

SCANNING AND ELECTRON MICROSCOPIC STUDY OF CANINE MEGAESOPHAGUS AND ITS MANAGEMENT

R.V.Suryawanshi¹, K.B.P. Raghavender², V.Gireeshkumar³ and M.Laxman⁴

^{1,2 and3}Department of Veterinary Surgery & Radiology, College of Veterinary Science,
Rajendranagar, Hyderabad-500030.Andhra Pradesh. India.

⁴Department of Veterinary Pathology, Ruska laboratories, CVSc, Hyderabad-030.

ABSTRACT: The present clinical study was carried on 18 clinical cases of megaesophagus reported to Campus Veterinary Hospital. The disease was encountered in Labrador (2 dogs), Golden Retriever (2 Dogs), German Shepherd (5 Dogs), Doberman (2 Dogs), Mongrel (3 Dogs), Pomeranian (2 Dogs), Cocker Spaniel (1 Dog) and Boxer (1 Dog) breeds of dogs. The mean age of occurrence was found to be 6.25 ± 0.88 years. Out of these 18 dogs, 11 were found to be males (61.12 %) and the rest were females (7 dogs; 38.88%). All these 18 dogs showed the signs of regurgitation of the food soon after food consumption. The haematological and biochemical studies showed that all the parameters studied were within the normal range, except for haemoglobin. Ultrasonography did not reveal any sort of esophageal pathology. Radiographic features of megaesophagus in the seven dogs were air filled dilated esophagus and tracheoesophageal stripe sign. Esophagography clearly revealed generalized distention of esophagus in all the 18 dogs while endoscopy revealed markedly dilated, flaccid esophagus. Among the three treatment regimens tested, Metoclopramide combined with feeding the dogs in an upright position from an elevated platform improved the esophageal function to the maximum extent. Post mortem examination of the seven dogs died or euthanized showed severe dilatation and thinning of the esophageal wall, while histopathological examination showed scanty muscle bundles, Infiltration of polymorphonuclear. Scanning electron microscopy revealed destruction of blood vessels, loss of normal architecture and direction of inner circular as well as outer longitudinal muscle fibers while, transmission electron microscopic examination showed complete loss of cellular architecture, complete loss of architecture of myoneuronal plate at the neuromuscular junction suggestive of megaesophagus is a neuromuscular disorder.

Keywords: Megaesophagus, Dog, Regurgitation, Neuromuscular, Electron-microscopy, Endoscopy.

INTRODUCTION

Regurgitation and vomiting is common clinical sign of many diseases of the esophagus and stomach (Guilford and Strombeck 1996; Ramprabhu *et al.*, 2001; Ettinger and Feldman 2005; Raekallio *et al.*, 2006; Luna *et al.*, 2007 and Harvey 2008) in addition to being one of the symptoms of many infectious and other diseases. Megaesophagus occurs as a congenital disorder that becomes clinically apparent at or shortly after weaning or it can occur as an acquired disorder in a previously normal adult. Acquired megaesophagus can be secondary to a variety of diseases that because neuromuscular dysfunction or it can occur as a primary disorder for which the cause is unknown (idiopathic megaesophagus). Keles *et al.*, (2007) and Johnson *et al.*, (2009) stated that megaesophagus is a condition characterised by decreased or absent esophageal motility that usually results in diffuse dilatation of the oesophagus.

Watrous (2002) studied that generalised megaesophagus may be due to congenital (hereditary) or acquired (idiopathic), although it may be secondary to one of many possible causes which include chest trauma, tetanus, autoimmune disease, lead toxicity, myasthenia gravis, polymyositis, polymyopathy, hypoadrenocorticocism, thymoma, esophagitis and GDV. Regurgitation is the most common clinical sign observed with megaesophagus Guilford (2005); Glazer and Walters (2008) and Johnson *et al.*, (2009). The present study was carried out to investigate the pathophysiology, symptomatology, management and causation of megaesophagus in dogs as an emerging and challenging disease to companion practitioners.

MATERIALS AND METHODS

Present clinical study was carried on 18 cases out of 42 with history of chronic regurgitation (42.85%). The symptoms exhibited by these cases were recorded. All dogs were underwent routine physical, clinical, haematological, biochemical, radiographic and endoscopic examination prior to medical treatment. These 18 cases of megaesophagus were divided into three groups by randomly assigning 6 dogs to each group. In first group (n=6) were subjected to Cisapride at the rate of 0.5mg/kg body weight orally, twice a day with upright position feeding. In second group, (n=6) with Metochlopramide at the dose rate of 0.4mg/kg body weight, orally, twice daily, with upright position feeding. While in third group (n=6) dogs were kept on elevated platform feeding without any medication. All owners were advised that medication will be needed permanently. Out of 18 cases seven dogs were died from megaesophagus during the course of the study were examined for necropsy, histopathological, scanning and electron microscopic examinations.

