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Rhodesian Ridgebacks have an increased risk to develop benign prostatic hyperplasia

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Abstract

Benign prostatic hyperplasia (BPH) is an age-dependent primarily non-inflammatory enlargement of the accessory gland in the intact dog. The aim of the present study was to control a previously raised suspicion of a breed-related higher incidence of BPH in dogs of the Rhodesian Ridgeback breed. For this, 18 Labrador Retrievers/LR and 20 Rhodesian Ridgebacks/RR were assigned to the age groups 18–24 months (n = 12), 25–48 months (n = 13) and 49–72 months (n = 13). Prostate gland status was determined by rectal palpation, B-mode ultrasound, calculation of the prostate gland volume and semen analysis regarding haemospermia and was classified according to blood plasma concentrations of canine prostate-specific arginine esterase (CPSE) (normal ≤ 60 ng/ml, increased ≥ 61 ng/ml; Pinheiro et al., 2017). Concentrations of testosterone, 5α-dihydrotestosterone and estradiol were analysed in peripheral blood serum or plasma for detecting breed-specific conditions regarding the endocrine metabolism. Prostatic volume was significantly larger in RR irrespective of the CPSE status. In RR, BPH occurred more frequently and started at an earlier age compared with the LR. Breed-related specificities in steroid metabolism in the RR were indicated by correlations of 5α-dihydrotestosterone and estradiol with age and of testosterone with prostate gland volume. Although the incidence of sonographic signs of BPH and haemospermia did not fit with normal and increased CPSE concentrations, a breed-specific higher incidence of BPH in the RR breed could be clearly verified.

KEYWORDS

canine prostate-specific arginine esterase, haemospermia, sexual steroids, ultrasound

1 | INTRODUCTION

The oval to spherical canine prostate gland is a bilobed structure encircling the proximal urethra (Smith, 2008). It reaches its normal size with completion of sexual maturity. Maturation and function directly depend on testicular steroid hormones, especially on testosterone and estrogens (Kawakami, Tsutsui, & Ogasa, 1991). Benign prostatic hyperplasia (BPH) is a combination of hypertrophy (increase in growth) and hyperplasia (increase in cell number) of the secretory glandular epithelium and stromal components. It is a common spontaneous age-dependent primarily not inflammatory enlargement of the accessory gland and due to this the

most frequent prostatic disease in the intact dog, in most cases showing a subclinical course (Berry, Coffey & Ewing, 1986; Berry, Strandberg et al., 1986; Johnston, Kamolpatana, Root-Kustritz, & Johnston, 2000). Early studies performed in Beagles indicate that hyperplasia of glandular epithelial cells may already start by the age of 2.5 years, with an increasing tendency to develop cystic hyperplasia from four years onwards (Berry, Coffey, & Ewing, 1986; Berry, Strandberg, et al., 1986; Brendler et al., 1983; Lowseth, Gerlach, Gillett, & Muggenburg, 1990). Regarding pathogenesis of BPH, it is clear that it begins with a change in the testosterone and oestrogen metabolism, leading to an alteration in the androgen:oestrogen ratio secreted by the testes (Bamberg-Thalen & Linde-Forsberg, 1993). In this regard, estrogens promote BPH by enhancing androgen receptors (Trachtenberg, Hicks, & Walsh, 1980). An increase in enzymatic reduction in testosterone to 5α-dihydrotestosterone (5α-DHT) within the glandular tissue is considered to be the main trigger of cell hyperplasia (Ewing, Berry, & Higginbottom, 1983; Ewing et al., 1984; Geller, 1989a,1989b; Isaacs & Coffey, 1981, 1984). The latter is expressed by an almost fourfold increase in the tissue concentrations of 5α-DHT compared with the physiological situation (Lloyd, Thomas, & Mawhinney, 1975).

During ejaculation, the prostatic secretion is delivered from the two glandular lobes into the pelvic part of the urethra. Its normal appearance is watery and clear. Admixture of blood to the secretion (haemospermia) may indicate BPH with or without accompanying chronic prostatitis (Johnston et al., 2000; Krawiec & Heflin, 1992).

