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Radiographic evaluation and comparative efficacy assessment of stanozolol and prednisolone acetate in canine tracheal collapse[#]

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Abstract

Canine tracheal collapse is a progressive, enduring and fatal respiratory insufficiency syndrome of dogs, which causes agony to the pet and pet parents alike. The disease is characterised by cartilage flaccidity and flattening of the tracheal rings and causes paroxysmal 'goose honking cough'. In this study, efficacy of stanozolol and prednisolone acetate in healing tracheal collapse was studied, which was assessed by radiographic findings. It was found that stanozolol at a dose of 0.15 mg/kg twice daily for 30 days followed by a tapering dose was more effective than prednisolone acetate in the medical management of tracheal collapse. Even though, there are reports on single dog breeds with tracheal collapse, studies on both brachycephalic and normocephalic dog breeds were not traceable. Moreover, no previous study has evaluated the comparative efficacy assessment of stanozolol and prednisolone acetate in canine tracheal collapse.

Keywords: *Tracheal collapse, Stanozolol, Prednisolone acetate, comparative therapeutic response*

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Respiratory system in dogs, as in most mammals, plays a vital role in gaseous exchange, supplying the body with oxygen and removing carbon dioxide. It comprises various organs and structures that work together to facilitate this process. The 'wind pipe' or trachea is a long flexible hollow organ, composed of C- shaped cartilaginous rings and consists of cervical and thoracic portions. It bifurcates at the level of carina into the principal (mainstem) bronchi. The trachea can be affected by both infectious and non-infectious conditions. The most important non-infectious condition affecting the trachea is its collapse, which can even lead to fatality.

It affects most of the breeds of dogs and often becomes a severe problem in brachycephalic breeds (De Lorenzi, 2009). Canine breeds like Yorkshire terriers, Pomeranians, Pugs, Poodles, Maltese, and Chihuahuas are over-presented with tracheal collapse (Macready *et al.,* 2007). The actual etiology of tracheal collapse is still controversial. Not a single or, specific cause has been identified in all cases of tracheal collapse so far. However, certain dogs with inherent abnormalities in their cartilage are predisposed to this condition (Wickens, 2011). The diagnosis of tracheal collapse involves several techniques and radiography can be used for the preliminary diagnosis of this condition. According to Maggiore (2014), the plain radiographic technique had been widely used as a diagnostic aid in primary tracheal disorders.

Table 1. Clinical signs of 24 dogs affected with tracheal collapse

Clinical signs	No. of dogs affected	Per cent
Cough	24	100
Dyspnoea	10	41.66
Syncope	2	8.33
Tachypnoea	24	100
Cyanosis	12	50
Nasal discharge	24	100

Table 3. Overall breed wise distribution of dogs with tracheal collapse

Breeds	No of dogs (Per cent) (N=24)
Pug	2 (8.33)
Pomeranian	6 (25)
Shih-tzu	1 (4.16)
Terrier	1 (4.16)
Miniature Pinscher	1 (4.16)
Cocker Spaniel	1 (4.16)
Labrador retriever	8 (33.33)
Mixed breed	4 (16.66)

Swathi *et al.* (2019) documented arterial blood gas analysis values of Labrador retriever dogs with upper (tracheal collapse) and lower respiratory tract diseases. Abnormal mean values in partial pressure of oxygen, oxygen saturation and alveolar-arterial (A-a) gradient was noticed as the significant evidence of hypoxemia in dogs with diseases of upper and lower respiratory tract.

Stanozolol is an anti-androgen that has been used in the long-term management of dogs suffering from tracheal collapse. It stimulates procollagen production and enhances synthesis of protein and chondroitin sulfate. An experimental study has documented the potential effect of stanozolol in canine tracheal collapse (Adamama-Moraitou *et al.,* 2011). In canine patients with tracheal collapse, conventionally, prednisolone was used as a first line treatment (Amith *et al.,* 2021). Therefore, this study compared the therapeutic effects of

Table 2. Clinical signs shown by dogs with tracheal collapse (group wise comparison)

Clinical signs	No. of dogs affected per cent			
	Group I (N=12)		Group II (N=12)	
	Group Ia	Group Ib	Group IIa	Group IIb
Cough	6 (100)	6 (100)	6 (100)	6 (100)
Dyspnoea	2 (33.33)	2 (33.33)	2 (33.33)	4 (66.66)
Syncope	2 (33.33)	0 (0)	0 (0)	0 (0)
Tachypnoea	6 (100)	6 (100)	6 (100)	6 (100)
Cyanosis	3 (50)	3 (50)	2 (33.33)	4 (66.66)
Nasal discharge	4 (66.66)	1 (16.66)	2 (33.33)	5 (83.33)

stanozolol and prednisolone acetate in treating tracheal collapse in dogs.

