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# Morphophysiological Study of Gastrointestinal Tract of the Donkey (*Equus asinus*)

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## Abstract

In most of the developing countries, donkeys are used to carry goods and water and to guard herds as a livestock guardian. Donkeys possessed a good digestive system and are being offered only low-cost fibers diet like hay and straw. Despite the biological potential of the donkey, only a few studies have focused on the morphophysiological aspects of their digestive system. A series of tubular organs and associated glands are present in the digestive system. Although generally the morphology of the donkey digestive system is comparable to the horse, few dissimilarities exist among such species. In this chapter, we tried to highlight the anatomy, histology and physiology of the digestive system of domestic donkeys including tongue (mucosa, papillae, muscle, taste buds), teeth, pharynx, esophagus, stomach, saccus cecus, descending part, an ascending part and transversal part of the duodenum, jejunum, ileum, cecum, colon (right dorsal and ventral; left dorsal and ventral), rectum and anal canal. The microarchitecture of the tunica mucosa, next to the lumen, is focused upon. Morphology of the large accessory digestive glands viz. salivary gland, liver and pancreas were also highlighted. These structures are situated away from the gut-tubular system but are attached to its lumen through their specified duct system. Furthermore, peculiar microstructures of the internal layers, immune system and microbiome of the gut were correspondingly highlighted in the chapter.

**Keywords:** anatomy, histology, physiology, oral cavity, stomach, intestine, glands, microscope, donkey

## 1. Introduction

### 1.1 Donkey in the past, present and future perspective

Donkey (*Equus africanus asinus*) belongs to Equidae family like horse and is domesticated in most parts of the world [1]. The African wild ass living wildly has been declared as wild ancestor of today's donkey. The life span of donkeys is generally ranging from 25 to 50 years. It is known to have been used as a beast of burden. "The first findings of donkey came from ancient art and archeological records. Donkeys bred and produced mule's offspring which were used by the Spanish during their occupations and defeats. By the same time the donkeys got progressively significance in America and were used for shipping gold around

mountainous mines. Although the world has move toward the mechanization, but donkey (an ancient animal) is being used as biological vehicle and is known as beast of burden. In arid and semi-arid places, it serves man in carrying luggage and used for transportation purpose [2]. It can live on low quality high fiber diet and scarce amount of water. It can bear harsh climatic conditions. It is the source of bread and butter for the poor laborer of the developing country and is called the horse of poor man available in low price. It is the source of earning on daily bases for many poor families. Along with mules, donkeys are also used as a means of transportation by those armed forces who are deployed in the large mountainous areas [3]. Donkeys are used to guard sheep, as they are more inclined to stand and fight than to run from a predator. They provide a means of transportation for agricultural goods, building materials, droughts, tracking carts and riding humans themselves all over the world. Donkey is also named as and will remain an inexpensive horse [2]. It can go places where cars and other vehicles cannot go, so it can be used for transportation such as hilly areas [3]. In addition, Donkey milk is used as an alternative to human breast milk, as it has many of the same important qualities viz. low in fat contents, promotes healthy intestinal flora, have anti-inflammatory properties, contains immune enhancing compounds which protect the body against pathogen [4]. Donkey meat is eaten in many places of world. Italy is the largest consumer of donkey meat in Europe [5]. Donkey meat is considered tastier than horse meat and is a delicacy in most of the Chinese restaurants. Donkey meat burgers are a favored way of eating the meat and are eaten in Canada and Mexico for example. Donkey milk components are used in making cosmetics soaps and skin creams. Donkey is also used as test animal in pharmacokinetics [4]. Donkey hide gelatin (aka ass hides glue) is used in traditional Chinese medicine to treat bleeding dizziness insomnia and dry cough and a source of raw material for Shoes company. Donkeys also played role in reproduction and produced fertile or infertile mules which helped and used by humans for many purposes [2]. Based on loyal behavior, such animal holds a position as noticeable companions and guard of pet animals. Scientists of the advance countries like America are planning to use donkey in Artificial Intelligence by modifying its brain function and so it can be used in secret missions. Due to its importance, veterinarians and other researchers are interested in discovering further benefits out of it. Only a few studies highlighted and focused the anatomical and physiological aspects of the donkey alimentary system. Unlike other individuals, such animals are being offered low-cost fibers diet [1]. High fiber forage diets are better digested by donkeys than horses. Donkeys are said to possess a better digestive system than horses, it comprises of a series of tubular organs and associated glands [6]. Its function is to cut down the ingested complex food materials and converted into the valuable energy source and removing the wasted portion for maintaining the health and growth of the organism. The digestive system of the donkey is explored in detail in this chapter.

## **2. Anatomy and physiology of the digestive system of donkey**

The donkey gut can be apportioned into two segments including the foregut made up of the stomach and small intestine and hindgut or large intestine is consists of cecum and colon. The overall digestive system is a hollow tube, starting with the mouth and oral cavity leading through to the anus with structures including the esophagus, stomach, small and large intestine, rectum and the anal canal in between [7]. The digestive glands like liver, pancreas and salivary glands are also

associated with the system. The entry of the gut (buccal cavity) is surrounded by the lips anteriorly and are present posterior to the nostrils, by the cheeks and teeth laterally, by the hard and soft palate dorsally, by the movable tongue ventrally which is present in the floor, and posteriorly it opens into the pharynx. The structures in the oral cavity include the tongue, teeth and gums, salivary glands, palates and immune tissues.