Canine prostate-specific arginine esterase (CPSE) makes >90% of the proteins in canine prostatic secretion (Chapdelaine, Dubé, Frenette, & Tremblay, 1984; Chevalier, Bleau, Robert, & Chapdelaine, 1984; Isaacs & Shaper, 1985) and is the most abundant kallikrein secreted by the canine prostate gland (Dubé, 1994). Both its synthesis in prostatic epithelial cells and secretion are androgen-dependent (Frenette, Dubé, & Tremblay, 1983; Frenette, Dubé, & Tremblay, 1985; Isaacs & Sharper 1985; Juniewicz et al., 1990). Significantly higher CPSE concentrations in blood serum of dogs with BPH have been found compared with those with healthy prostate glands and in dogs with other prostatic diseases like bacterial prostatitis and prostate gland carcinoma (Bell et al., 1995; Klausner, Johnston, & Bell, 1995; Lévy et al., 2009, 2014). By means of this, blood serum CPSE concentrations may serve as a valuable indicator for early diagnosis of BPH and for controlling the effect during medical therapy of BPH (Alonge, Melandri, Leoci, Lacalandra, & Aiudi, 2018; Gobello, Castex, & Corrada, 2002; Gobello & Corrada, 2002; Pinheiro et al., 2017). CPSE concentrations of ≤50 ng/ml (Alonge et al., 2018), ≤60 ng/ml (Pinheiro et al., 2017) and ≤90 ng/ml (Holst et al., 2017) were set as threshold values for dogs with healthy prostate glands using different test systems, especially for identifying BPH in asymptomatic dogs.

Krawiec and Heflin (1992) direct attention to an increased prevalence of prostate diseases in medium- and large-sized dogs, especially in Dobermans and German Shepherds, without special reference to BPH. An early enlargement of the prostate gland in Rhodesian Ridgebacks is described in our previous publication (Wolf et al., 2012). A significantly larger prostatic volume and significantly higher CPSE concentrations were found in comparison with dogs of other breeds with a similar body weight. Rhodesian Ridgebacks have not been considered in any other study so far. Therefore, the aim of our present study was to conduct in-depth research on this breed; we investigated selected criteria in connection with BPH in Rhodesian Ridgeback dogs in comparison with Labrador Retrievers serving as a control group.

2 | MATERIALS AND METHODS

2.1 | Animals and experimental design

A total of 38 physically healthy intact male dogs of two selected breeds of comparative size and body weight were included in this study. Physical health included the absence of BPH-related clinical signs like haematuria, constipation and dysuria or problems with defaecation. These privately owned breeding dogs consisted of 18 Labrador Retrievers (LR) (37.2 ± 4.0 kg body weight) and 20 Rhodesian Ridgebacks (RR) (41.7 ± 3.7 kg body weight). The dogs were assigned to three age groups: I (18–24 months, RR: n = 6, LR: n = 6), II (25–48 months, RR: n = 6, LR: n = 7) and III (49–72 months, RR: n = 8; LR: n = 5).

Regarding the prostate gland status (healthy vs. benign prostatic hyperplasia/BPH), classification of dogs was performed in relation to the blood plasma CPSE concentration. A concentration of ≤60 ng/ml was equated with 'normal' (CPSEn), a concentration of ≥61 ng/ml with 'increased' (CPSEi), as published by Pinheiro et al. (2017), who validated the CPSE assay used in our study.

All dogs underwent a complete breeding soundness evaluation including morphological and sonographic examination of the testes and epididymides, semen collection and evaluation. Only dogs with normospermia (LR: n = 14, RR: n = 18) or dysspermia (LR: n = 4, RR: n = 2) were included in the study. Dysspermia consisted of a slightly increased percentage of morphologically abnormal spermatozoa as the only alteration in semen quality (Günzel-Apel, 2016). The prostate gland was examined by digital rectal palpation to assess the approximate size, symmetry or asymmetry, the consistency and the position as well as the presence of painfulness. Sonographic images of the glandular parenchyma were differentiated into 'hypoechoic, homogeneous' (normal) and 'hypoechoic, inhomogeneous' (suspected BPH degree 1) to 'inhomogeneous with small cysts' (suspected BPH degree 2). All sonographic examinations were performed in a standing position by means of the ultrasound machine Logic 5 Pro (General Electric Medical Systems GmbH, Solingen, Germany) using a 6.0 MHz micro-convex transducer. If necessary, the hair coat in the suprapubic area was clipped. Coupling gel was applied to the skin to improve contact. The sonographic presentation of the prostate gland was performed as described by Ruel, Barthez, Mailles, and Begon (1998). Prostatic length and height were

measured in a sagittal direction. Length was defined as the maximum diameter along the urethral axis; height was defined as the maximum diameter vertical to the axis of length. To obtain transverse images of the prostate, the transducer was rotated 90 degrees to measure the height and width. Height was defined as the diameter of the gland on a line separating the two lobes and width as the maximum diameter vertical to the axis of the height.

Prostatic length, width and height were measured three times on maximum longitudinal and transverse sections of ultrasound images and mean values were calculated. Prostatic volume was estimated using the formula for the volume of an ellipsoid body (volume = length × width × height × 0.523) according to Ruel et al. (1998).