Materials and methods

Twenty-four dogs of different age, sex and breed with clinical symptoms of cough, dyspnoea, tachypnoea, syncope, cyanosis and nasal discharge presented to Veterinary hospitals under Kerala Veterinary and Animal Sciences University were subjected to detailed clinical examination (Table 1 and Table 2). These dogs were divided into two major groups (twelve dogs each), Group I dogs were subjected to treatment with stanozolol and group II dogs with prednisolone acetate. Each major groups were again subdivided into a and b**,** of which group a were brachycephalic breeds and group b consisted normocephalic breeds. Twelve apparently healthy dogs were selected (six each in brachycephalic and normocephalic breeds) as control and were compared with diseased animals. The overall breed distribution was shown in Table 3.

Radiographical assessment of tracheal dimensions was conducted as a preliminary diagnostic modality. All dogs were assessed for left lateral radiography of the neck and thorax. Collapse locations, tracheal lumen diameter to thoracic inlet distance ratio (Td/Ti) (Fig.1), thoracic tracheal luminal diameter to the width of proximal third of third rib ratio (TT/3R) (Fig.2) (Ingman *et al*., 2014) and vertebral heart score (VHS) (to rule out any cardiomegaly) were evaluated.

Group Ia and Ib were administered with oral stanozolol (Menabol^{R}), at a dose rate of 0.15 mg/kg body weight twice daily for 30 days (Adamama-Moraitou *et al*., 2011), oral glucosamine and chondroitin granules (Synopet^{R}) 4g once daily for 30 days, and oral etophylline and theophylline (Deriphylline^{R}) at a dose rate of 6mg per kg bodyweight, twice daily for 5 days. Group IIa and IIb were treated with oral prednisolone acetate at a dose rate of 0.5mg /kg body weight twice daily for the first five days, which was followed by a tapering dose for five more days along with oral glucosamine and chondroitin granules (Synopet^{R}), 4g once daily for 30 days, and oral etophylline and theophylline (Deriphylline^{R}) at a dose rate of 6mg per kg bodyweight, twice daily for 5 days. Amith *et al.* (2021) reported the oral supplementation of chondroitin sulfate along with steroids and angiotensin converting enzymes (ACE) inhibitors in the medical management of tracheal collapse in dogs. Jeung *et al.* (2019) documented a theophylline-based retrospective study and mentioned that clinical signs associated with tracheal collapse were found to be improved over the treatment period.

All parameters under this study were statistically analysed using the software SPSS software package version 24.0. Comparison between group I and II in both brachycephalic dogs and normocephalic dogs was done separately by using one-way ANOVA followed by Duncan Multiple range test. Similarly, before and after treatment (between day 0 and day 31) comparison was done by paired t-test. Sub groups were also analysed separately in each day and the results were compared by using independent t-test.

Results and discussion

The present study revealed the over presentation of tracheal collapse in Labrador retrievers and Pomeranians (Table 3) with a mean ± SE value of 8.33 ± 0.56 years for age of occurrence. The breed wise occurrence observed in the current study, is in concurrence with Marolf *et al.* (2007), who documented the occurrence of tracheal collapse in Labrador retrievers. The mean ± SE values of body weight was 10.65 ± 0.92 kg for brachycephalic breeds and 32.09 ± 0.98 kg for normocephalic breeds of dogs affected with tracheal collapse. These observations were in disagreement with Jeung *et al.* (2019), who documented the mean ± SD values of body weight in brachycephalic dogs as 4.51 ± 2.5 kg. Considering the history of illness, in group Ia, 66.66 per cent dogs had tracheal collapse for the last 2 - 4 years, which was followed by 16.66 per cent each for 3-6 months and 6 months-1-year durations. The details of duration with respect to each sub groups were given in Fig. 3. A similar study was conducted by Jeung *et al.* (2019), who recorded the duration of illness of tracheal collapse as one of the variables in the cough scoring scale developed by them. Even though, there

are reports on single dog breeds with tracheal collapse, studies on both brachycephalic and normocephalic dog breeds were not traceable.

