

Morphological and Histological Study on Vermiform Appendix in Rabbit, Goat and Human Being.

Jay Prakash Bharti¹, Saif Omar², Nawal Kishore Pandey³

¹Tutor, Department of Anatomy, Patna Medical College, Patna, Bihar, India.

²Associate Professor, Department of Anatomy, Katihar Medical College, Katihar, Bihar, India.

³Professor & Head, Department of Anatomy, Katihar Medical College, Katihar, Bihar, India.

ABSTRACT

Background: The vermiform appendix is a narrow blind tube projecting from the postero-medial wall of caecum. The name is derived from the Latin word "*Vermiforma*" meaning worm shaped or worm like. Anatomically, it is one of the mobile viscera of abdomen with an average length of 6-8cm. Microscopically, it consists of mucous, sub mucous, muscular and serous coats from inside out, and its surface epithelium being lined with a simple columnar type of cells including Goblet, Paneth and Argentaffine cells. Due to the presence of extensive lymphoid tissue in the mucous and submucous coats forming lymphoid follicles, it has been called 'Tonsil of the Abdomen'. This small structure without any known function in a human being has been regarded as a vestigial remnant of a better developed distal caecum in herbivores. However, in view of its rich blood supply and histological cyto differentiation, the vermiform appendix has been accepted as a complex and highly specialized organ rather than a degenerate vestigial structure. **Aim:** This study was performed to compare and contrast the morphology and histology of the vermiform appendix in three species of class *mammalia* with different dietary habits. **Methods:** Samples of fifty-three human vermiform appendices were collected out of which twenty-three were of human being, seventeen were of goats and thirteen were of rabbit. The samples were subjected to a naked eye examination along with routine histological staining and observation. **Results:** In man the range of length of vermiform appendix was from 5.4 -12.4 cm. histologically, all four layers were prominent. In goat the length of caecum was from 15 – 28 cm and microscopically all four layers were prominent. In the rabbit length of the vermiform appendix was from 8.5 – 12.5cm and all four layers were observed histologically. **Conclusion:** Morphological and histological differences as observed in vermiform appendix and caecum of three mammals in this study appear to be associated with their different dietary habits. Morphologically distinct vermiform appendix was found in human being and rabbit only as goat has a well-developed caecum. Rabbit revealed to possess a very large caecum acting as a fermentation tank but also a prominent and distinctive appendix with lymphoid aggregations.

Keywords: Appendix, Caecum, Goat, Rabbit, Vestigial.

INTRODUCTION

Vermiform appendix is found in all hominoid apes; including humans, chimpanzees, gorillas, orangutans and gibbons, and it exist to varying degrees in several species of New World and Old World monkeys.^[1,2] After making a comparative study of primate vermiform appendix, Scott (1980)^[3] has suggested that the caecum and vermiform appendix in man and anthropoid apes are more primitive than in monkeys. Structures similar to the hominoid vermiform appendix are present in few other mammals like rabbit, some rodents, South American opossum, wombat etc. After comparative analysis, other investigators have opined that caecal appendixes of human and these other mammals (e.g. rabbit, albino rat etc.) are derived from caecum independently; and these structures are not homologous as appendixes.^[4,5]

Name & Address of Corresponding Author

Dr Saif Omar
Associate Professor,
Department of Anatomy,
Katihar Medical College,
Katihar, India.
E mail: drsaifomar@gmail.com

It was mentioned that well-formed appendix is absent in some herbivorous mammals like goat, sheep, horse, ox etc.^[6] In vertebrate comparative

anatomy, the end (i.e. apex) of caecum of mammals that lack well-formed appendix has been considered structurally homologous to human vermiform appendix.^[5-8] In the human being, the vermiform appendix is a narrow, vermiform (worm like) tube, which arise from the postero-medial caecal wall, approximately 2 cm below the end of the ileum. Three taenia coli on ascending colon and caecum converge on the base of appendix and merge into its longitudinal muscle coat. Anterior taenia coli (*Taenia libera*) is usually distinct and can be traced to the base of appendix providing surgeons a guide to its location. It is mobile structure of abdomen. Its length varies from 2 to 20 cm, average length being 9 cm. Its diameter is about 5 mm. Normally, It is located in the right iliac fossa.^[9,10] Histological structure of the appendix conform to the general plan of the digestive tract consisting of i) mucosa, ii) sub-mucosa, iii) muscularis external and iv) serosa. Lymphoid follicles are initially present in the lamina propria of mucus, but they later become large and extend into the sub mucosa. Muscle coat consists of outer longitudinal layer and inner circular layer. Outer most is serous coat.^[5] In rabbit, caecum (about 50 cm long) ends in narrow vermiform appendix, which is about 10 cm in length. It has thick wall and much lymphoid tissue.^[9] Caecum of goat lacks vermiform appendix.^[11] In the present work, an attempt has