Semen collection was performed by digital manipulation in the presence of an oestrous teaser bitch as described by Günzel-Apel (2016). The watery pre-secretion (first fraction), representing the initial portion of prostatic secretion (England, Allen, & Middleton, 1990), the greyish to whitish sperm-rich fraction (second fraction) and the remaining watery prostatic secretion (third fraction) were collected in separate sterile glass vials. Semen analysis was performed regarding the macroscopic appearance of each ejaculated fraction (volume, consistency), determining the sperm concentration using a Thoma counting chamber and calculating the total sperm number by multiplying the volume and sperm concentration of the sperm-rich fraction. Sperm motility (percentages of progressively and locally motile as well as immotile spermatozoa) was estimated by phase contrast microscopy on a warming table at 38°C. The percentage of spermatozoa with damaged plasma membrane was determined using live–dead staining with eosin and the percentage of morphologically altered spermatozoa by phase contrast microscopy after immobilizing spermatozoa in 0.3 ml formol citrate (2.9 g trisodiumcitrate dihydrate, add 100 ml double distilled water, remove 4 ml and add 4 ml of approximately 35% commercial formaldehyde solution) (Günzel-Apel, 2016). All semen analyses were performed by the same person.

From each ejaculate, one sample either of pre-secretion (n = 30) or, if pre-secretion could not be obtained, of sperm-rich fraction (n = 8) was subjected to microbiological examination at the Institute of Microbiology, University of Veterinary Medicine Hannover, Germany. Only dogs with no or a low to medium degree of non-specific bacteria were included in the study.

Blood samples were collected from the left or right cephalic vein and left at room temperature for 20 min. Blood serum or blood plasma was separated by centrifugation at 3,030 × g for 10 min. The supernatant was divided into split samples of 0.5 to 1.0 ml according to the number of evaluated parameters. All samples were stored at −20°C until analyses.

2.2 | Analysis of canine prostate-specific arginine esterase (CPSE)

The CPSE concentrations in blood plasma were determined by means of a commercial enzyme-linked immunosorbent assay (ELISA) (Odelis®; CPSE, Virbac Tierarzneimittel GmbH, Bad Oldesloe, Germany), which had been validated by Pinheiro et al. (2017). Analyses were performed in accordance with the manufacturer's instructions. As the blood plasma sample of one LR of age group I had been lost, the calculation of CPSE concentrations in the LR was based on 17 instead of 18 samples.

2.3 | Hormone analyses

The testosterone (T) concentrations were analysed in blood serum using a competitive radioimmunoassay (Testosterone direct RIA-Kit, RIA Testosterone IM1119, direct, Immunotech s.r.o., Prague, the Czech Republic). The intra-assay CV was 5.7%. To determine the blood serum 5α-dihydrotestosterone (5α-DHT) concentrations, a competitive enzyme-linked immunoassay (ELISA) (Dihydrotestosterone (DHT) ELISA DE2330; Demeditec Diagnostics GmbH, Kiel, Germany) was used in accordance with the manufacturer's instructions. The intra-assay CV was 8.7%.

Concentrations of estradiol (E_2) were determined in blood plasma by a previously validated and since then repeatedly published RIA described by Hoffmann, Höveler, Hasan, and Failing (1992), Klein, Schams, Failing, and Hoffmann (2003) and Shenavai et al. (2010).

2.4 | Statistical evaluation

For statistical evaluation, the SAS® program (Statistical Analysis System®, SAS Institute Inc., Version Enterprise Guide® 7.1, Cary North Carolina, USA) was employed.

The descriptive statistic was performed by non-parametric one-factorial ANOVA. The Kruskal–Wallis test and the Wilcoxon's signed-rank test were used to compare the differences in the various parameters between groups regarding CPSE status (normal vs. increased), breed and age. Results are presented as mean ± SD. Differences between groups were assessed as being significant if $p < .05$. The Spearman's rank correlation coefficient was determined for characterization of related influences.

3 | RESULTS

3.1 | Clinical findings and CPSE concentrations in blood plasma

In all dogs included in the study, the testes and epididymides showed normal shape and consistency as well as normal sonographic appearance. At rectal digital palpation, only the caudal pole of the prostate gland could be reached due to the size of the dogs. It was detected in a pelvic position, both lobes being symmetric and of a normal, smooth elastic consistency. Symptoms of a painful disease of the prostate gland were missing.

