, prostatic cyst, prostatitis and prostatic neoplasia (Johnston et al., 2001). Since the canine prostatic diseases are symptomless in their onset and difficult to be diagnosed in their early stage, most of the times they are recognized at advanced stage (Johnston et al., 2000; Mantziaras et al., 2017). In men, due to the improved diagnostic tools, such as the serum prostate specific antigen (PSA) test, the early recognition of subclinical cases (with no clinical symptoms) increases the incidence of diagnosed prostatic diseases (Mukaratirwa and Chitura, 2007). For this reason, several authors focused their attention on the identification of canine serum marker similar to those routinely used in human medicine (Chapdelaine et al., 1984; Levy and Mimouni, 2009; Holst et al., 2017). Since '90, it was suggested that the Canine Prostatic Serum Esterase concentration (CPSE), that represents the major secretory product of the canine prostatic gland could be a useful diagnostic biomarker to identify dogs suffering from prostatic diseases (Chapdelaine et al., 1984; Bell et al., 1985). More recently several authors reported that higher CPSE was observed in dogs suffering from prostatic disorders such as benign prostatic hyperplasia (BPH), bacterial prostatitis or prostatic carcinoma (Teintflat et al., 2000; Levy and Mimouni, 2009; Wolf et al., 2012) and among these abnormalities, benign prostatic hyperplasia (BPH) represents the most common physio-pathological alteration of the gland (Levy et al., 2014). The diagnosis is usually based on clinical signs in combination with an enlarged gland. In clinical veterinary practice, the most commonly used methods to diagnose canine prostatic gland diseases are digital rectal examination and abdominal ultrasound (Newell et al., 1998; Mukaratirwa and Chitura, 2007; Mantziaras et al., 2017). Recently some authors suggested that the monitoring of local blood flow by Doppler or Contrast-Enhanced UltraSonography is helpful in differentiating prostatic physio-pathological conditions (Bigliardi and

Ferrari 2011; Russo et al., 2012; Troisi et al., 2015; Alonge et al., 2017). Cytological evaluation by fine-needle aspiration or biopsy of the prostate is usually performed to confirm the benign nature of the prostate volume enlargement (Kraft et al., 2005; Paclikova et al., 2006; Davidson and Baker, 2009; Levy et al., 2014). Nevertheless, some dogs might have an enlarged prostate without symptoms (Russo et al., 2012). For this reason, a prostate health-screening program could obtain a more reliable estimation of the prevalence of canine prostatic disorders and the CPSE might be a potentially suitable biomarker for this purpose in dogs, as in men. Thus, the aim of this study was to estimate the CPSE threshold to be used in clinical practice to early identify prostatic diseases in clinically asymptomatic dogs with just ultrasonographic signs of prostatic disorders.

MATERIALS AND METHODS

Animals

Nineteen dogs of different breeds (BW: 6-40 kg, age: 1-5 years) with no symptoms of prostatic diseases were included in this study. Each dog underwent clinical examination including a thorough history, and a rectal exploration of the prostate. All were healthy.

Procedure

In each dog, a venous blood sample was collected from the cephalic vein to measure serum CPSE (Speed CPSE, Virbac, Italy). Samples were stored at -20 °C until CPSE was analyzed using a commercial assay (Speed Reader, Virbac, Italy).

For the ultrasonography evaluation, dogs were positioned in lateral recumbency, transmission gel was applied, and two-dimensional, gray-scale, real-time ultrasound images were produced using a 5-7.5 MHz microconvex probe (MyLab™ClassC, Esaote Spa, Genua, Italy). Ultrasonographic prostate appearance, border and volume were evaluated as reported in the literature (Davidson and Baker, 2009). The ultrasonography exam allowed an accurate evaluation of the prostate for changes in its normal position, parenchyma, symmetry and shape.

The normal intact canine prostate gland is mainly located in the pelvic region and only the cranial part of the gland is located in the abdominal cavity. The echogenicity, similar to that in the spleen, is fairly uniform with a smooth, stippled texture. Its shape is symmetrically bilobed in the transverse plane and oval in the longitudinal plane (Gobello and Corrada, 2002; Davidson and Baker, 2009).

Dogs were grouped according to the presence (group A) or absence (group B) of ultrasonographic signs of prostatic disorders (i.e. altered appearance, border, cysts).

The ratio between the calculated prostatic volume (according to the formula published by Ruel *et al.*, 1998: volume = length x width x height x 0.523) (Ruel et al., 1998) and the estimated normal volume (according to the formula suggested by Sannamwong *et al.*, 2012: expected normal volume = 0.33 x BW (Kg) + 3.28) (Sannamwong et al., 2012) was assessed for each dog.