The normal and diseased wall of esophagus was subjected to scanning electron microscopic (SEM) and transmission electron microscopic (TEM) examination to study the morphological or structural as well as cellular changes of dilated part of esophagus were collected during the post-mortem examination study. The normal esophageal tissue samples were obtained from a dog that was euthanized due to irreparable trauma in an automobile accident.

Scanning electron microscopic (SEM) method: For SEM studies, the samples were transferred to vials and fixed in 2.5% glutaraldehyde in 0.05 M phosphate buffer (ph 7.2) for 24 hrs at 4^o and post fixed 2% aqueous Osmium tetroxide in the same buffer for 2 hrs. After the post fixation samples were dehydrated in a series of graded alcohol and dried to critical point with electron microscopy science CPD unit. Then the dried samples were mounted over the tubes with double sided conductivity tape. Finally, a thin layer of gold metal was applied over the sample using an automated sputter coater (JEOL JFC-1600) for about three minutes. Then the samples were scanned in the scanning electron microscope (Model: JOEL-JSM 5600, JAPAN) at various magnifications.

Transmission electron microscopic (TEM) method: Samples were fixed in 2.5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 24 hrs at 4^o and post fixed 2% aqueous Osmium tetroxide in the same buffer for 2 hours. Dehydrated in series of graded alcohols, infiltrated and embedded in araldite 6005 resin or spur resin (Spurr 1969). Ultra thin (50-70nm), sections were made with a glass knife on ultra microtome (Leice ultra cut UCT-GA-D/E-1/00), mounted on cooper grids and stained with saturated aqueous uranyl acetate and counter stained with Reynolds lead citrate. Viewed under TEM (Model: Hitachi, H-7500 from JAPAN) at required magnifications.

RESULTS AND DISCUSSION

Vomiting or regurgitation and weight loss were the most commonly recorded clinical signs in thirty (71.42%) dogs out of 42 cases. All 18 dogs showed persistent regurgitation of variable frequency and timing of regurgitation after feed. The regurgitated material was reported to be undigested food particles. In addition, there are so many other organ systems, whose diseases also cause vomiting (Guilford 1990; Lobetti 2000; Jergens 2005 and Glazer and Walters 2008). In effect, what this means is that dogs presented with vomiting as one of the complaints may actually be afflicted with disease of esophagus, stomach, kidney, liver etc., among several other diseases.

The disease was encountered in Labrador (2), Golden Retriever (2), German shepherd (5), Doberman (2), Mongrel (3), Pomeranian (2), Cocker Spaniel and Boxer breeds of each dog. Out of these 18 dogs, 11 were found to be males (61.12 %) and the rest were females (7 dogs; 38.88%). History revealed chronic weight loss in most of the dogs that had megaesophagus i.e. loss of 3 to 5 kg body weight over the past 2 to 3 months. While Richard *et al* (1967); Michael and Goldstein (2004) and Guilford (2005) stated that German shepherd dogs, breeds were more commonly affected with megaesophagus, Gualtieri (2001) and Michael and Goldstein (2004) reported that megaesophagus had no breed predisposition, as in the present study. The average age at which megaesophagus was diagnosed was found to be 6.25 ± 0.88 years in the present study with a range of 7 months to 13 years. Similar observations were made by Richard *et al*, (1967) and Michael and Goldstein (2004).

All dogs showed emaciation; hide bound condition, shrunken abdomen, prominent rib cage and slight to moderate pain and discomfort at cervical and abdominal area on palpation. Mean duration of the clinical signs prior to presentation was approximately three months (range 2-4 months). The symptoms of megaesophagus recorded in the present clinical study were in concurrence with the reports of Richard *et al* (1967); Sherding *et al* (1999); Gualtieri (2001) and Johnson *et al* (2009). Physical examination also revealed that these dogs consumed food very eagerly when offered were extremely hungry. The time of regurgitation of undigested food was found to be immediately after to several hours after feeding were recorded in present study and Sherding *et al* (1999); Gualtieri (2001) and Michael and Goldstein (2004) also reported that regurgitation occurred at varying times after consumption of food.

The hematological and biochemical studies showed that all the parameters studied were within the normal range, except for haemoglobin (8.74 ± 0.12 g/dl). Gualtieri (2001) and Jergens (2005) also reported normal levels of all the hematological and biochemical parameters. Ultrasonography of the cervical esophagus failed to reveal any indication of any sort of esophageal pathology in any of the dogs. Similarly Washababu (1996) and Kealy and McAllister (2005) stated that ultrasonography does not have a traditional role in the diagnosis of esophageal diseases.