Sonographic findings in relation to CPSE concentrations ≤60 ng/ml and ≥61 ng/ml are summarized in Table 1. In LR, ultrasound examination of the prostate gland revealed no signs

TABLE 1 Sonographic findings (US) and haemospermia in Labrador Retriever and Rhodesian Ridgeback dogs classified according to CPSE concentrations ≤ 60 ng/ml and ≥ 61 ng/ml

	CPSE					
	≤60 ng/ml (normal)			≥61 ng/ml (increased)		
Labrador retriever (*n* = 18)						
Age (months)	18–24	25–48	49–72	–		
n	6	7	5	–		
%	100	100	100			
US *n*	0	0	3	–		
%[a]			60			
Degree 1 *n*	0	0	1	–		
%[a]			20			
Degree 2 *n*	0	0	2	–		
%[a]			40			
haemosp. *n*	1	1	2	–		
%[a]	16.7	14.3	40			
Rhodesian Ridgeback (*n* = 20)						
Age (months)	18–24	25–48	49–72	18–24	25–48	49–72
n	3	4	3	3	2	5
%	50	66.6	37.5	50	33.3	62.5
US *n*	1	2	1	1	1	5
%[a]	33.3	50	33.3	33.3	50	100
Degree 1 *n*	1	1	0	1	1	0
%[a]	33.3	25		33.3	50	
Degree 2 *n*	0	1	1	0	0	5
%[a]		25	33.3			100
haemosp. *n*	2	3	3	3	2	5
%[a]	66.6	75	100	100	100	100

[a]% of the respective age group.

of 'suspected BPH' in neither of the age groups 18–24 months or 25–48 months, but in three out of five dogs (60%) aged 49–72 months (one degree 1, two degree 2). In RR, two dogs in the age group I (33.3%, both degree 1), three in age group II (50%, two degree 1, one degree 2) and six in group III (75%, all degree 2) were affected.

Haemospermia as a possible indication of BPH was found in a total of 4 LR (22.2%) and 18 RR (90%) being distributed among all age groups (Table 1).

In all LR, CPSE concentrations were clearly below 60 ng/ml (16.1 ± 8.0, 4.6–38.9 ng/ml) and thus classified as CPSE normal (CPSEn). In the 20 RR dogs, CPSE concentrations of ≤ 60 ng/ml (16.6 ± 8.4, 6.8–37.1 ng/ml) and ≥ 61 ng/ml (180.9 ± 119.8, 71.3–401.0 ng/ml) were measured in equal parts (*n* = 10 each). Increased CPSE concentrations (CPSEi) were found in all three age groups (Table 1). This becomes obvious in the high mean plasma CPSE concentrations and the even higher standard deviations shown in Table 3.

TABLE 2 Concentrations of testosterone (*T*, ng/ml), 5α-DHT (ng/ml), estradiol (E_2, pg/ml) and CPSE (ng/ml) in blood serum or plasma of Labrador Retrievers (LR) and Rhodesian Ridgebacks (RR) with normal CPSE concentrations (CPSEn) and increased CPSE concentrations (CPSEi)

	CPSEn LR		CPSEn RR		CPSEi RR	
Parameter	mean	±*SD*	mean	±*SD*	mean	±*SD*
T	1.8	1.7	1.1	0.5	1.4	0.5
5α-DHT	1.3	1.0	1.6	0.9	1.8	1.4
E_2	26.3	9.0	24.9	7.4	24.2	5.7
E_2/*T* ratio	1.3[a]	0.5	1.4[ab]	0.5	1.6[b]	0.4
CPSE	16.1[a]	8.0	16.6[a]	8.4	180.9[b]	119.8

Note: E_2/*T* ratio: comparison between CPSEn LR and CPSEi RR: a/b $p < .05$; CPSE: comparison between CPSEn LR and CPSEi RR: a/b $p < .0001$, comparison between CPSEn RR and CPSEi RR: a/b $p < .001$

3.2 | Prostate gland volume and CPSE concentrations in relation to breed and age

In the RR with normal CPSE, the prostate gland volume was 52.9 ± 34.4 ccm and thus significantly larger than in the CPSEn LR (26.1 ± 11.4 ccm) ($p < .01$). Comparison of the prostate gland volume

TABLE 3 Concentrations of testosterone (*T*, ng/ml), 5α-DHT (ng/ml), estradiol (E_2, pg/ml), E_2/T ratio and CPSE (ng/ml) in blood serum or plasma of Labrador Retrievers (LR) and Rhodesian Ridgebacks (RR) of different age irrespective of the CPSE status