Statistical analysis

The normal distribution of CPSE data in the two groups was assessed and CPSE values were statistically compared (t-Test, $p<0.05$). The CPSE threshold in blood serum between groups was calculated by a Receiver Operating Characteristic analysis: web-based calculator for ROC curves (Eng, 2017).

RESULTS

As reported in table 1, 11 out of the 19 dogs examined, showed ultrasonographically altered prostate (group A, 2-5 years). Five dogs out from the eight dogs that showed cysts, presented also one or more other US abnormalities [altered parenchyma echogenicity (n.=3), asymmetrical lobes (n.=1), gland border alteration (n.=3)]. Three dogs presented altered echotexture/echogenicity and/or irregular gland borders. Finally, in the remaining eight dogs (group B, 1-3 years) prostate was considered normal. The calculated/estimated volume ratio resulted greater than 1.5 in all group A dogs and lower than this threshold in all the dogs of group B. The CPSE was statistically different

between groups ($p<0.0001$), being higher in group A (mean ± SD, 184.9 ± 126 ng/ml) than in group B (mean ± SD, 38.9 ± 22.1 ng/ml).

The cut-off for the CPSE threshold in blood serum between group A and B from the web-based calculator for ROC curves (Eng, 2017) resulted 52.3 ng/ml (AUC=0.974, SE 95.6%, SP 89.2%) (Fig. 1).

DISCUSSION

This study confirms that CPSE represents a useful tool to early detect prostatic disorders in dogs. Some authors have previously suggested that prostate size could be affected by age, breed, and body weight, but also by the emergence of pathologic processes, making the establishment of normal dimensions difficult because of the wide variety of canine sizes, breeds and conditions (O'Shea, 1962; Cartee and Rowels, 1983; Atalan et al., 1999; Smith, 2008; Freitas et al., 2015). Several studies were performed in order to correctly establish the actual prostatic volume, through the two dimensional ultrasonographic prostatic measures. (Ruel et al., 1998; Atalan et al., 1999; Kamolpatana et al., 2000; Gobello and Corrada 2002). Morever, it was stated that the ultrasonographic exam is a highly dependent diagnostic imaging modality whose measurements accuracy might depend by the operator's ability and experience (Leroy et al., 2013). Thus, recently the Computed Tomography exam of the prostate was proposed (Dimitrov et al., 2010; Lee et al., 2011; Pasikowsha et al., 2015). The Computed Tomography examination would allow a more precise and repeatable measurements inter-observers, but it is less accessible, more expensive and time consuming, and requires general anesthesia (Pasikowsha et al., 2015). On the other hand, the ultrasonographic exam of the canine prostate is non-invasive and fast and so it remains the diagnostic imaging tool of choice for the evaluation of this organ (Mantziaras et al., 2017). Anyway, for all these reasons, since a clear threshold measure to identify the normal volume of the canine prostate dogs does not exist, in the present study dogs were grouped in accordance to

presence/absence of other ultrasonographical abnormal findings (i.e. altered appearance, border, cysts).

Almost the 60% of the asymptomatic dogs presented at the ultrasonography an altered prostate (Group A) while the gland resulted echographically normal in the remaining 40% of the animals (Group B). This result confirms that prostatic disorders often remain asymptomatic, therefore may be under-estimated (Levy et al., 2014; Polisca et al., 2016; Mantziaras et al., 2017). Due to the lack of pathognomonical clinical signs, at least at their onset, in the general dog population prostatic disorders often go unnoticed, while these abnormalities are diagnosed more frequently in dogs presented for poor fertility evaluation (Polisca et al., 2016). In this last case, an ultrasonographic exam of the prostate is strongly recommended when a presumptive diagnosis of BPH is based on detection of blood in prostatic fluid of the ejaculate or in case of a presumptive diagnosis of chronic prostatitis based on signs of infertility or decreased libido (Johnston et al., 2000; Lopate, 2012).

Present results show that prostate affected by ultrasonographically detectable abnormalities show a volume at least 1.5 times greater than the normal expected volume. Many dogs do not exhibit clinical signs even in case of hyperplastic growth of the gland (Palmieri et al., 2014), most likely because the outward expansion of the canine prostate (McConnell, 1991) compared to the inward nodular growth that compress the urethra in men (McNeal, 1978). Recently, it was suggested that dogs with a prostatic calculated volume higher than 2.5 times greater than the normal expected volume present clinical signs and a CPSE concentration higher than 90 ng/ml (Holst et al., 2017).