## 2.1 Lips

The lips are the main prehensile organ in donkeys, they are lined by stratified squamous keratinized epithelium and comprise of skin, glands, hair follicles, and tactile hairs. Microscopically lips are composed of epidermis, subcutaneous tissues thick layer of connective tissue, orbicularis oris muscle fibers (of skeletal type). The labial glands and a layer of adipose tissue are present in lamina propria and submucosa. The lips of donkey are mobile which help in collection and direction of grass toward the incisors for cutting and the premolar and molar teeth for mastication [8].

## 2.2 Dental anatomy in donkeys

The equine tooth is made up of the same substances as human dentition i.e. cementum, enamel, dentine and pulp but the matrix is different, and our teeth are encapsulated in enamel whereas the donkey occlusal surface shows a cross section of all materials except pulp. Pulp is innermost layer contains vital structure like nerves, blood supply, lymphatics and bone forming cells the odontoblasts [9]. This soft structure is protected by outer layer. The next layer is which occupy main portion of the tooth but is less mineralized than enamel is the dentin. By this reason the dentin wears out more than enamel which is the hardest portion of the tooth. Enamel does not have ability to heal up if it is scratched and injured like the other tissue in tooth can, but it remains protected between cementum and dentin. Cementum is the outer layer and is similar to bone and assists connection between periodontal ligament and the tooth. This structure defends the tooth, maintaining it in the socket within the gum and provides care as the animal masticates. Only 11% of infundibula are completely cementum filled. The developing bone is externally lined by stratified squamous non keratinized epithelium followed by a primitive connective tissue. The incisors are located at the front of the mouth and are visible when you lift up the animal's lips [10]. The permanent incisors have crescent shaped depression called infundibulum that is filled with cementum. The incisors are used to cut the grass during grazing and also aid in assessing the animal age. Canine or bridal teeth are located between incisors and premolars. Lower canines are positioned more rostral than the upper ones. Male donkeys generally have four canines, but these generally do not fully develop in females. Wolf teeth are located just medial to first cheek teeth in both upper and lower jaws. They may be absent or four in number. Cheek teeth are most caudal group of teeth. Three molars and three premolars make up each row. Upper cheek teeth have infundibula that wear out with time. The molar are permanent teeth only. Based on closed location, collective premolars and molars performance is like a specific unit for the breakdown of food. Total number of equine teeth is as per the following formula:

$$\begin{aligned} \text{Deciduous I3 C0 P3/I3 C0 P3} &= 12 \text{ and the long - lasting are as } 3 \ 1 \ 3(4) \ 3/3 \ 1 \ 3 \ 3 \\ &= 20 \ (21) \end{aligned} \quad (1)$$

The deciduous incisor teeth are rounded at the top and are whiter in color while the long-lasting are adopting square shape at the margin of the gum and appears yellower. Teeth eruption processes are as follows.

Teeth	Date of deciduous teeth eruption	Date of permanent teeth eruption (years)
Central incisors	0–2 weeks	3–3.5
Middle incisors	5–8 weeks	4
Corner incisors	1 year	5–5.5

2.3 Donkey tongue

It is a strong muscular organ enclosed in thick mucosa. It is very sensitive organ with a groove between inner part of it which is connected to underlying tissue and a free part in front. This organ has spatula shaped having torus linguae (extended torus and muscular distinction), which is distinctive for Equidae. Stratified squamous keratinized epithelium lining the external surface followed by connective tissue and a layer of skeletal muscles [11]. The muscle layers are arranged in various forms which help in rotation of the tongue during feed mastication. Epithelium lining ventrally is the non-cornified. Filiform, fungiform, foliate and vallate papillae are present at three parts of tongue (apex, body, and base). All types of papillae are lined by partial to complete carnified epithelium. Filiform papillae are mechanical and almost cover major portion on the dorsal surface. It is short and thin at apex, pointed and rough at body, and elongated at the caudal portion. Fungiform mainly scattered at lateral surfaces, around the filiform and are round to lobulated. These are larger, wider, taller but less in number than filiform. The vallate papillae with circular grove and central spherical bulges are positioned caudally in the body and 3–4 times larger than fungiform. Group of foliate are located near the base of palato-glossal arch and are organized like leaves alienated by variable grooves. Fungiform and filiform are devoid of taste buds but vallate and foliate has taste buds. Basal cells are present at the base of each taste bud and act like the stem cells. As compared to the tongue of horses, the feature is the occasional occurrence of the dorsum cartilage (cartilago dorsi linguae) of the tongue. Lymphatic nodules are special aggregated lymphoid cells and are present dorsally. Small mucous secreting labial glands are also present in connective tissue, secretion of which moistens the oral mucosa. The connective tissue in the lamina propria is richly supplied with blood vessels, lymphatics, nerves and adipose tissue.

2.4 Cheek of donkey

The structure of the cheek is like that of lips, designed principally of buccinator muscles and contains some minor glands (salivary). The powerful muscles of cheek help in mastication, grinding and mixing of food. These includes masseter, pterygoid medially, pterygoid laterally [12]. These muscles get their nerve innervation from the mandibular branch of the trigeminal nerve. Furthermore, cheek muscles involved in closing the mouth through elevation of the mandible [13].

2.4.1 Masseter muscles

These muscles have wide multipennate muscles with numerous tendinous connections. In the donkey, it is the largest muscle involved in mastication. It is grouped into; “Proper (first, second superficial, middle and deep) masseter



coatings and improper masseter muscles groups (zygomatico-mandibularis and maxillo-mandibularis)". Its main function comprises of the movement for chewing, achieved by the masseter group (proper) and definite shutting of the oral cavity is executed by the improper type of masseter group [14]. The improper muscle moves the mandible in lever style. Arteries of masseter muscle include masseteric artery, transverse facial artery, buccal artery, facial artery. The arrangement of the vessels which supply blood to the masseter muscle defined its importance.