Radiographic features of megaesophagus in these seven dogs were recorded to be air filled dilated esophagus, extending throughout the length of the esophagus tracheoesophageal stripe sign was consistently noticed in these seven cases. Watrous (2002) and Guilford (2005) also reported that plain radiography is not always diagnostic method of megaesophagus, but may, in some cases prompt further examination is required. Esophagography showed generalised dilatation of esophageal wall (fig.1) throughout its course. This could be confirmed even in the seven dogs that did not show any radiographic signs of megaesophagus on plain radiography. These observations are in corroboration with the findings of Hoenig *et al* (1990); Sherding *et al* (1999); Gualtieri (2001); Lavin (2003); Keles *et al* (2007) and Rousseau *et al* (2007).



Fig.1: Esophagram with barium sulphate showing severe dilatation of the entire-esophagus. The contour of the esophagus indicated its flaccidity.

Esophagoscopy revealed markedly dilated, flaccid esophagus extending from the cranial cervical region to the gastroesophageal sphincter and pooling of retained fluid, saliva and fermenting fluid in the lumen, in the most dependent segment of the esophagus in most of the dogs. In present study common problem encountered that was always puzzling during the endoscopy is the fact that whether the esophagus appears dilated due to megaesophagus or due to its insufflation with air. Gualtieri (2001) and Jergens (2005) and also encountered some difficulty in confirming megaesophagus by endoscopy.

To sum up, the results of the present study clearly indicated that contrast radiography of the esophagus was the most accurate and reliable method in diagnosing the presence and extent of megaesophagus than other methods. Similar observations were also made by Hoenig *et al* (1990); Sherding *et al* (1999); Gualtieri (2001); Keles *et al* (2007) and Rousseau *et al* (2007). Among the three treatment regimens tested, Metoclopramide combined with feeding the dogs in an upright position from an elevated platform improved the esophageal function to the maximum extent. The number of times of regurgitation was lesser in these dogs. Improvement in the physical condition was also reported by the owners in five of the six dogs treated with Metoclopramide combined with feeding the dogs in an upright position from an elevated platform. Hoenig *et al* (1990); Michael and Goldstein (2004) and Johnson *et al* (2009) also reported similar findings. However, the observations of Jergens (2005) are not in agreement with the present study, who reported Cisapride to be better for this purpose in cats. This could be due to some species related variations and need further investigations.

Out of 18, three dogs died and four dogs were euthanized during the course of the treatment due to malnutrition, generalised weakness and dehydration. The post mortem examination of esophageal wall revealed dilated, thin, congested mucosal surface, congested lungs with ecchymotic or patchy haemorrhagic spots over, suggestive of aspiration pneumonia. Histopathologically esophageal wall showed scanty muscle bundles, infiltration of polymorphonuclear cells with submucosal congestion and enlargement of submucosal glandular pattern with epithelial irregularity. Similar findings collaborate with Richard *et al* (1967) Barber *et al* (1983), Hoenig *et al* (1990) and Keles *et al* (2007) in dogs with megaesophagus.

Scanning electron microscopic examination of the normal esophagus showed comparatively more muscle mass and intactness of all the concerned layers of the esophagus (fig.2), as was also not reported in the present literature. On the contrary, scanning electron microscopy of the esophageal tissues from dogs affected with megaesophagus revealed destruction of blood vessels, loss of normal architecture and direction of inner circular as well as outer longitudinal muscle fibers (fig.3). Similar findings also reported by Barber *et al* (1983) were a reduction in thickness of the mucosa, inner muscular layer (circular layer) and outer muscular layer (longitudinal layer) of colt esophagus affected with megaesophagus.

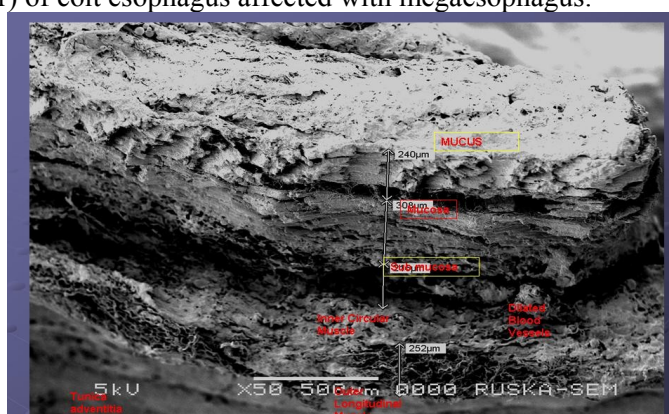


Fig.2: Photomicrograph showing the scanning electron microscopic (SEM) structure of normal thoracic esophagus in dog (6kVx50µm).