In the present study the concentration of CPSE was significantly associated with the presence of prostatic abnormal findings that could be identified by the ultrasound exam. Although, a histological confirmation of prostatic disease would have been desirable to definitively confirm the diagnosis, it is nearly impossible also in everyday routine practice when no one owner would agree to perform a more invasive procedure in an asymptomatic and apparently healthy dog, such as the prostate biopsy. On the other hand, the CPSE is a known marker for prostatic secretion, that can be easily assessed by a simple blood serum sample. It constitutes more than 90% of seminal proteins in

dogs, but its exact role in the different prostatic disorders is not yet completely understood (Frenette et al., 1987; Gobello et al., 2002). Recently, it was suggested that the CPSE is secreted in the form of prostasomes (prostate granules) to semen during ejaculation. The activity of CPSE is regulated by the level of available zinc that is of great importance for maintaining the normal functions of the prostate and the spermatozoa. (Mogielnicka-Brzozowska et al., 2015). The production of CPSE is regulated by androgen hormone (testosterone), it can be inhibited by the surgical castration or anti-androgen treatment (Frenette et al., 1983; Isaacs and Sharper; 1985; Juniewicz et al., 1990) or promoted by the exogenous androgen administration following the surgical castration (Frenette et al., 1983; Isaacs and Sharper; 1985). Thus, the canine CPSE and the human PSA, even though their different biological activity (CPSE: trypsin-like; PSA: chymotrypsin-like), are under identical hormonal regulation (Dube et al., 1986; Clement, 1989). Moreover, for this reason the CPSE seems to be a promising diagnostic tool for the detection of prostatic disorders in a "prostate health screening program" similarly to PSA in human medicine (Gobello et al., 2002).

Recently, it was suggested that, in dogs over the 40% of the maximum expected longevity for the breed, a preventive ultrasonographic screening program would be advisable (Mantziaras et al., 2017). In that study, the authors reported a strong probability to detect prostatic abnormal findings by ultrasonographic exam irrespective of clinical evidence (Mantziaras et al., 2017).

CONCLUSIONS

Results of the present study indicate that serum concentration higher than 50 ng/ml in asymptomatic dogs is associated with ultrasonographic alterations and increased prostate size (volume by 1.5 times greater than the normal size). In clinical practice, since the onset of prostatic disorders, such as BPH, often remains asymptomatic until aggressive therapy is required, the rapid assessment of CPSE could be suitable like a preventive screening tool to select preventively those animals that would require further more accurate, more time-consuming and more expensive evaluations before clinical signs appear.

DECLARATIONS - Ethical Guidelines committee

The present study was performed in accordance with the ethical guidelines of the animal welfare committee. Institutional Review Board approval of the study was obtained by the University of Bari "Aldo Moro", Ethic Committee DETO, Italy (Protocol N° 35/17 DETO; 26/06/2017). Procedures with animals were performed following good veterinary practice for animal welfare according to national laws in force (D.Lgs 116/92). Informed owner consent was obtained.

Authors' contributions

All authors contributed to design the study, collect, analyze the data and draft the paper. All authors have approved the final version.

Competing Interests

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

REFERENCES

Alonge, S., Melandri, M., Fanciullo, L., Lacalandra, G., Aiudi, G. (2017) Prostate vascular flow: the effect of the ejaculation on the power doppler ultrasonographic examination. Reprod Domest Anim, DOI: 10.1111/rda.13078;

Atalan, G., Holt, P. E., Barr, F. J. (1999). Ultrasonographic estimation of prostate size in normal dogs and relationship to bodyweight and age. J Small Anim Pract, 40, 110–122;

Bell, F. W., Klausner, J. S., Hayden, D. W., Lund, E. M., Liebenstein, B. B., Feeney, D. A., Johnston, S. D., Shivers, J. L., Ewing, C. M., Isaacs, W. B. (1985). Evaluation of serum and seminal plasma markers in the diagnosis of canine prostatic disorders. J Vet Intern Med, 9, 149–153;

Bigliardi, E., Ferrari, L. (2011). Contrast-enhanced ultrasound of the normal canine prostate gland. Vet Radiol Ultrasound, 52, 107-110;

Cartee, R. E., Rowels, T. (1983). Transabdominal sonographic evaluation of the canine prostate. Vet Radiol, 24, 156–164;

Chapdelaine, P., Dube, J. Y., Frenette, G., Tremblay, R. R. (1984). Identification of arginine esterase as the major androgen-dependent protein secreted by dog prostate and preliminary molecular characterization in seminal plasma. J Androl, 5, 206-210;

Clement, J. A. (1989). The glandular kallikreinin family of enzymes: tissue-specific expression and hormonalregulation. Endocrinol Rev, 10, 39-419;