	18–24 months				25–48 months				49–72 months			
	LR *n* = 6		RR *n* = 6		LR *n* = 7		RR *n* = 6		LR *n* = 5		RR *n* = 8	
Parameter	mean	±*SD*	mean	±*SD*	mean	±*SD*	mean	±*SD*	mean	±*SD*	mean	±*SD*
T	1.5	0.9	1.0[a]	0.4	1.9	2.4	1.2	0.5	2.0	1.3	1.5[b]	0.5
5α-DHT	1.7	1.5	1.2[a]	1.1	0.9	0.6	1.3	0.6	1.3	0.7	2.3[b]	1.3
E_2	27.2	9.6	23.8	8.3	28.5	7.5	22.5	4.6	22.0	10.4	26.8	6.3
E_2/T ratio	1.5	0.3	1.3	0.8	1.2	0.6	1.5	0.2	1.0	0.6	1.6	0.2
CPSE	10.7	4.8	99.5	151.3	16.5	5.1	67.3	73.4	20.9	11.0	121.8	127.1

Note: T - RR: 18–24 mo – 49–72 mo a/b $p = .05$; 5α-DHT: 18–24 mo - 49–72 mo a/b $p = .06$

of the RR with increased CPSE (81.3 ± 38.1 ccm) with that of the total number of dogs with CPSEn (35.7 ± 25.5 ccm) and the LR alone also revealed significant differences ($p < .0001$) (Figure 1). The difference between the glandular volume in the RR with normal and increased CPSE was, however, non-significant ($p = .08$).

Comparison of the prostate gland volume in relation to age revealed a steady increase in the LR dogs with significant differences between the youngest group I, on the one hand, and groups II and III, on the other hand ($p < .05$) (Figure 2). Comparison of the LR with the CPSEn RR revealed a significantly larger prostate gland volume in the RR in age group I ($p < .05$) (Figure 2). Consideration of age-related development of the prostate gland volume showed a similar process in the RR with CPSEn as in the LR up to four years of age (Figure 2). However, a significant increase occurred in the oldest age group, where the prostate gland volume was as large as in the corresponding RR with CPSEi. Differences between the prostate gland volume of RR with CPSEn and CPSEi in age groups I and II were not significant, probably due to the small number of dogs per age group.

Considering the entire group of dogs included in the study, significant correlations were found for breed on the one hand, and prostate gland volume, CPSE status and plasma CPSE concentration on the other hand (Table 4). Prostate gland volume was also correlated with age with regard to the total number of LR and RR (CPSEn and CPSEi), the CPSEn LR and the total number of CPSEn LR and RR as well as with regard to the entire group of RR (CPSEn and CPSEi) but not to the RR with CPSEi. Positive correlations with age were found for the CPSE concentration in the dogs with normal CPSE (LR and RR), but not in the RR group alone. Furthermore, CPSE was correlated with prostate gland volume in both the entire group of dogs (LR and RR) and the entire group of RR irrespective of the CPSE status.

3.3 | Hormone concentrations in relation to breed and age

Despite markedly lower serum concentrations of *T* especially in the RR with CPSEn, the difference turned out to be non-significant, probably due to the large variation in *T* values in the LR (Table 2). On the other hand, tendentially higher 5α-DHT concentrations were found in the RR compared with the LR. Plasma concentrations of estradiol did not differ regarding breeds and the CPSE status of the RR (Table 2). However, the E_2/T ratio was significantly

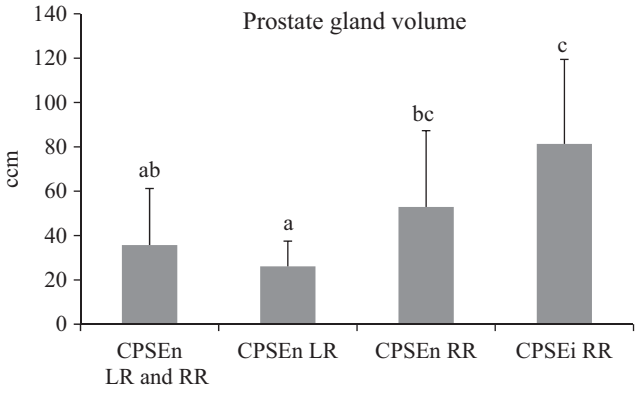

FIGURE 1 Prostate gland volume (mean ± *SD*) in dogs with normal and increased concentrations of canine prostate-specific arginine esterase (CPSEn, CPSEi). a:c and ab:c $p < .0001$, a:bc $p < .01$. LR = Labrador Retriever, RR = Rhodesian Ridgeback