The main clinical symptoms observed in the current study were cough, dyspnoea, tachypnoea, syncope, cyanosis and nasal discharge. Similar observations were made by Jeung *et al.* (2019). Radiographical interpretation revealed collapse at the cervico-thoracic junction in 75 per cent (18 out of 24) dogs with decreased Td/Ti and TT/3R ratios (Ingman *et al*., 2014) at day 0. Cardiomegaly was observed in most of the dogs with increased VHS values (Table 4). Nelson (2003) enlisted cardiac diseases, bronchitis, recent intubation with endotracheal tube, cervical trauma, respiratory diseases and hyperadrenocorticism as the major causes of tracheal collapse in dogs.

Therapeutic effects of stanozolol and prednisolone acetate were monitored on day 31. Group I and group II dogs showed significant changes in clinical signs after therapy and it was found that only three dogs of group I, showed cough thereafter. But, in group II, all the dogs had mild or moderate cough with duration less than a minute on day 31. Similarly, there was significant reduction in other clinical signs in both the groups (Table 5). A recent, retrospective theophylline-based study had mentioned the importance of cough in brachycephalic dog breeds with tracheal collapse (Jeung *et al.*, 2019), but studies on both brachycephalic and normocephalic dog breeds were not traceable.

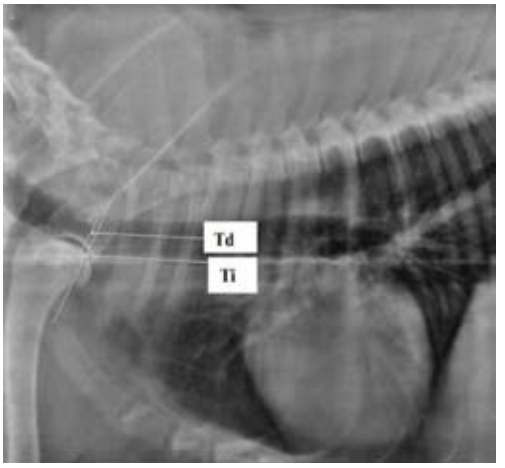

Fig. 1. Lateral thoracic radiograph of a Labrador retriever - measurement of Td/Ti ratio

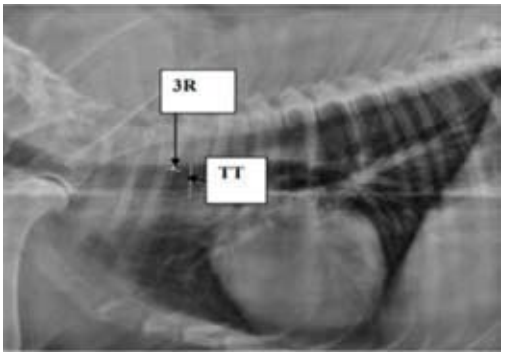

Fig. 2. Lateral thoracic radiograph of a Labrador retriever - measurement of TT/3R ratio

Pre and post treatment appearance of tracheal collapse (Fig. 4 and 5) and tracheal dimensions of dogs (n=24) were evaluated radiographically. Diameter of the tracheal lumen got increased after the therapy with stanozolol