Davidson, A. P., Baker, T. W. (2009). Reproductive Ultrasound of the dog and tom. Top Companion Anim Med, 24, 64-70;

Dimitrov, R., Yonkova, P., Vladova, D., Kostrov, D. (2010). Computed tomography imaging of the topographical anatomy of canine prostate. Trakia J Sci, 8, 78-82;

Dube, J. Y., Lazure, C., Tremblay, R. R. (1986). Dog prostate arginine esterase is related to human prostate-specific antigen. Clin Invest Med, 9, 51-54;

Eng, J. (2017). ROC analysis: web-based calculator for ROC curves. Baltimore: Johns Hopkins University. Available from: http://www.jrocfit.org;

Freitas, L.A., Pinto, J. N., Silva, H. V. R., Machado da Silva, L. D. (2015). Two-dimensional and Doppler sonographic prostatic appearance of sexually intact French Bulldogs. Theriogenology, 83, 1140–1146;

Frenette, G., Dube, J. Y., Lacoste, D., Tremblay, R. R. (1987). Radioimmunoassay in blood plasma of arginine esterase: the major secretory product of dog prostate. Prostate, 10, 145:152;

Frenette, G., Dube, J. Y., Marcotre, J. R., Tremblay R. R. (1983). Effects of castration and steroid synthesis on the activity of some hydrolitic enzymes in the dog prostate. Prostate, 4, 206-210;

Gobello, C., Castex, G., Corrada, Y. (2002). Serum and seminal markers in the diagnosis of disorders of the genital tract of the dog: a mini-review. Theriogenology, 57, 1285-1291;

Gobello, C., Corrada, Y. (2002). Noninfectious prostatic diseases in dogs. Compend Contin Educ Vet, 2, 99-107;

Holst, B. S., Holmroos, E., Frilling, L., Hanas, S., Langborg, L. M., Franko, M. A., Hansson, K. (2017). The association between the serum concentration of canine prostate specific esterase (CPSE) and the size of the canine prostate. Theriogenology, 93, 33-39;

Isaacs, W., Sharper, J. H. (1985). Immunological localization and quantification of the androgen dependent secretory protease of canine prostate. Endcrinology, 117:1512-1520;

Johnston, S. D., Kamolpatana, K., Root-Kustritz, M. V., Johnston, G. R. (2000). Prostatic disorders in the dog. Anim Reprod Sci, 60-61, 405-415;

Johnston, S. D., Root-Kustritz, M. V., Olson, P. N. (2001). Disorders of the canine prostate. In: Johnston, S. D., Root-Kustritz, M. V., Olson, P. N. (Eds.) Canine and feline theriogenology. Saunders Co, Philadelphia; 2001 pp 337–355;

Juniewicz, P. E., Barbolt, T. A., Egy, M. A., Frenette, G., Dube, J. Y., Tremblay, R. R. (1990). Effects of androgen and antiandrogen treatment on canine prostatic arginine esterase. Prostate, 17, 101-11;

Kamolpatana, K., Johnston, G. R., Johnston, S.D. (2000). Determination of canine prostatic volume using transabdominal ultrasonography. Vet Radiol Ultrasound, 41, 73-77;

Kraft, M., Brown, H.M., LeRoy, B. (2005). Citology of the canine prostate. Ir Vet J, 5, 320-324;

Lee, K. J., Shimizu, J., Kishimoto, M., Kadohira, M., Iwasaki, T., Miyake, Y. I., Yamada, K. (2011). Computed tomography of the prostate gland in apparently healthy entire dogs. J Small Anim Pract, 52, 146-151;

Leroy, C., Conchou, F., Layssol-Lamour, C., Deviers, A., Sautet, J., Concordet, D., Mogicato, G. (2013). Normal canine prostate gland: repetibility, reproducibility, observer-dependent variability of ultrasonographic measurements of the prostate in healthy intact beagles. Anat Histol Embryol, 42, 355-361;

Levy, X., Mimouni, P. (2009). Hyperplasie benigne de la prostate: actualites. Le point veterinaire, 293, 39–43;

Levy, X., Nizanski, W., von Heimendahl, A., Mimouni, P. (2014). Diagnosis of common prostatic conditions in dogs: an update. Reprod Domest Anim, 49 (Suppl 2), 50-57;

Lopate, C. (2012). The problem stud dog. Vet Clin North Am Small Anim Pract, 42, 469-488;

Mantziaras, G., Alonge, S., Faustini, M., Luvoni, G. C. (2017). Assessment of the age for a preventive ultrasonographic examination of the prostate in the dog. Theriogenology, 100, 114 – 119;

McConnell, J. D. (1991). The pathophysiology of benign prostatic hyperplasia. J Androl, 12, 356–363;