been made to study morphological and histological differences in the vermiform appendix / caecum of three mammals: human being, rabbit and goat having different dietary habits. Human being is omnivorous (dependent mainly on well-boiled food). Rabbit is herbivorous and coprophagous whereas goat (a ruminant) is obligating herbivorous.

MATERIALS AND METHODS

After obtaining prior approval of the Institutional Ethics Committee of Katihar Medical College this research work was undertaken. Three species of class *mammalia* with different dietary habits were selected. They were human being (an omnivorous), rabbit (a herbivorous belonging to group of coprophagous animals) and goat (a herbivorous). Fresh human vermiform appendices were collected from the operation theatre of Department of Surgery, Katihar Medical College & Hospital, Katihar. Samples of caecum of goat along with the terminal part of the ileum and beginning of ascending colon were collected from a butcher shop in the local meat market from goats sacrificed for commercial purpose. No goat was sacrificed, particularly for this research work. Samples of vermiform appendix along with caecum, terminal part of the ileum and part of the ascending colon of rabbit were collected from the respective departments of Pharmacology and Physiology of Katihar Medical College. No rabbit was sacrificed, particularly for this research work. Rabbits that were sacrificed in both the previously mentioned departments during routine academic teaching were used.

Instruments used were:

- Tray of the size 18 inch × 15 inch
- Dissecting forceps
- Scalpel
- BP blade
- Gloves
- Measuring scale
- Glass jar
- Bucket
- Sony cyber shot digital still camera
- Glass slide and cover slip
- Light Microscope
- Microtome

Chemicals used were:

- Formalin
- Eosin
- Hematoxylin
- Normal saline
- Hydrochloric acid
- Distilled water

Twenty-three human vermiform appendices were collected from patients undergoing laparotomy in operation theatre of the Department of Surgery, Katihar Medical College. Their positions were noted in situ. Tissues from these samples were fixed at 10% formal saline. The lengths of all human vermiform appendices were measured against a ruler. Small pieces of 5 mm length from all human vermiform appendices were fixed at 10% formal saline. Specimens were labelled for histological studies. Thirteen vermiform appendices along with caecum, terminal part of the ileum and part of ascending colon were collected from rabbits previously mentioned. Specimens thus obtained were cleaned in running tap water and then gently laid on blotting paper. They were then spread on white paper. Measurements of length (of each border and central part of caecum) and breadth (at three places: at 2 cm below its beginning, at the middle and at 2cm above the end of caecum) were taken. The mean of the three measurements in each case was recorded as the length and breadth of the caecum. In case of the vermiform appendix, length of the central part of the vermiform appendix was recorded, whereas measurement of breadth was recorded in the middle part of the vermiform appendix. Small pieces 10 mm x 5 mm from caecum and small pieces 5 mm in length from vermiform appendix were cut and fixed in 10% formal saline. Specimens were labelled for histological studies. Seventeen caecum along with the terminal part of the ileum and beginning of ascending colon of goats were collected according to the previously described methodology. They were cleaned under running tap water, laid on blotting paper and then spread over white paper. Measurements of breadth were taken at 3 different places (at 2cm below its beginning, at the middle and at 2cm above the end of caecum). Lengths were measured at right and left borders; and at the central part of caecum. Mean of the three measurements in each case was recorded as length and breadth of the caecum of the goat. Small pieces of tissue 5 mm x 5 mm from caecal apex of each specimen were fixed in 10% formal saline. Specimens were labelled for histological studies. Tissues fixed in 10% formal saline of each specimen as stated above were properly trimmed and processed through graded alcohol for dehydration, cleared in xylene and embedded in paraffin. The tissue sections of 5 μ thickness were obtained by manually operated rotary microtome. These sections were stained by Harris' Hematoxylin and Eosin for general histological observations.

RESULTS

A total of 53 specimens were examined and out of them 23 specimens were of human, 17 specimens

were of goat and 13 specimens were of rabbit which were studied in this present work.