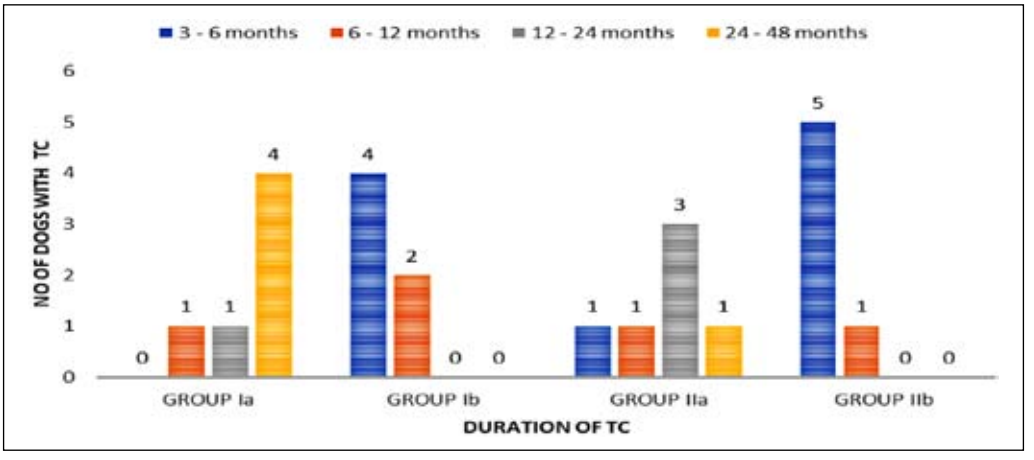

Fig. 3. History of illness of tracheal collapse

Table 4. Pre and post treatment comparison of radiographic findings of brachycephalic dog breeds (Group Ia and IIa)

Variables	Day	Group Ia	Group IIa	Control	F-value (p-value)
Td/Ti ratio	Day 0	0.12 ± 0.005^{b}	0.1267±0.007^{b}	0.18 ± 0.01^{a}	37.38**(<0.001)
	Day 31	0.15 ± 0.01^{b}	0.132 ± 0.006^{b}	0.18 ± 0.01^{a}	17.91**(< 0.001)
	t-value (p-value)	4.503**(0.006)	2.236^{ns} (0.076)	0^{ns} (1)	
TT/3R ratio	Day 0	0.82 ± 0.06^{b}	0.81 ± 0.08^{b}	2.07 ± 0.03^{a}	153.92**(< 0.001)
	Day 31	0.82 ± 0.06^{b}	0.91 ± 0.08^{b}	2.07 ± 0.03^{a}	124.37**(< 0.001)
	t-value(p-value)	0^{ns} (1)	0.970^{ns} (0.377)	0^{ns} (1)	
VHS	Day 0	12.00 ± 0.14^{a}	12.30 ± 0.21^{a}	10.02±0.08^{b}	67.15**(< 0.001)
	Day 31	11.83 ± 0.10^{a}	12.25 ± 0.23^{a}	10.02±0.08^{b}	64.05**(< 0.001)
	t-value (p-value)	2.076^{ns} (0.093)	0.999^{ns} (0.364)	0^{ns} (1)	

*** Significant at 0.01 level(p<0.01); * Significant at 0.05 level (p<0.05); ^{ns} non-significant (p>0.05); Means having different letter as superscript differ significantly within a row*

Table 5. Status of improvement in the clinical signs over the treatment period

Parameter	Day 0 (before treatment)				Day 31 (after treatment)			
	Group I (No f dogs with per cent)		Group II (No f dogs with per cent)		Group I (No f dogs with per cent)		Group II (No of dogs with per cent)	
	Ia	Ib	IIa	IIb	Ia	Ib	IIa	IIb
Cough	6 (100)	6 (100)	6 (100)	6 (100)	2 (33.33)	1 (16.66)	6 (100)	6 (100)
Dyspnoea	2 (33.33)	2 (33.33)	2 (33.33)	4 (66.66)	0 (0)	0 (0)	2 (33.33)	2 (33.33)
Syncope	2 (33.33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tachypnoea	6 (100)	6 (100)	6 (100)	6 (100)	0 (0)	0 (0)	4 (66.66)	0 (0)
Cyanosis	3 (50)	3 (50)	2 (33.33)	4 (66.66)	0 (0)	0 (0)	0 (0)	0 (0)
Nasal discharge	4 (66.66)	1 (16.66	2 (33.33)	5 (83.33)	0 (0)	0 (0)	0 (0)	0 (0)

Table 6. Pre and post treatment comparison of radiographic findings of normocephalic dog breeds (Group Ib and IIb)