McNeal, J. E. (1978). Origin and evolution of benign prostatic enlargement. Invest Urol, 15, 340–345;

Mogielnicka-Brzozowska, M., Kowalska, N., Fraser, L., Kordan. W. (2015). Proteomic characterization of zinc-binding proteins of canine seminal plasma. Reprod Domest Anim, 50, 1017-1021;

Mukaratirwa, S., Chitura, T. (2007). Canine subclinical prostatic disease: histological prevalence and validity of digital rectal examination as a screening test. J S Afr Vet Assoc, 78, 66-68;

Newell, S. M., Neuwirth, L., Ginn, P. E., Roberts, G. D., Prime, L. S., Harrison, J. M. (1998). Doppler ultrasound of the prostate in normal dogs and in dogs with chronic lymphocytic-lymphoplastic prostatitis. Vet Radiol Ultrasound, 39, 332-334;

O'Shea, J. D. (1962). Studies on the canine prostate gland. I. Factors influencing its size and weight. J Comp Pathol, 72, 321–31;

Palmieri, C., Lean, F. Z., Akter, S. H., Romussi, S., Grieco, V. (2014). A retrospective analysis of 111 canine prostatic samples: histopathological findings and classification. Res Vet Sci, 97,568-573;

Paclikova, K., Kohout, P., Vlasin, M. (2006). Diagnostic possibilities in the management of canine prostatic disorders. Vet Med, 51, 1-13;

Pasikowsa, J., Hebel, M., Nizanski, W., Nowak, M. (2015) Computed tomography of the prostate gland in healthy intact dogs and dogs with benign prostatic hyperplasia. Reprod Domest Anim, 50, 776-783;

Polisca, A., Troisi, A., Fontaine, E., Menchetti, L., Fontbonne, A. (2016). A retrospective study of canine prostatic diseases from 2002 to 2009 at the Alfort Veterinary College in France. Theriogenology, 85, 835-840;

Ruel, Y., Barthez, P. Y., Mailles, A., Begon, D. (1998). Ultrasonographic evaluation of the prostate in healthy intact dogs. Vet Radiol Ultrasound, 39, 212-216;

Russo, M., Vignoli, M., England, G. C. (2012). B-mode and contrast-enhanced ultrasonographic findings in canine prostatic disorders. Reprod Domest Anim, 47(Suppl 6), 238-242;

Sannamwong, N., Saengklub, N., Sriphuttathachot, P., Ponglowhapan, S. (2012). Formula derived prostate volume determination of normal healthy intact dogs in comparison to dogs with clinical BPH. In: England, G., Kutzler, M., Comizzoli, P., Nizanski, W., Rijsselaere, T., Concannon, P., eds. 7th international symposium on canine and feline reproduction. Whistler, Canada, p 226;

Smith, J. (2008). Canine prostatic disease: a review of anatomy, pathology, diagnosis, and treatment. Theriogenology, 70, 375–383;

Teintflat, M., Miller, I., Loupal, G., Thalhamme, J. G., Gemeiner, M. (2000). Quantitative determination of canine prostatic-specific protein and its clinical relevance. Tierarztlinche Praxis Ausgabe Kleimtiere, 28, 127-131;

Troisi, A., Orlandi, R., Bargellini, P., Menchetti, L., Borges, P., Zelli, R., Polisca, A. (2015) Contrast-enhanced ultrasonographic characteristics oft he diseased canine prostatic gland. Theriogenology, 84, 1423-1430;

Wolf, K., Kayacelebi, H., Urhausen, C., Piechotta, M., Mischke, R., Kramer, S., Einspanier, A., Oei, C. H., Guenzel-Apel, A. (2012). Testicular steroids, prolactin, relaxin and prostate gland markers in peripheral blood and seminal plasma of normal dogs and dogs with prostatic hyperplasia. Reprod Domest Anim, 47(Suppl 6), 243-246.

Figure 1. The cut-off for the CPSE threshold in blood serum between group A and group B from the web-based calculator for ROC curves (Eng, 2017).

Table 1. Canine Prostatic Specific Esterase serum concentration and measurements of the prostate in the 19 dogs ultrasonographically examined grouped according Presence (Group A) or absence (Group B) of abnormal ultrasonographic findings (altered appearance, cysts or irregular borders).