#### *2.4.2 Pterygoid muscles*

These muscles routed through the bottom of the skull via mandible medially. Pterygoid muscles accompany the masseter during function. Upon bilateral contraction the pterygoid muscles caused elevation of the mandible and upon performing unilateral action they attract the mandible sidewise of the contracting muscle [13]. Its lateral portion is capable to direct the rostral direction of the mandible, particularly when the oral cavity is opened.

#### *2.4.3 Temporal muscle*

The temporal muscle is originated from the temporal crest and occupies the temporal fossa. It is inserted on the coronoid process of the mandible. Its functions include elevations of the mandible and help other muscles mutually during mastication's [13].

#### *2.4.4 Digastric muscle*

These caudal and rostral bellied muscles are not the actual muscle of mastication but may also add partially to the jaw movements during opening to the oral cavity. It prolongs among the process of paracondylar of the occiput and the mandible medially [13]. The facial nerve innervates the caudal part while the mandibular nerve innervated the rostral part. The lateral portion is formed from the extension of the caudal belly, attached on the mandibular angle and attracts the mandible bone backward. Below the basihyoid bone it develops the rostral belly, which attaches medially to the mandible body. This muscle opens the oral cavity by pushing the mandible [11].

### **2.5 Palate**

The palate has mucosa on both oral and nasal sides and has soft and hard parts. Oral mucosa is lined by tough cornified squamous epithelium. Hard palate is formed by union of palatine, maxillary, and incisive bones with no muscles [13]. Soft palate is a muscular structure made of intrinsic paired palatine muscles paired extrinsic tensor and levator veli palatine muscles along with palatine glands present in it. Trigeminal nerve supply to the palate and glosso-pharyngeal and vagus nerve supply the muscles of soft palate. Lymphoid follicles are present in the lamina propria along with FCT [14].

### **2.6 Pharynx of donkey**

Pharynx is about 15 cm in an adult animal. It is present at the back (posterior) of the mouth and is located between the skull at base and the initial two cervical vertebrae at dorsal portion and ventrally the larynx. On lateral side, two pairs of palatopharyngeal arches are present from the soft palate to esophagus. Wall of

pharynx consist of striated muscles. It includes the nasopharynx which is entrance to auditory tubes, oropharynx and laryngopharynx [15]. Nasopharyngeal mucosal epithelium is composed of pseudostratified columnar epithelium with goblet cells. Lymphoid follicles can also be seen in the lamina propria and submucosal area. Nasopharynx is innervated by cranial nerves V, IX, X, XII. It is also composed of sensory receptors of glossopharyngeal and trigeminal nerves. During swallowing, the soft palate is raised which divides pharynx into dorsal and ventral sections [13]. It plays important role in deglutition. It serves as pathway of food from mouth to esophagus. It consists of rostral constrictor muscles including the hypopharyngeal, pterygoid, and palatopharyngeus. The stylopharyngeus are the muscles responsible to dilate the area while pterygopharyngeal muscle and palatopharyngeal muscles shorten the pharynx. Palatopharyngeal muscles also close the pharyngeal arch. There are some tactile receptors which detected the air flow and cause dilatation of the air way by stimulating the gag reflex. Augmented action of such receptors stabilizing the muscles which improves the dilatation of upper respiratory tract and prevent it from being collapsed. It is advised that glossopharyngeal nerves should never locally anesthetized otherwise there be dysfunction of oropharyngeal muscle which may causing collapse of dorsal nasopharynx and ultimately inspiratory obstruction in exercising donkey. Failure of pharynx or neuromuscular activities will result into the severe respiratory disorders.

## **2.7 Tonsils**

This tissue is responsible for defense, located at the rare area of the throat. A tissue of soft lymphoid follicle like the lymph nodes surrounded by a layer of stratified squamous epithelium. The mucosa invaginates deep in the lamina propria forming crypts and fundi [11]. Both diffuse and nodular arrangements of the lymphoid tissues are present.

## **2.8 Esophagus**

The length of the esophagus depends upon the body of animal. It consists of cervical, thoracic and abdominal parts. It moves lateral to trachea as moving down and becomes ventral again at thoracic inlet. Unique feature of donkey esophagus is its pigmentation at different parts. Esophageal obstruction is also common in donkey due to different anatomical entrance to stomach [16]. Cervical part of the esophagus is located dorsal to trachea and ventral to cervical vertebrae and the thoracic part is located dorsal to sternum, medially in the thoracic cavity. The esophagus ranges from 125 to 200 cm in length in average adult animal. It lies dorsally on trachea in the cranial third then turned toward left in the middle third of the neck [17]. In the area of thoracic inlet, it lies ventrally to the trachea. Under microscope its wall is divided into mucosa, submucosa, muscularis and tunica adventitia. Mucosa is the innermost part of the esophagus toward lumen and is lined by keratinized stratified squamous epithelium. In the lamina propria, the glands are present along with a layer of FCT and some lymphoid follicles, blood vessels and other vasculature. Lower layer of the mucosa is surrounded by smooth muscle called muscularis mucosae. The submucosa contains elastic fibers, adipose tissue and seromucous gland. The muscularis externa is composed of skeletal muscle in the proximal two-thirds and turns to smooth muscle in the distal third. The skeletal muscle layers are adapted in inner circular and outer longitudinal arrangements. The cervical pleura and peritoneum add to tunica adventitia in all portions of the esophagus. The loose attachments of the esophagus with the adjacent tissue permit the neck movement during swallowing. At abdominal portion the esophagus has a serosal covering.