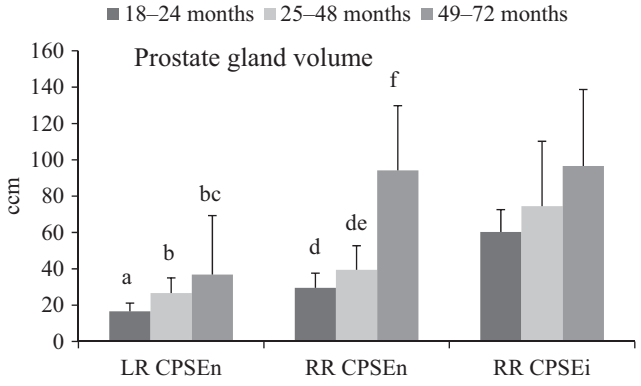

FIGURE 2 Prostate gland volume (mean ± *SD*) in Labrador Retrievers (LR) and Rhodesian Ridgebacks (RR) of different age with normal and increased concentrations of canine prostate-specific arginine esterase (CPSEn, CPSEi). LR CPSEn: a/b and a/c $p < .05$; RR CPSEn: d/f and e/f $p < .05$; LR CPSEn versus. RR CPSEn - 18–24 mo: a/d $p < .05$; 49–72 mo: bc/f $p < .05$

TABLE 4 Correlations of breed, age and prostate gland volume (PGV) with concentrations of CPSE, testosterone, 5α-dihydrotestosterone and estradiol in blood plasma or blood serum regarding the CPSE status (CPSEn—normal concentration, CPSEi—increased concentration)

	total (CPSEn and CPSEi)		total LR (CPSEn)		total CPSEn (LR and RR)		total RR (CPSEn and CPSEi)		RR (CPSEi)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
breed	Prostate gland volume									
	.67	<.0001	–	–	–	–	–	–	–	–
	CPSE status									
	.58	<.0001	–	–	–	–	–	–	–	–
	CPSE									
	.44	<.01	–	–	–	–	–	–	–	–
age	Prostate gland volume									
	.63	<.0001	.80	<.0001	.71	<.0001	.59	<.01	–	–
	CPSE									
	.38	<.05	.5	<.05	.43	<.05	–	–	–	–
	5α-dihydrotestosterone									
	–	–	–	–	–	–	–	–	.70	<.05
	Estradiol									
	–	–	–	–	–	–	–	–	.76	<.05
PGV	CPSE									
	.59	≤.0001	–	––	–	–	.50	<.05	–	––
	testosterone									
	–	–	–	–	–	–	.65	<.01	–	–

Note: The degrees of association between breed, age and prostate gland volume (left side vertical column) and the parameters listed in the corresponding horizontal lines were calculated using the Spearman's rank correlation test.

higher in the RR with increased CPSE compared with the CPSEn LR ($p < .05$) (Table 2).

Age-related concentrations of steroid hormones and CPSE of both breeds irrespective of the CPSE status are summarized in Table 3. Differences in concentrations of serum *T* and 5α-DHT, detected in RR, both between the youngest and oldest age groups, were close to significant (Table 3).

Correlations of peripheral steroid hormone concentrations were limited to the RR breed. 5α-DHT and estradiol were correlated with age in RR with CPSEi, whereas *T* was correlated with prostate gland volume in the entire group of RR regardless of the CPSE status (Table 4). A close to significant correlation was detected between the E_2/T ratio and the prostate gland volume when considering the entire group of 38 dogs ($r = .32$ $p = .053$).

4 | DISCUSSION

Studies regarding the incidence and appearance of canine BPH have been mostly performed in canine populations including a large variety of breeds. Detailed breed-specific features have not been published so far. Data on Rhodesian Ridgeback dogs (RR) are completely missing. Due to a suspected breed disposition resulting from a previous study (Wolf et al., 2012), the main focus of the present study was on the RR breed. Our study concentrated on dogs which were intended to be used for breeding or had already started their breeding career. In order to avoid multifactorial influences of different breeds, we chose the Labrador Retriever as control group because its body weight is similar to RR dogs. The dog ages were restricted to three defined groups with the upper limit of 72 months (6 years) to obtain information about the early incidence and development of BPH in the two breeds.

4.1 | Clinical findings, prostate gland volume and CPSE concentration regarding BPH in relation to breed

Sonographic symptoms of BPH were found in three of the oldest LR and in a total of 11 RR, these being distributed among all age groups (Table 1). Furthermore, in age group III (49–72 months), a higher incidence of inhomogeneous prostatic tissue with small cysts (degree 2) occurred in RR ($n = 6$) compared with the LR ($n = 2$). These findings, together with the markedly higher incidence of haemospermia in the RR ($n = 18$, 90%) compared with the LR ($n = 4$, 22.2%), point to a breed disposition to develop cystic BPH in the RR. Moreover, based on the high incidence of sonographic findings with or without haemospermia in age groups I and II of the RR regardless of the CPSE status, a breed-related tendency to develop BPH earlier becomes obvious.