Dog	Age years	Breed	BW Kg	CPSE ng/ml	Abnormal findings	H cm	L cm	W cm	Ruel et al 1998	Sannamwong et al 2012	Volume ratio	Group
1	1	weimaraner	29	11.49	no	1.88	2.14	2.60	5.47	12.85	0.43	B
2	1	dachshund	6	47.26	no	1.86	2.23	2.45	5.31	5.26	1.01	B
3	1.35	pointer	20	45.00	no	3.19	2.33	3.03	11.78	9.88	1.19	B
4	1	mongrel	30.4	78.36	no	2.65	3.87	3.28	17.59	13.31	1.32	B
5	3	weimaraner	31.5	49.10	no	3.08	3.43	3.32	18.34	13.68	1.34	B
6	2	weimaraner	34	38,96	no	3,77	2.81	3.71	20.53	14.50	1.42	B
7	3	weimaraner	30.5	9.65	no	3.08	3.79	3.11	18.99	13.35	1.42	B
8	1.4	pointer	20	31.88	no	3.18	2.53	3.40	14.31	9.88	1.45	B
9	2.5	labrador	34	75.52	yes	2.89	3.85	3.85	22.37	14.50	1.54	A
10	3	husky	25.5	178.39	yes	3.50	2.67	3.75	18.33	11.70	1.57	A
11	2.5	bracco	28	52.30	yes	2.97	3.40	3.80	20.07	12.52	1.60	A
12	2	weimaraner	34	289.51	yes	3.55	3.23	4.14	24.83	14.50	1.71	A
13	5	mongrel	37	120.95	yes	3.42	3.22	4.76	27.42	15.49	1.77	A
14	4	rottweiler	40	200.62	yes	3.00	3.96	4.91	30.51	16.48	1.85	A

15	4	rottweiler	40	236.27	yes	3.56	3.45	4.85	31.15	16.48	1.89	A
16	2.4	chow chow	30	115.54	yes	3.01	4.20	4.00	26.45	13.18	2.01	A
17	5.5	german sh.	36.5	171.93	yes	3.28	4.98	4.61	39.38	15.33	2.57	A
18	3.5	bouledogue	12.8	92.47	yes	2.80	3.70	3.95	21.40	7.50	2.85	A
19	4	amstaff	32.5	500.00	yes	3.44	5.73	5.77	59.48	14.01	4.25	A

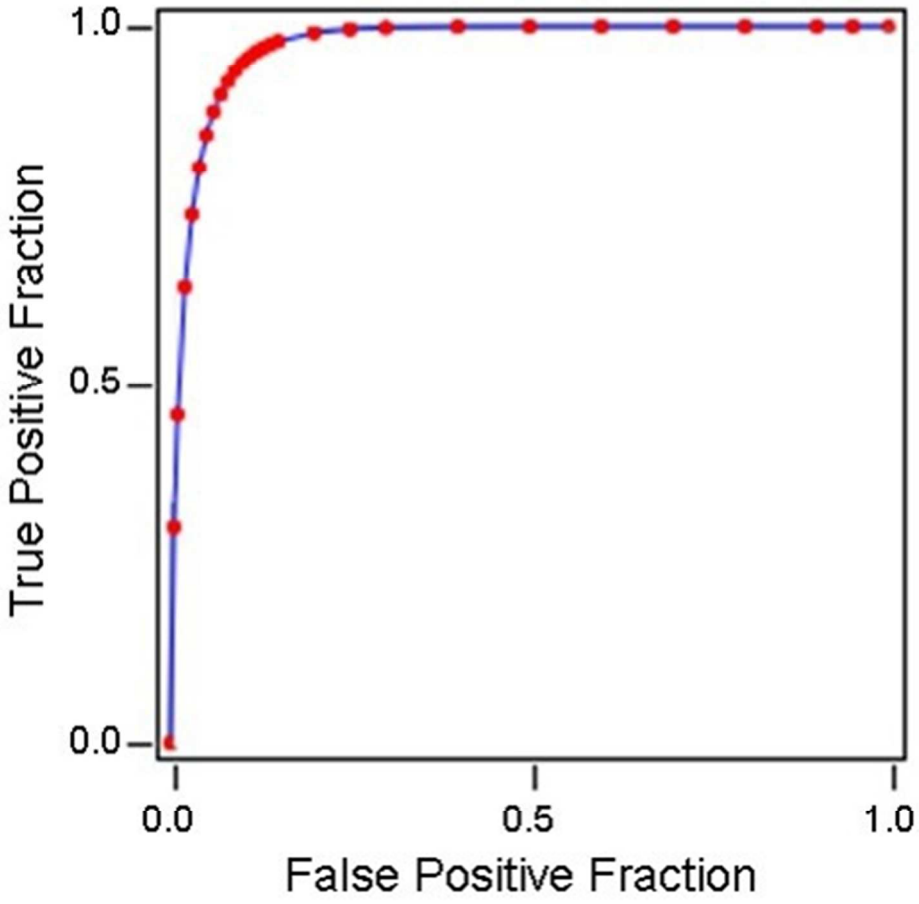

Figure 1. The cut-off for the CPSE threshold in blood serum between group A and group B from the web-based calculator for ROC curves (Eng, 2017).