The function of esophagus is to provide pathway to partially digested food into the stomach. There is no digestion in esophagus [18].

## 2.9 Stomach

Lower portion of the esophagus and the stomach lies toward right side in the abdominal cavity. Between esophagus and stomach there occurs a junction i.e., esophagus-gastric junction [17]. The stomach has three sections, saccus caecus, fundic and pyloric regions. The saccus caecus is a non-glandular portion on stomach and is located close to the esophagus entrance in the stomach [16]. It is situated ventrally to the diaphragmatic left crus and is underneath the dorsal portion of 16th and 17th ribs. It relates to pancreas, present behind the great colon extension and situated laterally to spleen bases. This portion is covered by keratinized stratified squamous epithelium. The keratin layer thickness differs with degree of stomach distension, age and diet of the animal. The lamina propria normally has plasma cells, lymphocytes, mast cells and neutrophils. The muscularis mucosa is continuous and the submucosa holds nerves plexus and lymphatics. The muscularis externa is comprised smooth muscle arranged in three layers viz. oblique (inner), circular (middle) and longitudinal (outer) layers. Between the inner circular and outer longitudinal layers of muscle there is the myenteric nerve plexus. In this area the HCL initially combine with the ingested food mass and reduces the prior process of fermentation that initiated with the discharge of sugars (soluble) from the food in donkey's oral cavity. It is imperative that in the stomach the fermentation is very sparse because it leads to the gas formation. In donkey there is a slight experience to belch or otherwise to dispel collecting gas. Histologically, the lining epithelium at junction of stomach is abruptly transit to columnar epithelium from stratified squamous form. This junction acts like a valve that does not allow acidic contents of stomach to enter in esophagus. Due to any abnormality, this junction is not performing its proper function; it can result in reflux esophagitis. Externally diaphragmatic crura and internally C-shaped sling fibers of stomach make it possible to perform its pinchcock like action. Grossly we can say that proximal cardiac portion of stomach and distal end of esophagus makes this muscular junction. The stomach of donkey is like horses in its conformation. The average weight of an empty stomach in donkey is 1.5 kg. The comparative stomach capacity of donkey is 14 and the caecum and colon is about 80, whereas ruminants have the stomach capacity around 80 and that of caecum and colon is only 13. Hence the stomach of donkey and caecum of large ruminants are similar. Donkeys have monogastric type of small stomach that bounds the feed portion which can be got at a time. It attempts incessant foraging as numerous slight feedings are superior than few big meals since the stomach starts to unfilled when it is 2/3 full irrespective the food is processed or not in the stomach. The mucosa has folds which flattened when the stomach fills and has gastric pits and glands. The cardiac glandular region of the stomach has short, coiled tubular glands that are lined by simple cuboidal epithelium. Proper gastric (fundic) regions of the stomach is containing straight, branched tubular glands of which narrow neck, long body and dilated blind ended fundus [19]. The pyloric region has deeper pits. The mucosal glands are lined by the chief (zymogen secreting) and parietal cells (acid secretion) along with mucous neck cells. The chief cells are larger in number while the parietal cells are larger in size. Although the digestion by microbial happens in caecum and colon in donkeys while stomach temporarily stores food because of its emptying behavior. Overall compared to other animals, larger area of the donkey's stomach is covered by the non-glandular regions [20]. A small amount of food is digested in the stomach and then goes to caecum and colon for microbial digestion.



## 2.10 Intestine

It is positioned ventral to the vertebral column in the abdominal cavity and has the following three parts. The duodenum is the initial and shortest portion of the small intestine located at left side of the abdominal cavity [17]. Duodenum joins the jejunum and the stomach together and is divided into the following four parts:

1. Superior (first) part also called ampulla duodeni
2. Descending (second) part
3. Horizontal (third) part
4. Ascending (fourth) part

**Superior part of duodenum:** It is in interaction with the liver through the visceral surface and forming ampulla which is a dilated portion and a sigmoid flexure. The initial curve of the flexure is dorsally convex and the other also called cranial flexure is ventrally convex which provide the site of attachment for body of the pancreas. The first 2 cm of superior part of duodenum, immediately distal to the pylorus has mesentery and is mobile. This free part called the ampulla (duodenal cap). The distal 3 cm of the superior part have no mesentery and are immobile because they are retroperitoneal. The duodenal superior segment ascends from pylorus and is overlapped by the liver. Peritoneum covers its anterior aspects, but it is bare of peritoneum posteriorly, except for the ampulla.