In dogs, the sanguineous prostatic fluid most commonly occurs secondary to BPH (England & Allen, 1992; Memon, 2007).

Blood admixture may be due to an increased vascularization of the hypertrophic canine prostate gland that has been verified by Doppler ultrasonography (Günzel-Apel, Möhrke, & Poulsen Nautrup, 2001) and contrast-enhanced ultrasonography (Troisi et al., 2015). The latter research study found a characteristic change in vascular architecture with an enhancement of blood vessels in BPH. The increase in vascularization may result in vascular leakage or haemorrhage into the gland and excretion of blood through the secretory ducts into the urethra. Chronic prostatitis as the cause of haemospermia can be largely ruled out in this study, as only dogs with no bacteria or a low to medium degree of non-specific bacteria in pre-secretion or semen were selected. Other possible sources of blood admixture, such as penile tumour or accidental trauma of the penis or prepuce during semen collection (Johnston et al., 2000; Rijsselaere, Soom, Maes, Verberckmoes, & Kruif, 2004), can also be excluded.

In addition to the increased frequency of clinical findings, the significantly larger mean prostate gland volume found in RR with CPSEn (52.9 ccm) compared with the LR with CPSEn (26.1 ccm) may indicate a breed-related feature. This is supported by the significant correlation of the prostate gland volume with breed (Table 4). However, in the CPSEn RR, the prostate gland volume varied from 20.2 to 130.2 ccm compared with the situation in the LR (11.1–50.7 ccm). This discrepancy is due to the fact that 40% of the RR with CPSE ≤ 60 ng/ml showed sonographic signs of BPH and/or haemospermia.

As expected and described by Ruel et al. (1998), a correlation between prostate gland volume and age was found in all groups of dogs, except for the CPSEi RR (Table 4). In the LR, the breed not showing clinical signs of BPH in age groups I and II, the significantly larger prostate gland volume seen in the 25- to 48-month-old dogs compared with the younger age group (Figure 2) may represent early hyperplasia of glandular epithelial cells. This is described as starting by the age of 2.5 years, with an increasing tendency to develop cystic hyperplasia from four years upwards (Berry, Coffey, & Ewing, 1986; Berry, Strandberg, et al., 1986; Lowseth et al., 1990). The latter can be assumed as a reason for the significant increase in prostatic size in the 49- to 72-month-old LR. In two out of five dogs, this was combined with the sonographic finding of an inhomogeneous tissue echotexture and small intraprostatic cysts.

In the 18- to 24-month-old RR with CPSEn, a breed-related stronger shaping of the prostate gland became obvious in the significantly larger glandular volume in comparison with the LR in age group I (Figure 2). However, in the 49- to 72-month-old CPSEn RR, the increase in prostate gland volume was 3.2 times that of the youngest CPSEn RR and 5.7 times that of the youngest LR, thus being identical with that of the CPSEi RR in age group III (Figure 2). These growth ratios are within the range of the twofold to 6.5-fold increase in prostatic volume given for dogs with BPH compared with dogs with a normal prostate (Johnston et al., 2000). Therefore, in the RR dogs a primarily BPH-related prostatic growth is obvious. This assessment is supported by the missing correlation of prostate gland volume with age in the CPSEi RR.

The correlations of the CPSE status and plasma CPSE concentration with breed detected in the entire group of dogs (Table 4) is assessed as an additional indication of a breed-related increased incidence of BPH in the RR breed.

4.2 | CPSE concentrations as indicator of BPH

In the present study, we used the CPSE concentration of 60 ng/ml as a threshold CPSE value to characterize the prostate gland status. This value had been set by Pinheiro et al. (2017), who validated the same test system regarding cytological criteria of BPH. Holst et al. (2017) set the threshold of CPSE at 90 ng/ml, based on a 2.5-fold increase in the prostatic size compared with the size of younger dogs. In our study, values between 60 ng/ml and 90 ng/ml were only detected in three of the RR combined with haemospermia (18–24 months n = 2, 49–72 months n = 1). In the remaining seven RR, CPSE values ranged from 138.2 to 401.0 ng/ml. On the other hand, Alonge et al. (2018) suggested setting the threshold level at 50 ng/ml for clinically asymptomatic dogs (aged 1 to 5 years) showing ultrasonographic alterations and increased prostatic size (the volume being 1.5 times greater than normal). As in our study, CPSE concentrations of all dogs with CPSEn were < 50 ng/ml (LR: 4.6–38.9 ng/ml; RR 6.8–37.1 ng/ml), the grouping would have remained the same.