Human vermiform appendix: [Table 1] shows the incidence of different positions of vermiform appendix in 23 cases. The percentage of position of the vermiform appendix is 60.80% retrocaecal, 34.74% pelvic and 4.34%. Pre-ileal, Post-ileal and Subcaecal varieties were not found.

Table 1: Positions of Vermiform Appendix.

Position	Number (n)	Percentage (%)
Retrocaecal	14	60.80
Pelvic	8	34.74
Pre-ileal	1	4.34
Post-ileal	0	0.00
Subcaecal	0	0.00

[Table 2] shows length of 23 vermiform appendices in present study was found to be ranging between 5.4 cm to 12.4 cm. Average length (Arithmetic mean) stood at 7.92 (+/- 1.87) cm.

Table 2: Length of Vermiform Appendix

S. No.	Length (cm)	Standard Deviation (+/-)	Coefficient of Variation	Range
1	5.4	1.87	23.6	5.4 – 12.4
2	7.8			
3	6.2			
4	7.1			
5	8.1			
6	6.8			
7	8.3			
8	5.8			
9	9.1			
10	8.0			
11	7.1			
12	9.5			
13	7.0			
14	6.0			
15	10.1			
16	10.5			
17	7.9			
18	6.4			
19	12.4			
20	7.7			
21	6.5			
22	11.8			
23	6.8			

Sections of the human vermiform appendix showed same coats as those of the intestine: mucous, sub mucous, muscular and serous. The mucous coat was seen lined by simple columnar epithelium with plenty of goblet cells. Under low magnification (10X) intestinal glands (Crypts of Lieberkuhn) were observed in the lamina propria. They are short and straight. They were not placed very close to each other in the lamina propria.

Muscularis mucosae were distinctly seen. The well developed submucous coat was seen with large number of lymphoid follicles. Two distinct layers were in the muscular coat. Outer longitudinal muscular fibres appeared as uniformly thick layer which invested the whole organ. Inner circular muscle fibres formed a thicker layer than the outer longitudinal fibres. A distinct layer of connective tissue was seen the outer longitudinal and inner circular muscle fibres. The serous coat was seen as complete investment for the vermiform appendix except along the narrow line of attachment of mesoappendix.

Caecum and Vermiform Appendix of Rabbit:

Vermiform appendix and caecum in case of rabbit were found in the right flank of the abdomen. Caecum was identified by the presence of pouch like dilatations. The vermiform appendix was identified by its smooth wall and as distal continuation of caecum in the form of a narrow blind ending tube. [Table 3] shows length of the vermiform appendix of 13 rabbits ranging from 8.5 to 12.5 cm. Average length stood at 10 cm.

Table 3: Length of Vermiform Appendix in Rabbits.

S. No.	Length (cm)	Standard Deviation	Coefficient of Variation	Range
1	9.0	1.35	13.5	8.5-12.5
2	9.8			
3	12.5			
4	9.4			
5	12.3			
6	9.1			
7	12.0			
8	9.2			
9	8.8			
10	9.2			
11	9.6			
12	10.0			
13	8.5			

[Table 4] shows breadth of the vermiform appendix of 13 rabbits ranging from 0.6 to 1.4 cm. Average breadth stood at 0.91 cm.

Table 4: Length of Vermiform Appendix in Rabbits.

S. No.	Breadth (cm)	Standard Deviation	Coefficient of Variation	Range
1	0.7	0.23	25.2	0.6-1.4
2	1.0			
3	1.4			
4	0.9			
5	1.2			
6	0.7			
7	1.2			
8	0.8			
9	0.7			
10	0.8			
11	0.9			
12	1.0			
13	0.6			

[Table 5] shows length of the caecum of 13 rabbits was found to be ranging from 30.6 to 44.8. Average length stood at 37.5 cm.

Table 5: Length of Caecum in Rabbits.

S. No.	Length (cm)	Standard Deviation	Coefficient of Variation	Range
1	34.6	3.98	10.4	30.6-44.8
2	39.3			
3	44.8			
4	37.0			
5	41.4			
6	35.0			
7	41.7			
8	36.0			
9	32.5			
10	36.0			
11	38.2			
12	40.5			
13	30.6			

[Table 6] shows breadth of the caecum of 13 rabbits ranging from 1.6 to 2.5 cm. Average breadth stood at 1.94 cm.

Table 6: Breadth of Caecum in Rabbits.