Variables	Day	Group Ib	Group IIb	Control	F-value (p-value)
Td/Ti ratio	Day 0	0.15 ± 0.01^{b}	0.127 ± 0.008^{b}	0.50 ± 0.09^{a}	17.56**(< 0.001)
	Day 31	0.18 ± 0.01^{b}	0.130 ± 0.007^{b}	0.50 ± 0.09^{a}	16.25**(< 0.001)
	t-value (p-value)	6.708**(0.001)	1.581^{ns} (0.175)	0^{ns} (1)	
TT3R	Day 0	0.82 ± 0.08^{b}	0.93 ± 0.10^{b}	2.30 ± 0.09^{a}	84.84**(< 0.001)
	Day 31	0.92 ± 0.04^{b}	0.97 ± 0.10^{b}	2.30 ± 0.09^{a}	102.29**(< 0.001)
	t-value (p-value)	1.369^{ns} (0.229)	1.000^{ns} (0.363)	0^{ns} (1)	
VHS	Day 0	12.08 ± 0.12^{a}	12.26 ± 0.15^{a}	10.32 ± 0.04^{b}	92.05**(< 0.001)
	Day 31	11.90 ± 0.09^{a}	11.80 ± 0.14^{a}	10.32 ± 0.04^{b}	85.85**(< 0.001)
	t-value (p-value)	3.379* (0.020)	2.843*(0.036)	0^{ns} (1)	

*** Significant at 0.01 level(p<0.01); * Significant at 0.05 level (p<0.05); ns non-significant (p>0.05); Means having different letter as superscript differ significantly within a row*

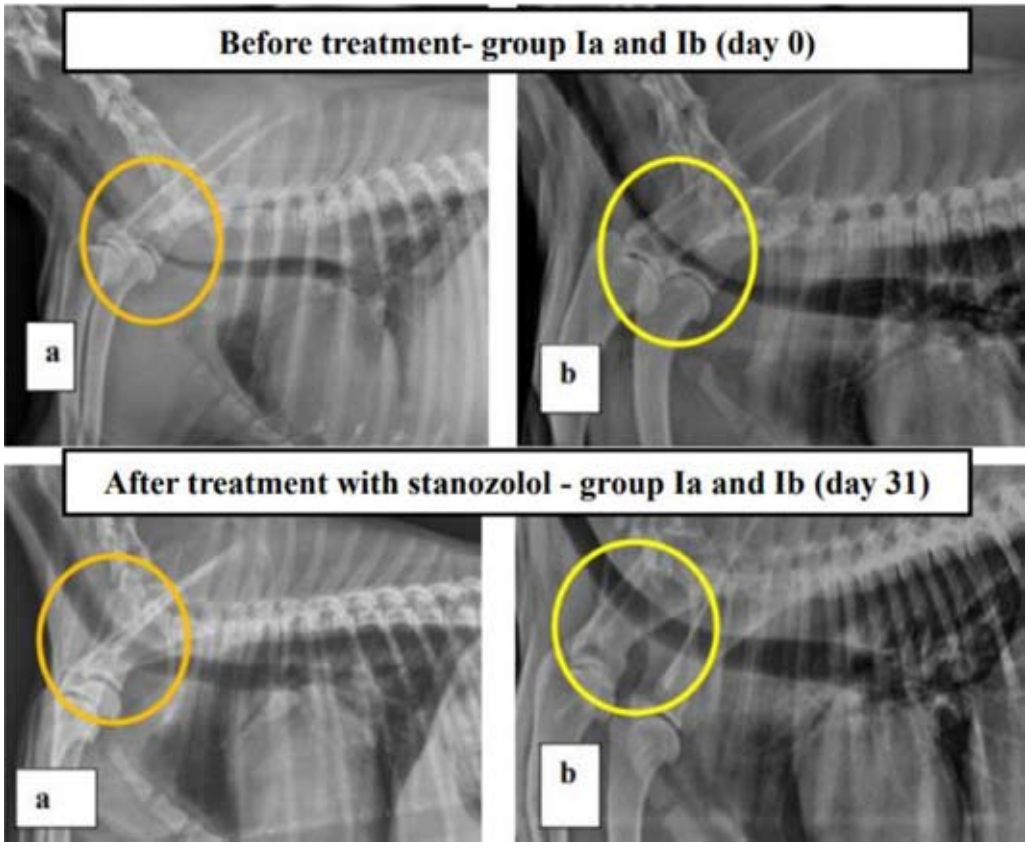

Fig. 4. Left lateral thoracic radiographs of tracheal collapse (effect of stanozolol therapy in group Ia and Ib dogs)