101x97mm (128 x 128 DPI)

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

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To the Editor of Reproduction in Domestic Animals

Bari, October 11th , 2017

Dear Editor,

please find enclosed the REVISED manuscript:

"CANINE PROSTATE SPECIFIC ESTERASE (CPSE) AS AN USEFUL BIOMARKER IN PREVENTIVE SCREENING PROGRAM OF CANINE PROSTATE: CPSE THRESHOLD VALUE ASSESSMENT AND ITS CORRELATION WITH ULTRASONOGRAPHIC PROSTATIC ABNORMALITIES IN ASYMPTOMATIC DOGS."

by Salvatore ALONGE, Monica MELANDRI, Raffaella LEOCI,

Giovanni Michele LACALANDRA, and Giulio AIUDI

The REVISED manuscript and "Point to Point reply to reviewers" (see below) have been approved by all co-authors.

Correspondence regarding the paper should be directed to the following address:

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Thank you for your attention.

Yours sincerely,

Salvatore Alonge

Manuscript ID RDA-OA-Sep-2017-0367

entitled "CANINE PROSTATE SPECIFIC ESTERASE (CPSE) AS AN USEFUL BIOMARKER IN PREVENTIVE SCREENING PROGRAM OF CANINE PROSTATE: CPSE THRESHOLD VALUE ASSESSMENT AND ITS CORRELATION WITH ULTRASONOGRAPHIC PROSTATIC ABNORMALITIES IN ASYMPTOMATIC DOGS"

The paper has been revised according to the Reviewer's suggestions.

We thank for the constructive criticism which contribute to improve the quality of the paper.

Referee(s)' Comments to Author:

Referee: 1

Comments to the Author

The article is interesting and has clinical application of the marker CPSE

Introduction

Line 45 : Specify the validity of ultrasonography in the diagnosis of prostate diseases also associated with the use of contrast agent such as indicated in the article CONTRAST-ENHANCED ULTRASOUND OF THE NORMAL CANINE PROSTATE GLAND Bigliardi E. Veterinary Radiology & Ultrasound 2011, Doppler ultrasound of the prostate in normal dogs and in dogs with chronic lymphocytic-lymphoplasmocytic prostatitis. Newell SM, Veterinary Radiology & Ultrasound 1998

Authors: Please, see line 46, this text was added.

In clinical veterinary practice, the most commonly used methods to diagnose canine prostatic gland diseases are digital rectal examination and abdominal ultrasound (Newell et al., 1998; Mukaratirwa and Chitura, 2007; Mantziaras et al., 2017). Recently some authors suggested that the monitoring of local blood flow by Doppler or Contrast-Enhanced UltraSonography is helpful in differentiating prostatic physio-pathological conditions (Bigliardi and Ferrari 2011; Russo et al., 2012; Troisi et al., 2015; Alonge et al., 2017).

Material and Methods

Line 66: the common position for ultrasonography evaluation of prostate is dorsal recumbency that allows the evaluation of both lobes, constant relationships with surrounding structures and similar projections

Authors: The patient was accurately examined in the lateral recumbency in order to minimize the necessary restraint.

Line 68: the 7,5 MHz probe in dog with BW >25 kg is not appropriate (5 Mhz)

Authors: We agree, the line was corrected 5-7.5 MHz (lines 73)

Line 70: Specify the parameters used to evaluate the alterations of ecogenicity, symmetry, position etc.

Authors: Parameters (and literature) were specified (see lines 77-80).

The normal intact canine prostate gland is mainly located in the pelvic region and only the cranial part of the gland is located in the abdominal cavity. The echogenicity, similar to that in the spleen, is fairly uniform with a smooth, stippled texture. Its shape is symmetrically bilobed in the transverse plane and oval in the longitudinal plane (Gobello and Corrada, 2002; Davidson and Baker, 2009).

Result

Line 88: Specifies how many dogs had alterations related to changes in ecogenicity, cysts, gland border alteration, and so on.

Authors: More detailed results were reported (see lines 95-100).

As reported in table 1, 11 out of the 19 dogs examined, showed ultrasonographically altered prostate (group A, 2-5 years). Five dogs out from the eight that showed cysts, presented also one or more other US abnormalities [altered parenchyma echogenicity (n.=3), asymmetrical lobes (n.=1), gland border alteration (n.=3)]. Three dogs presented altered echotexture/echogenicity and/or irregular gland borders. Finally, in the remaining eight dogs (group B, 1-3 years) prostate was considered normal.