The major duodenal papilla is a rounded projection at the beginning portion of the mutual pancreatic and bile duct into the duodenum and is the primary source of bile and other enzymes secretion that ease the process of digestion. Mucosa forming protruding papillary folds at ampulla where the lining epithelium transitions from common gut surface type to pancreatobiliary type like distal ducts. The lamina propria mucosa contains infrequent plasma cells, lymphocytes and mast cells. Little ductless mucous glands ductules lie beneath the mucosa. Sphincter of Oddi represented by smooth muscles possibly ranged into mucosal surface folds and might have some neighboring acini (pancreatic), but typically the islets are not seen nearby major papillae. The development of major duodenal papilla begins with evaginations of the gut tube lies caudal to the stomach. The dorsal mesogastrium and the ventral mesogastrium pancreatic buds are formed. Few of the epithelium fail their associations to the emerging pancreatic duct system and lead to develop into the endocrine portion in the form of islets of Langerhans in pancreas. The minor papilla (duodenal) is positioned typically about 2 cm ventroproximal to the major duodenal papilla. Jejunum is the longest portion in the small intestine. It is situated in the middle part of the intestine [21] and is present in abdominal left side. A large number of digestive glands are present in the jejunum responsible for releasing buffers and enzymes into the gut lumen. In this largest luminal absorptive area, most of minerals and nutrients are absorbed [22]. Ilium is the last part of the small intestine and is present also in the abdominal left side and is the final section of small intestine. The ileocecal fold is situated between the antimesenteric side of the ileum and the tenia dorsalis of the cecum. Its role is to absorb all the remaining bile salts vitamin B12, and other digested stuffs that were available un-absorbed in the lumen. Ileal and cecocolic ostia generally have a small opening or orifice. A muscular layer circular in shape is the sphincter which is connection of the ileum and the cecum called ileal ostium (ileocecal valve). During dissection of the gastrointestinal tract of donkey, these are the macroscopic structures. In the terminal portion

of the gastrointestinal tract (GIT) at the cecal basis, the ostia (ileo-ceco-colic) are detected undoubtedly [23]. The ileal ostia inhibit the large intestinal luminal contents (rich in bacteria) refluxes back to the small intestine. The Peyer's patches located at the ileal submucosal tunics are the distinguishing histological items [24]. The ileocecal and cecocolic folds (peritoneal) set the cecum with other intestinal portions. Through the *ceco-colic ostium* the substances present in the ceca are drained directly into the colon (ventral). Gas accompanied ingesta are also eliminated across this ostium. Mucosa of the small intestine is lined by simple columnar epithelium. It covers the longest villi and the highest number of Goblet cells related to other parts of small intestine [22]. Sub-mucosa of duodenum contains Brunner's gland that secrete a serous secretion. Two layers, circular (inner) and longitudinal (outer) arrangements of muscularis externa and the outer serosa is present in its wall. The cecum is a portion of large intestine having pouch-like region present in pelvic portion of abdominal cavity located laterally and inferior to the ileum [17]. It is a very large chamber. The cecum has comparatively thicker mucosa, lined by simple absorptive columnar epithelium having plentiful goblet cells and entero-endocrine cells. Its lamina propria and muscular mucosae is identical to that of small intestine and the glands are packed tightly and lengthier. They lack Paneth cells. The cecum further absorbed the salt and remaining digested fluids through its thick mucosa and also add mucous to the remaining intra luminal contents [20]. The colon is present in abdominal cavity [17] and pushes all other organs cranially to thoracic part of abdominal cavity. The hindgut of the equine keeps similar job to that of other animals' large intestine viz. retention, further mixing and forward movement of the intraluminal contents. Such cecal movement is based on forced contractions of the wall.

## 2.11 Immune cells of the intestine

Lamina propria lymphocytes are B-cells that secrete IgA (Antibody A). IgA comes into lumen through epithelial cells; here it performs the function of adhesion and invasion of bacteria. Intraepithelial lymphocytes are present in the basolateral spaces between luminal epithelial cells [25]. Microfold cell (M-cell) is present in mucosa-associated lymphoid tissues [26]. Its main objective is to conveyance luminal antigen to the cellular immune system. Intestinal macrophages are heterogeneous and have the ability to locate and engulf bacteria [24], virus, fungi and parasites. Intestinal macrophages are mainly located in sub-epithelial area. Activated macrophages are important source of cytokines (IL-10). These prevents large intestine from excessive inflammation during bacterial infections. Paneth cells are present just beneath the intestinal stem in intestinal gland (crypts) in colon. These cells produce great amount of alpha defensins and other antimicrobial peptides such as secretory phospholipase A2 and lysozymes.

## 2.12 Colon of donkey

The ascending colon is splatted into left dorsal, left ventral, right dorsal and right ventral portions by the flexures (sternal flexure, pelvic and diaphragmatic). Location of the sternal flexure linking to the pair portions of the ventral colon [18]. In the border between dorsal and ventral colon the pelvic flexure is located, and the diaphragmatic flexure location is between the pair portions of dorsal colon. The ascending mesocolon is attached with ventral and dorsal parts. Ventral and dorsal colon is similar in length and is a part of the gastrointestinal tract of donkey. The transverse colon is positioned between the descending and ascending colon. The descending colon has extended mesocolon (descending).