S. No.	Breadth (cm)	Standard Deviation	Coefficient of Variation	Range
1	1.8	0.26	13.6	1.6-2.5
2	2.0			
3	2.5			
4	1.8			
5	2.2			
6	1.8			
7	2.2			
8	1.8			
9	1.6			
10	1.8			
11	2.0			
12	2.1			
13	1.6			

Sections of the vermiform appendix of rabbit showed four layers as those of the intestine: mucous, sub mucous, muscular and serous. The mucous coat was seen lined by simple columnar epithelium. Its mucosa appeared to be smooth and continuous with flask shaped glands and crypts of Lieberkuhn. Mucous coat was full of tall conoid lymphoid follicles which were closely packed. Each lymphoid follicle was associated with flask shaped gland. Muscularis mucosae were not distinctly seen. Well developed submucous coat was seen.

Muscle coat was thin. Serous coat formed a complete investment of the vermiform appendix.

Caecum of Goat:

[Table 7] shows length of the caecum of goat ranging from 15.0 to 28.0 cm. Average length stood at 22.11 cm.

Table 7: Length of Caecum in Goat.

S. No.	Length (cm)	Standard Deviation	Coefficient of Variation	Range
1	23.5	3.61	16.3	15.0-28.0
2	15.0			
3	22.5			
4	21.5			
5	23.0			
6	22.5			
7	26.5			
8	18.5			
9	22.0			
10	28.0			
11	21.0			
12	25.5			
13	24.5			
14	19.5			
15	17.0			
16	27.0			
17	18.5			

[Table 8] shows breadth of the caecum of goat ranging from 2.0 to 6.5 cm. Average breadth stood at 4.29 (+/- 2.13) cm.

Table 8: Breadth of Caecum in Goat.

S. No.	Breadth (cm)	Standard Deviation	Coefficient of Variation	Range
1	5.0	2.13	50.7	2.0-6.5
2	2.5			
3	5.0			
4	4.5			
5	5.0			
6	5.0			
7	6.0			
8	3.5			
9	4.5			
10	3.5			
11	4.0			
12	5.5			
13	4.0			
14	2.0			
15	4.0			
16	6.5			
17	2.5			

Sections of the caecum of goat showed four layers. They (from inside outward) were tunica mucosa (mucous coat), tela sub mucosa (sub- mucous coat), the tunica muscularis (muscle coat) and the tunica serosa (serous coat). Tunica mucosa consisted of simple columnar epithelium, lamina propria with intestinal glands and lamina muscularis (muscularis mucosae). Intestinal glands in caecum were oval, tortuous and straight. Lamina muscularis consisted

of inner circular and outer longitudinal layers of smooth muscle. Tela sub mucosa was well developed. Tunica muscularis is composed of inner circular and outer longitudinal layers of smooth muscle fibres. Tunica serosa is a loose connective tissue layer with collagen and elastic fibres.

DISCUSSION

Out of the three mammals (human being, goat and rabbit) in the present study, the well-defined vermiform appendix was revealed in human beings and rabbit only. In the goat, no vermiform appendix was revealed. It is interesting to note that in many herbivorous mammals, caecum is large and its size is proportionate to the amount of plant matter in a given organism's diet. The caecum is large in size in obligate herbivores mammals (like goat, sheep, cow, horse etc) and devoid of vermiform appendix. It may also be noted that a vermiform appendix is not unique to humans. It is found in all hominoids apes, including humans, chimpanzees, gorillas, orangutans, and gibbons; and it exist to some degrees in several species of New World and Old world monkeys.^[6,12]

Human vermiform appendix:

With regard to various positions of human vermiform appendix, in this study the incidence of retro-caecal position (60.80%) was highest followed by pelvic position (34.74%). This result corresponds with the findings of Wakeley (1933) conducted in England and conducted in Nigeria, where retro-caecal position was higher followed by pelvic position.^[13] However, this result does not correspond with findings of Neal et al (1979) conducted in Zambia in which pelvic position was the commonest (43%).^[14] It also does not correspond with findings of Gopalipour et al (2003)^[7] conducted in Gorgan, Iran where pelvic position (33.3%) was highest followed by retro-caecal position (32.4%). With regard to the length of the human vermiform appendix, in this study, the average length of vermiform appendix 7.92 (+/- 1.87 cm) as observed is close to the figure (9 cm)^[15] and the range of the length (5.4 cm to 12.4 cm) is also within the limit of figures (2 cm to 20 cm) mentioned in the above stated text book of Anatomy. This result did not coincide with the findings of Gopalipour et al (2003)^[7] who reported the average length of vermiform appendix 6.61 cm in male and 6.06 cm in female in Gorgan population of Iran. Sections of the human vermiform appendix showed same coats as those of intestine: mucous, sub mucous, muscular and serous. All features conform to normal histological appearance of human vermiform appendix and is in agreement with text book descriptions.^[5,16-21]