If the Authors have evaluated the levels of acid phosphatase in relation with CPSE can be useful.

Authors: We appreciate your suggestion but unfortunately these Acid Phospatase levels have not been evaluated in this study thus we cannot describe any relation between them and the CPSE level.

Referee: 2

Comments to the Author

General comments:

This is submission which lies perfectly in the scope of the journal. Scientifically elaborated clinical work. Authors analysed the CPSE blood concentration in the group of normal dogs and prostatomegalic dogs, both groups asymptomatic. It was proved that the concentration of CPSE in dogs with prostatomegaly is far higher. The cut off values of CPSE between normal/abnormal group is 50 ng/mL. Interesting observation. Interesting especially for practitioners, bacause it may be helpful in daily work-for example for screening examinations.

The drawback of this submission is that authors did not performed histological confirmation of BPH. It would be more professional to add such a confirmation. But we have to know that it would be nearly impossible in routine practice. No one owner would agree to perform prostate biopsy in asymptomatic dog.

Authors: We agree and we have added the following sentences (lines 147-152).

In the present study the concentration of CPSE was significantly associated with the presence of prostatic abnormal findings that could be identified by the ultrasound exam. Although, a histological confirmation of prostatic disease would have been desirable to definitively confirm the diagnosis, it is nearly impossible also in everyday routine practice when no one owner would agree to perform a more invasive procedure in an asymptomatic and apparently healthy dog, such as the prostate biopsy. On the other hand, the CPSE is a known marker for prostatic secretion, that can be easily assessed by a simple blood serum sample. It constitutes more than 90% of seminal proteins in dogs, but its exact role in the different prostatic disorders is not yet completely understood (Frenette et al., 1987; Gobello et al., 2002).

And also another suggestions. Authors did not compare nor mentioned about recent publication of Pasikowska et al., who used CT and tried to establish some measuring system aiming similarly to authors of this submission.

Authors: We agree and we have added these sentences (see lines 114-124).

This study confirms that CPSE represents a useful tool to early detect prostatic disorders in dogs. Some authors have previously suggested that prostate size could be affected by age, breed, and body weight, but also by the emergence of pathologic processes, making the establishment of normal dimensions difficult because of the wide variety of canine sizes, breeds and conditions (O'Shea, 1962; Cartee and Rowels, 1983; Atalan et al., 1999; Smith, 2008; Freitas et al., 2015). Several studies were performed in order to correctly establish the actual prostatic volume, through the two dimensional ultrasonographic prostatic measures. (Ruel et al., 1998; Atalan et al., 1999; Kamolpatana et al., 2000; Gobello and Corrada 2002). Morever, it was stated that the ultrasonographic exam is a highly dependent diagnostic imaging modality whose measurements accuracy might depend by the operator's ability and experience (Leroy et al., 2013). Thus, recently the Computed Tomography exam of the prostate was proposed (Dimitrov et al., 2010; Lee et al., 2011; Pasikowsha et al., 2015). The Computed Tomography examination would allow a more precise and repeatable measurements inter-observers, but it is less accessible, more expensive and time consuming, and requires general anesthesia (Pasikowsha et al., 2015). On the other hand, the ultrasonographic exam of the canine prostate is non-invasive and fast and so it remains the diagnostic imaging tool of choice for the evaluation of this organ (Levy et al., 2014; Mantziaras et al., 2017). Anyway, for all these reasons, since a clear threshold measure to identify the normal volume of the canine prostate dogs does not exist, in the present study dogs were grouped in accordance to presence/absence of other ultrasonographical abnormal findings (i.e. altered appearance, border, cysts).

I have to say that also some biochemists (for example Mogielnicka-Brzozowska) published recently papers focused on analysis of CPSE by use proteomic methods and mentioned about would be also aimful, interesting for readers and may scientifically deepen Discussion. I found mentioned publications as very inspirating and I suggest to add them tomreferences, cause authors cited a little older publications on CPSE.

Authors: We thank for the proper suggestion, we added this sentence (see lines 154-157).

Recently, it was suggested that the CPSE is secreted in the form of prostasomes (prostate granules) to semen during ejaculation. The activity of CPSE is regulated by the level of

available zinc that is of great importance for maintaining the normal functions of the prostate and the spermatozoa. (Mogielnicka-Brzozowska et al., 2015).

Specific comments:

It is exception that I have no comments. Text is carefully prepared and I agree with all theses of authors. The language is correct. The tile, aim, M&M and results are adequately written. I attached the file with only one simple mistake in spelling

Authors: Thank you, we added your correction (see line 132).