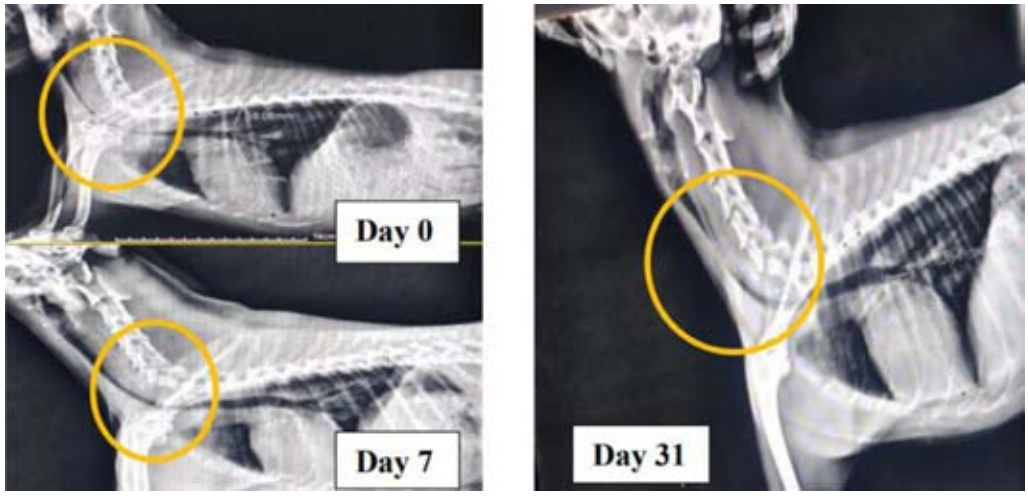

Fig. 5. Left lateral thoracic radiographs with therapeutic effects of prednisolone acetate therapy (group IIa)

in group Ia and Ib and a normal airway passage has been created. Whereas, in group IIa and IIb, there was no clear airway passage and the collapse remained the same at the cervico-thoracic junction. The effect of stanozolol alone in canine tracheal collapse were endoscopically studied by Adamama-Moraitou *et al.* (2011). The therapeutic effect of prednisolone acetate with oral supplementation of chondroitin sulfate and angiotensin converting enzyme (ACE)

Table 7. Pre and post treatment comparison of radiographic findings of subgroups

Variable	Group	Day	Group a	Group b	t-value(p-value)
Td/Ti ratio	I	Day 0	0.12 ± 0.005^{b}	0.15 ± 0.01^{b}	3.656** (0.004)
		Day 31	0.15 ± 0.01^{b}	0.18 ± 0.01^{b}	3.371** (0.007)
		t-value (p-value)	4.503**(0.006)	6.708**(0.001)	
	II	Day 0	0.1267 ± 0.007^{b}	0.127 ± 0.008^{b}	0^{ns} (1)
		Day 31	0.132 ± 0.006^{b}	0.130 ± 0.007^{b}	0.176^{ns} (0.864)
		t-value (p-value)	2.236^{ns} (0.076)	1.581^{ns} (0.175)	
TT3R	I	Day 0	0.82 ± 0.06^{b}	0.82 ± 0.08^{b}	0^{ns} (1)
		Day 31	0.82 ± 0.06^{b}	0.92 ± 0.04^{b}	1.384^{ns} (0.197)
		t-value (p-value)	0^{ns} (1)	1.369^{ns} (0.229)	
	II	Day 0	0.81 ± 0.08^{b}	0.93 ± 0.10^{b}	1.007^{ns} (0.338)
		Day 31	0.91 ± 0.08^{b}	0.97 ± 0.10^{b}	0.483^{ns} (0.639)
		t-value (p-value)	0.970^{ns} (0.377)	1.000^{ns} (0.363)	
VHS	I	Day 0	12.00 ± 0.14^{a}	12.08 ± 0.12^{a}	0.455^{ns} (0.659)
		Day 31	11.83 ± 0.10^{a}	11.90 ± 0.09^{a}	0.520^{ns} (0.614)
		t-value (p-value)	2.076^{ns} (0.093)	3.379* (0.020)	
	II	Day 0	12.30 ± 0.21^{a}	12.26 ± 0.15^{a}	0.156^{ns} (0.879)
		Day 31	12.25 ± 0.23^{a}	11.80 ± 0.14^{a}	1.717^{ns} (0.117)
		t-value (p-value)	0.999^{ns} (0.364)	2.843* (0.036)	