Fig.3: Photomicrograph showing the scanning electron microscopic (SEM) structure of dilated thoracic esophagus in a dog with megaesophagus (5kVx50µm)

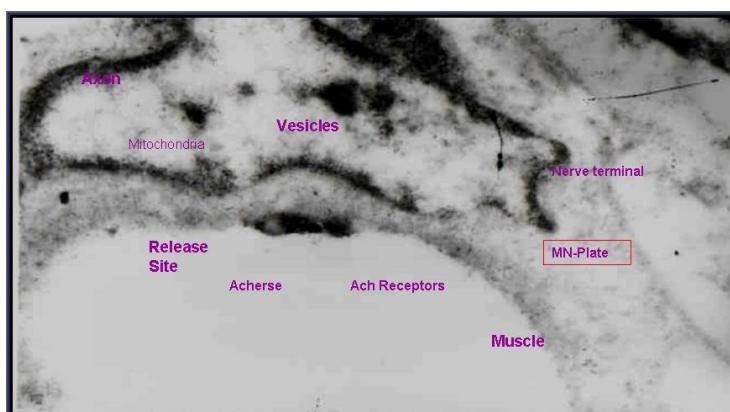


Fig.4: Electron microscopic examination normal neuro-muscular junction (NMJ) showing with synaptic cleft (single arrow) and vesicles with myoneuronal plate (double arrow) (21480X52).

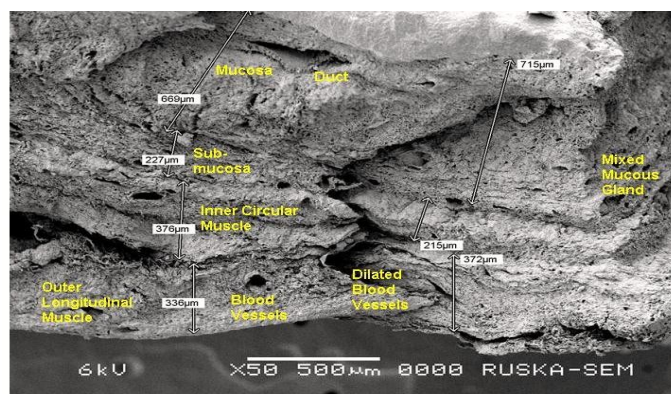
The layer wise thickness the different layers of the megaesophagus tissues was measured against the normal esophageal wall (Table.1). The transmission electron microscopic examination of dogs with megaesophagus showed dilated myofibrillar bundles, condensed nucleus, complete loss of cellular architecture (Fig.4) and vacuolization between muscle fibers bundles, karyolysis. All dogs showed the complete loss of normal shape of mitochondria characterised by swollen and condensed mitochondria with thick electron dense material. Neuromuscular junction showed complete loss of architecture of myoneuronal plate with thick synaptic cleft and vesicular degeneration as compare to normal NMJ (5). While Richard *et al* (1967) and Johnson *et al* (2009) also stated that megaesophagus is a neuromuscular disorder; there are very few electron microscopic studies on megaesophagus. The available literature supports the present findings (Randelia *et al* 1990; Ross *et al* 1995 and Lemasters 2009). Further investigation and treatment like regenerative medicine (stem cell) will counteract the condition in companion animals.

Summary

It was concluded that, the most common malady affecting the esophagus in dogs was found to be megaesophagus, characterised by chronic regurgitation occurred immediately to several hours after feeding with undigested food. Disease could be easily diagnosed by esophagography and esophagoscopy and this can be reasonably well managed by using metoclopramide and feeding the dogs in an upright position from an elevated platform. The disease is considered to be a neuromuscular disease as seen during scanning and transmission electron microscopic studies as it also required further investigation and treatment like regenerative medicine (stem cell therapy).

Table.1. Mean values of scanning electron microscopic (SEM) measurements of esophageal wall thickness (μm) of the megaesophagus dogs (n=7).

Tissue layers	Megaesophagus dogs(n=2)							Mean \pm S.E	Normal Dog (n=1)
	Case 1	Case 2	Case 3	Case4	Case5	Case6	Case7		
Mucosa (μm)	240	257	249	273	298	257	310	269.14 \pm 9.84	669
Submucosa (μm)	308	315	324	307	342	310	302	315.42 \pm 5.15	227
Inner circular muscularis (μm)	216	210	198	241	221	211	203	214.28 \pm 5.30	376
Outer longitudinal muscularis (μm)	252	235	268	246	238	245	248	247.42 \pm 4.06	336

**Fig.5:** Electron microscopic examination of megaesophagus showing completely destroyed neuromuscular junction (arrow) (12530X52).**Acknowledgement:**

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