This portion of large intestine has typical similar histological structures to that of cecum including mucosa, submucosa, muscular and serosa. Extensive mucus layer and crypts in the mucosa supports the feces passage. Colon is the lengthiest segment of large intestine and collects nearly entire digested material from the cecum, absorbs the remaining nutrients and water, and permits the drainage of feces to the rectum [7]. The roll of ascending colon is to absorb the remaining water and other key nutrients from the indigestible material, solidifying it to form stool. The waste material (feces) temporarily stored in the descending colon will finally be emptied into the rectum [17]. The rectum is present in pelvic cavity and is dorsal to reproductive tract. It lies between the terminal portion of colon and anus. It is usually found empty except when there is movement of feces with the help of mass movement through large intestine. It may also happen when animal is in the state of hyper aesthesia. Rectum is situated dorsal to genital and urinary tracts. Hence, it is also used for palpation. There is recto-genital pouch at dorsal side of rectum. It is the place where rectum and vagina in female and urethra in male are attached. Meso-rectum is the ligament that is attached to rectum. The rectum has pressure sensitive cells that are activated when it is filled with feces. These special cells are involved in initiation of defecation reflex. This starts the forceful contraction of rectal muscles and internal anal sphincter relaxation. This is the way that feces are passed out. Donkey lacks the ability to control the external anal sphincter. Hence whenever stretch receptors are activated there is a sure or confirmed defecation reflex. In the rectum the columnar epithelium with goblet cells turn to stratified squamous epithelium at recto-anal junction. Circular muscles of tunica muscularis form the internal anal sphincter while that of the other anal sphincter (external) is made up of skeletal muscles that are somewhat of voluntary control. The most terminal portion of the lower GIT is the anal canal which lies between the verge of the anal portion in the perineum below and above the rectum (below the level of the pelvic diaphragm) and located in triangular perineum of left and right ischioanal fossa and ultimately it open into the anus. On the basis of the structure, anal canal may be apportioned into two segments (lower and upper) separated by pectinate line or dentate line. Mucosa of the zona columnaris (upper zone) is lined by simple columnar epithelium and the elevation of the mucosa layer produces a valve. It is supplied by superior rectal artery (a branch of the inferior rectal artery). The lower zone is divided into two smaller zones, separated by a line known as Hilton line. The stratified squamous non-keratinized epithelium lining the zona hemorrhagica while the zona cutanea lined stratified squamous keratinized epithelium which blend with the perianal skin. The inferior rectal artery supplies this zone. Anal gland is small gland near the anus in many mammals [27]. Sebaceous gland at the lining of the anal glands secretes some liquid. The medium number of the anal glands in each anus is ranging from (3–10) 85% anal glands were found in the sub mucosa, 7% extended to the internal smooth muscle sphincter and only 2% in the intersphincteric space. Hence these anal glands found in sacs form in the anus and these secrete special types of hormones that encourage the other members of that species of opposite sex.

### **2.13 Microbial digestion of rough and fibrous food in colon of donkey**

Like rumen of the ruminants the microbial digestion mostly accomplished the cecum and colon of the equines. The stomach of ruminants and the large intestine of the donkey are therefore functionally similar. The donkey although is not more efficient in digestive process (grazing) compared to ruminants but has a combination of a large cecum and colon where the process of absorption and fermentation happens. Bacterial counts remain higher in equines where most of the fibrous and

rough food digestion occurs [28]. The higher counts of hemicellulytic and cellulytic bacilli are present in the donkey cecum and in colon the luminal bacterial counts are even more. The intestinal microflora may prevent infection by fighting with pathogens. It is a complex ecosystem containing many bacterial species, protozoa, fungi and yeast. There are five types of microbes present in large intestine includes proteolytic bacteria that cause breakdown of protein, lactic acid bacteria that digest starch, protozoa make volatile fatty acids, cellulytic bacteria and yeast/fungi that digest/break fibers and few vitamin-B producing bacteria. The bacteria that is present in it includes Lactobacillus & Firmicutes in the ileum, Lachnospiraceae, Ruminococcaceae, Bacteroidetes and Spirochetes in the proximal part of large intestine and Prevotellaceae in the distal part of large intestine etc. The donkey receives much of its dietary supplement through hydrolysis and by fermentation of these microflora.

### **3. Fecal ball formation**

First of all, animal eat food and its whole digestion process is like other animals. Mastication of food occurs after prehension. Digestion depends on good food grinding by teeth. During mastication saliva is produced and it depends on food which type of food is eaten by donkey. In stomach digestion is minimal and its main function is liquefaction of food then food is drained into small intestine. However, there are many types of enzymes released by stomach. Food particles are broken by gastric acid that produce by stomach. While protein digestion is due to enzyme pepsin. Pancreas release an enzyme called amylase, when food drains into duodenum part of small intestine. This enzyme is less produced in donkeys, so digestion of starch is minimal. The end product of protein is amino acids, done by enzyme released such as pepsin, and it absorb into blood [29]. Volatile fatty acids are produced by process of fermentation and then blood absorbs it. Actually, this volatile fatty act as source of energy. The proteins that remain undigested in large intestine are broken down by enzyme released by microbes. Ammonia is produced by this protein and it is beneficial for growth of beneficial bacteria [20]. Water is absorbed by large intestine, when whole grinded food enters into colon, more reabsorption occurs, and semi-solid feces formed. In colon end step occur as formation of fecal ball and then move into rectum and then anus [7].

### **4. The nervous system of the gastrointestinal tract**

The digestive process like gut motility, absorption, secretion and the blood flow is influenced by the nervous system [30]. Although there is a bit links between the CNC and the digestive system, but the gut is capable of having their own nervous system called as the enteric nervous system (ENS). Like the spinal cord, this system holds numerous neurons. This system alongside with parasympathetic and sympathetic nervous systems establish the autonomic nervous system. The prime constituents of the ENS based on two neurons plexuses (networks) which is implanted along the length of gut wall. The submucosal networks embedded in the submucosa while the myenteric plexus is positioned in muscular externa which regulates motility of the gut. Its key function is in-sensing the intraluminal situation, controlling the mucosal epithelium function and regulating the gut blood flow. In esophagus the submucosal plexus are spars and its function are minimal. Sensory neurons of the mucosa and muscularis receive information from sensory receptors. Almost five diverse mucosal receptors are being known to act to the stimuli