*** Significant at 0.01 level(p<0.01); * Significant at 0.05 level (p<0.05); ns non-significant (p>0.05); Means having different letter as superscript differ significantly within a row*

Table 8. Asymptomatic period of dogs with TC – after treatment

Groups	Duration of asymptomatic period
Group Ia	8 months
Group Ib	4 months
Group IIa	15 days
Group IIb	1 month

inhibitors was studied by Amith *et al*. (2021) and successfully managed a case of tracheal collapse in a dog. Whereas, the present study is based on radiographic interpretations with both brachycephalic and normocephalic dog breeds and there are no previous studies which documented the comparative effects of stanozolol and prednisolone acetate in the respective dog breeds. The common radiographical findings observed were interstitial pattern, interstitial infiltration and biventricular or right ventricular enlargement in dogs with tracheal collapse and this is in concurrence with the study of Tangner and Hobson (1982). Even though there are reports on pulmonary functions, studies on analysis of tracheal dimensions and echocardiography in relation to tracheal collapse of both brachycephalic and normocephalic dog breeds is not traceable. Statistical analysis of tracheal dimensions and VHS values revealed better response with stanozolol in respective dog breeds of the present study.

The mean Td/Ti ratio recorded at day 0 from group Ia and group IIa dogs were 0.12 ± 0.005 and 0.1267 ± 0.007, respectively and revealed a statistical reduction in the mean values (p<0.001) than that of control group (0.18 ± 0.01). Likewise, mean Td/Ti ratio recorded from group Ib and group IIb dogs with tracheal collapse was 0.15 ± 0.01 and 0.127 ± 0.008 respectively and revealed statistically significant reduction (p<0.001) when compared to control group (0.5 ± 0.09) at day 0. Group 1a showed an increase in mean ± SE value of Td/Ti ratio which was statistically significant (p=0.006) over the treatment period. However, for group IIa, this ratio was non-significant at day 31. Subgroup

comparisons revealed a better performance of group Ib dogs (statistical increase with p=0.001) after the stanozolol therapy, whereas group IIb, this ratio was non-significant. All comparisons showed non-significant TT/3R ratios in all groups. Likewise, VHS values were significant in group b, under that Ib and IIb showed decreased values after treatment and which was statistically significant at 0.05 level. Detailed statistical analysis reports were given in Tables 4, 6 and 7. Similar observations were made by Ingman *et al.* (2014), but documents on both brachycephalic and normocephalic dog breeds were not studied yet.

Considering the asymptomatic period, the duration was highest for group Ia and the details were given in table 8. Similarly, Jeung *et al.* (2019) reported asymptomatic periods of tracheal collapse after a theophylline-based study of tracheal collapse.

Based on the clinical evaluation and radiographical interpretation, it can be inferred that stanozolol had a better therapeutic response in normocephalic dogs vis-à-vis prednisolone acetate.

Stanozolol is a synthetic antiandrogen derived from testosterone and shows higher affinity towards glucocorticoid receptors than androgens. The glucocorticoid receptor block reduces tissue catabolic activity with low stanozolol doses. *Invitro* studies reported the effect of stanozolol on collagen synthesis in fibroblastic cell cultures which is cytokine TGFß-1 (transforming growth factor beta 1) mediated. TGFß-1 facilitates the proliferation of chondrocytes and deposition of extracellular matrix. The potential action of stanozolol in canine tracheal collapse is induced by an increased TGFß-1 synthesis by tracheal chondrocytes and fibroblasts (Dondi *et al.,* 2002). Oral administration of stanozolol showed a significant change in both clinical symptoms and radiographical findings in dogs affected with tracheal collapse.

Conclusion

The present study concluded that lateral radiographic techniques can be used for the preliminary diagnosis of tracheal collapse in dogs. Oral stanozolol was found to be effective as the first line treatment along with other supportive medications for a long-term benefit in the management of tracheal collapse in dogs. Though, prednisolone acetate was beneficial in short term amelioration of clinical signs, it was non-responsive in long term management.

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Conflict of interest

The authors declare that they have no conflict of interest.

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