The suitability of the blood plasma CPSE concentration as an indicator of BPH was evaluated in a large variety of breeds excluding the Rhodesian Ridgeback breed (Gobello & Corrada, 2002; Gobello et al., 2002; Lévy et al., 2009, 2014; Holst et al., 2017; Pinheiro et al., 2017; Alonge et al., 2018). In our study, the correlations of CPSE with age and prostate gland volume (Table 4) may confirm this assessment. However, the discrepancy between CPSE concentrations and the presence of BPH-related clinical findings (Table 1) contradicts the statement of Pinheiro et al. (2017) that the CPSE test can accurately differentiate between dogs with and without BPH. Even in the LR with CPSEn, a combination of signs indicating BPH was detected in three out of the five 49- to 72-month-old dogs. In the RR, the discrepancy between CPSE concentrations and the presence of BPH-related sonographic signs and haemospermia was even stronger. Thus, the only use of the peripheral CPSE concentration as indicator of BPH in clinically asymptomatic dogs cannot be recommended in general and especially not in this breed. This may not only be true for the CPSE assay, used in our study, but also for other test systems (Alonge et al., 2018; Holst et al., 2017).

4.3 | Hormone concentrations regarding BPH in relation to breed

The comparison of blood steroid concentrations between the LR and RR with CPSEn as well as between RR with CPSEn and CPSEi (Table 2) did not reveal any significant difference, confirming the results of Wolf et al. (2012). However, it must be considered that in both studies only a single blood sample was taken from each dog,

which can only roughly represent the testicular endocrine function. It is well known that testosterone is secreted in a pulsatile manner following the superordinate secretion of LH and GnRH at the pituitary and hypothalamic level (De Coster, Beckers, Wouters-Ballman, & Ectors, 1979; Günzel-Apel, Brinckmann, & Hoppen, 1990; De Palatis, Moore, & Falvo, 1978). Nevertheless, the positive correlation between prostate gland volume and serum testosterone in the entire group of RR (Table 4) may point to a causal connection with the breed-related incidence of BPH. This assessment is underlined by the close to significantly higher mean concentrations of testosterone and 5α-DHT measured with increasing age in the RR (Table 3). Moreover, the correlation between 5α-DHT and age in the RR CPSEi group (Table 4) confirms this. Elevated 5α-DHT concentrations in prostatic tissue are reported for dogs with BPH, representing an increased intraprostatic reduction of testosterone into 5α-DHT due to an increased 5α-reductase activity (Ewing et al., 1983, 1984; Isaacs & Coffey, 1981). Furthermore, the significantly higher E_2/T ratio observed in RR with CPSE concentrations ≥61 ng/ml compared with the LR (Table 2) confirms the results of our previous study (Wolf et al., 2012), where the E_2/T ratio was significantly higher in dogs with BPH than in those with a healthy prostate gland. This finding may indicate a shift in testicular steroid synthesis, which is known to play a role in the pathogenesis of canine BPH (Isaacs & Coffey, 1981). In addition, the positive correlation of estradiol with age in the RR CPSEi group (Table 4) may be the result of the breed-specific earlier and higher incidence of BPH.

5 | CONCLUSIONS

The results of the present study confirm the previously suspected breed disposition for BPH in Rhodesian Ridgebacks. It becomes obvious in the frequency and early incidence of cystic BPH and/or haemospermia as well as the significant BPH-related increase in prostate gland volume. These findings must be considered with regard to a higher risk of developing acute or chronic prostatitis. The latter may turn out to be the reason for a progressive decline in fertility resulting in an early termination of the breeding career. Therefore, a special focus should be given to the prostate gland even in young breeding dogs of the RR breed in order to avoid unnoticed development of a severe chronic prostatic disease. The examination must include rectal palpation, sonographic imaging and sonographic measurement of the prostate gland. Furthermore, in the case of breeding dogs, semen collection and evaluation with regard to the presence of blood admixture must be carried out. Determining the CPSE concentration in blood serum or plasma is considered to be an additional feature when characterizing the prostate gland health status. To the best of our knowledge, this is the first report on a breed-related prevalence of canine BPH.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Günzel-Apel designed study, supervised experimental part and corrected manuscript to final version. Werhahn Beining recruited patients, performed ultrasound examinations and blood sampling, analysed data and drafted paper. Urhausen performed clinical examinations. Wolf contributed to semen collections and evaluations. Schmicke was involved in analyses of CPSE, T and 5-alpha-DHT. Rohn supported statistical analyses. Schuler was involved in analysis of estradiol.

DECLARATIONS—ETHICAL GUIDELINES COMMITTEE

The study was approved by the Lower Saxony State Office for Consumer Protection and Food Safety, Germany (Reference no. 33.19–42502–04-15/1885). Informed owner consent was obtained.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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