including chemical, thermal, mechanical and osmotic origin. The chemoreceptors are sensitive to intraluminal glucose, acid, and amino acids. The muscular sensory receptors are reacting to all kinds of tension and stretch. The ENS are collectively gathering the evidence on condition of the gut wall and its luminal contents and motor neurons controlling the intraluminal absorption and secretion along with gut motility. Motor neurons act directly on many effector cells, including secretory cells viz. parietal, chief, enterocytes, mucous, gut endocrine, pancreatic exocrine cells and the smooth muscle cells [31]. The interneurons of the intestine are liable for assimilating information from sensory neurons and delivering it to motor neurons. In autonomic nervous system the T5, T6, T7, T8 make greater splanchnic nerve of which splanchnic ganglion and celiac ganglion are formed that further form celiac plexus (that supplies the stomach). The T11, T12 make least splanchnic nerve of which superior enteric plexus and inferior enteric plexus are formed that further innervates intestines. The L1, L2, L3 also innervates intestines. Sympathetic nervous system includes S2, S3, S4 forms pelvic nerve that supplies intestines and C10 innervates both stomach and the intestines. Ganglions including celiacomesenteric ganglion and caudal mesenteric ganglion and the lumbosacral plexus (hypogastric nerve) also innervates stomach.

## **5. Accessory glandular structures**

All three major salivary glands are composed of either serous acini, mucous acini, or a combination of both. While parotid gland is largest of the three. All glands function is to produce saliva to moisturize the mouth and assist in the breakdown of carbohydrates in the mouth. The submandibular gland is the primary source of basal saliva secretion [32], while the parotid gland is the main source of stimulated saliva secretion. Salivary glands also play a crucial immunologic role as their secretions contain many immunoglobins, namely IgA, that help fight bacteria and other foreign antigens in the oropharyngeal environment. The sublingual glands lie inferolateral to the tongue, below the floor of the mouth and above the mylohyoid muscle [18]. Sublingual tissue is also palpable and is an oval shaped when sectioned transversely, however, its shape is longitudinal and lentiform when sectioned parallel to the body of the mandible. The sublingual gland differs from the other major salivary glands, because it lacks intercalated or striated ducts, so the saliva secretes directly through the ducts of Rivinus. These ducts empty along an elevated ridge called the plica fimbriata formed by the sublingual folds, which are oblique to the frenulum linguae bilaterally. The sublingual duct of Bartholin joins Wharton's duct to form the draining orifice on each side of the lingual frenulum. The sublingual tissue is predominantly a mucous gland, however, is considered a mixed serous and mucous gland. It is made up of mainly mucous acini with serous demilunes at periphery. It is the only unencapsulated major salivary gland. Sublingual tissue primarily produces a thick mucinous fluid and lubricates the oral cavity which allows for swallowing, initiating digestion, buffering pH, and dental hygiene. It retains both serous and mucous acinar cells while parotid salivary glands possess predominantly serous acini and produces watery fluid [17]. Myoepithelial cells are present around the acinar cells. The mandibular is tubule-acinar seromucous gland. The myoepithelial cells are present around the secretory units. Cells of the mucous acini have a pale-staining foamy cytoplasm which pushed the nuclei toward the basal lamina. While, the serous cells cytoplasm has zymogen granules (markedly eosinophilic) and their nuclei having rounded shape. The secretory acini which is made up of collection of secretory cells are categorized into serous and mucous category. The serous acini have just spherical shaped serous cells and

the mucous acini have only tubular shaped mucous cells. The sero-mucous (mixed) acini hold a combination of mucous and serous cells [6]. In histological set sections of the tissue, the swell mucous cells push the serous cells into a marginal area forming cap like structure recognized as serous demilune (demilune = “half-moon”). The submandibular gland obtains the supply of blood from lingual and facial arteries and emptied by shared lingual and facial veins. The parotid is supplied through the carotid artery (external) and its terminal branches including the superficial temporal and the maxillary artery and emptied by the retromandibular veins. The sublingual glands receive its blood supply from the submental and sublingual arteries.

## 6. Liver

Liver is the principal gland having no gall bladder. Its left side lobe is divided further. It is found underneath the diaphragm and protected by the ribs. It is covered by a fibrous connective tissue capsule, known as Glisson's capsule, which penetrates deep into the organ parenchyma to form septa that divide the main organ into lobes. The liver is formed from an invagination of the digestive tube during the embryonic development; therefore, it is an epithelial derivative [6]. The cellular organization of the liver is relatively simple based on the repetition of a basic structure called hepatic lobule. Lobules are separated from each other by connective tissue. The morphology of lobules is like polygonal prisms, of about 1–2 mm in diameter, and, in cross-sections, the lobules are similar to a hexagon containing a central vein of large diameter [33]. Hepatocytes represent more than 75% of the liver and are organized in anastomosed layers, or trabeculae. These layers of hepatocytes are usually one-cell thick and fused together to form a complex structure similar to a sponge. Small diameter sinusoids run between the layers of hepatocytes [34]. Between the endothelium of sinusoids and hepatocytes there are free-cellular spaces known as perisinusoidal spaces or spaces of Disse. Hepatocytes release two types of substances: endocrine toward sinusoids and exocrine toward the bile canaliculi. Hepatocytes are relatively large (around 20–30  $\mu\text{m}$ ) with a rounded nucleus, some are binucleated, and most of them are tetraploid. Hepatocytes are epithelial cells polygonal in shape having rich eosinophilic granular cytoplasm and centrally placed large spherical nuclei with conspicuous nucleolus. Hepatocytes having two nuclei are also common. Cells having numerous SER and RER, several mitochondria and Golgi apparatus. Hepatocytes are arranged in form of cords and the cells are separated by sinusoidal spaces called sinusoids. These are capillaries and are lined by flattened nucleated endothelial cells. The portal tirades constituted hepatic artery, portal vein and bile duct within the connective tissue are located at the portal areas between nearby lobules [35]. Portal triads are constituted by a branch of the portal vein (venule), a branch of the hepatic artery (arteriole) and a bile duct. In addition, lymphatic vessels and nerve fibers are found in the portal areas. The bile ducts of the portal triad collect the exocrine content, or bile, produced by the hepatocytes. Bile flows in the opposite direction to the blood that runs through the sinusoidal capillaries, in other words, it is directed from the hepatocytes to the bile ducts of the periphery of the hepatic lobule (the portal areas). This is possible because the plasma membranes of adjoining hepatocytes form interconnected spaces, the bile canaliculi, which are organized in an anastomosed network that finally fuses with the bile ducts. Ito cells (stellate cells/lipocytes) exist in space of Disse (between hepatocytes and endothelial cells). Kupffer cells are round in shape positioned in the sinusoids at vascular space within sinusoids [36]. Oval cells (pluripotent stem cells) are also present in the liver. Short lived lymphocytes (pit

cells) are situated in the sinusoids. The coeliac artery branched in hepatic artery and portal vein is fashioned by tributaries draining the pancreas, digestive tract and spleen [37]. Blood flows from the portal areas into the central vein lined by simple squamous epithelium. The blood vessels, nerves and bile duct leave and enter the liver at the hepatic porta.

## **7. Pancreas**

The pancreas is encapsulated and lobulated organs having both the endocrine and exocrine portions. The color of the pancreas of the donkey is reddish cream. The connective tissue stroma divides the parenchyma into various lobules having secretory units and the intralobular duct. The pancreas is triangular, tubuloacinar gland and is present aside from the duodenum [35]. It consists of a body, right lobe, and left lobe. The pancreas has pyramidal acinar cells. Apical portions of these cells have secretory granules (zymogen granules). Exocrine portion produces several enzymes while the Islets of Langerhans are the endocrine portion of this gland. Alfa, beta and delta cells of the islets produces glucagon, insulin and somatostatin respectively [36]. Glandular tissue from the caudal end of the right lobe extended over the portal vein to the left lobe thus forming a ring. The pancreas secretes digestive enzymes into duodenum such as amylase, lipase and trypsin through pancreatic duct. These enzymes digest the carbohydrates, lipids and protein part of feed. The main pancreatic ducts which empties into the duodenum is the extension of interlobular duct, intralobular duct, and intercalated duct. The body of the pancreas received its blood supply from pancreatic branches of the gastroduodenal artery, the first branch was the larger one and originated from the gastroduodenal artery just after its origin from the hepatic artery, the second smaller branch has originated just before the gastroduodenal artery distributed into cranial pancreaticoduodenal and right gastroepiploic arteries. The left lobe received its blood supply from hepatic and splenic artery. The right lobe established its supply of blood from the cranial mesenteric artery.

## **8. Mucosa associated lymphatic tissues**

Mucosa-associated lymphoid tissue (MALT) defends the body from gut invasion of pathogens. The mucosae of the respiratory, urinary and digestive tracts often have few aggregated lymphocytes called MALT or lymphoid follicles [38]. It is situated in different portions of the body viz. nasopharynx, lungs, breast, thyroid, eye, salivary glands, skin and GI tract. MALT is made-up of B and T lymphocytes, macrophages and plasma cells. In the case of intestinal MALT, there are also M cells that take antigen from the lumen and deliver it to the lymphoid tissue. MALT constitutes about 50% of the lymphoid tissue in animal body and its components are sometimes divided into the following areas/types: GALT (lymphoid tissue associated with the intestine. Peyer patches are a component of GALT, which is found in the lining of the small intestine), BALT (lymphoid tissue associated with bronchi), NALT (nasal associated lymphoid tissue), CALT (conjunctiva-associated lymphoid tissue), LALT (lymphoid tissue associated with the larynx), SALT (skin-associated lymphatic tissue), VALT (lymphoid tissue associated with vulvovaginal) and TALT (lymphoid tissue associated with testicular). It can also be distinguished by the degree of tissue organization: O-MALT (lymphoid tissue associated with the organized mucosa), D-MALT (diffuse lymphoid tissue of the mucosa). The MALT that is not organized as a mass, tissue or organ anatomically identifiable separately macroscopically (like

the O-MALT mentioned above) is diffuse MALT. Due to its function during food intake, the mucous membrane is superficially slim and performed as permeable barrier in the body. Likewise, its permeability and delicateness make it susceptible to infection, and in fact most infectious agents that enter the body practice this way. GALT as protection mostly depends on plasma cells that produce antibodies. The lymphatic tissue associated with the intestine is found throughout the intestine and histopathology is the better option to study those [39]. Like thymocytes, GALT containing intestinal Peyer patches (lymphoid follicles made up of lymphocytes) are responsible to safeguard the animal health from the gut luminal side [40, 41].

## 9. Conclusion

The morphophysiological study both (gross and microscopic) of the gastrointestinal tract and associated structures of domestic donkeys are very important to document. The microstructure of internal luminal layer of the gut, luminal ecosystem, immunity and function of the gut is highlighted in this chapter.

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