Vermiform appendix and caecum of the rabbit:

Length of the vermiform appendix of rabbit as recorded in this work was found to be variable, which is evident from wide S.D. (standard deviation), wide C.V. (coefficient of variation) and wide range [Table 3]. Average length 10 (+/- 1.35) cm as recorded in this work is similar to the length of appendix cited by Vidyarthi (1978)^[22] and Jordan and Nigam (1996).^[9] Breadth of the vermiform appendix as noted in this work is also variable and its variability is evident from wide S.D., C.V. and range [Table 4] Average breadth was 0.91 (+/- 0.23) cm (approximately 1 cm). Data on breadth could not be found in a textbook or in literature, but it is less than the breadth of appendix (1.10 cm) as cited by Prasad (1990).^[16] Length of the caecum of rabbit as recorded in this work was found to be variable, which is evident from wide S.D., wide C.V. and wide range [Table 5]. Average length 37.5 (+/- 3-98) cm as recorded in this work is much less than the length of caecum (46 cm) cited by Vidyarthi (1978); and (50 cm) cited by Jordan and Nigam (1996).^[9,22] Breadth of caecum of rabbit as noted in this work is also variable and variability is evident from wide S.D., C.V. and range [Table 6]. Average breadth 1.94(+/- 0.26) cm (approximately 2 cm) is much less than the breadth of caecum (2.5 cm) as cited by Vidyarthi (1978) and is similar to finding of Prasad (1990).^[16,22] Histological section of vermiform appendix of rabbit revealed all the features of mucous coat (i.e. simple columnar lining epithelium, smooth appearance of mucosa, presence of flask shaped glands in association with tall conoid lymph follicles, presence of crypts of Liberkuhn etc.) which are similar to observations of Blackwood et al (1973).^[2]

Caecum of goat:

Length of the caecum of goat as recorded in this work was found to be variable which is evident from wide S.D., wide C.V. and wide range [Table 7]. Average length 22.11(+/- 3.61) cm as recorded in this work is more than the average length of the caecum (20 cm) cited by North (2004); and much less than the average length (28 cm) cited by Prasad (1990).^[16] Breadth of caecum of goat as noted in this work is also variable and variability is evident from wide S.D., C.V. and range [Table 8]. Average breadth 4.29 (+/- 2.13) cm is close to the average breath (5 cm) mentioned by North (2004) and is similar to finding of Prasad (1990).^[16] Histological section of caecum of goat revealed all features (i.e. simple columnar epithelium lining the mucous coat with Goblet cells, closely packed intestinal glands which are oval, tortuous straight in shape. Lamina muscularis consisting of inner circular and outer longitudinal layers. Tela sub mucosa consisting of collagen and elastic fibres with blood vessels. Tunica muscularis composed of well defined inner circular and outer longitudinal

layers of smooth muscle fibers which are similar to the observations of Kadam et al, (2007) and Kadam et al, (2011).^[11,12]

Morphological differences in vermiform appendix / caecum of humans, rabbit and goat:

Average length of the caecum of human is 6 cm^[23,24] is far less than the average length of caecum of rabbit (37.5 cm) and also average length of caecum of goat (22.11) as observed during this work. Average length of the human vermiform appendix and average length of vermiform appendix of the rabbit as found in this work are 7.92 (+/- 1.87) cm and 10 (+/- 1.35) cm respectively. The average breadth of the caecum of human is 7.5 cm^[24] is much more than average breadth of caecum of rabbit (1.94 cm) and also average breadth of caecum of goat (4.29 cm) as observed during this work. Considering the average body weight of human 70 kg, average body weight of rabbit as 2 kg and average body weight of goat as 15 kg in Kosi region, ratio of the body weight of human (H) to body weight of rabbit (R) stand at 35 to 1, whereas same of human (H) to goat (G) stand at 4.6 to 1 and same of goat (G) to rabbit (R) stand at 7.5 to 1. Considering the average length of caecum / vermiform appendix of human, rabbit and goat as observed in this work, different ratios obtained are as follows:

- (a) Length of caecum of human (LCH) : length of caecum rabbit (LCR)=0.16:1.
- (b) Length of caecum of human (LCH) : length of caecum of goat (LCG) = 0.27:1
- (c) Length of caecum of goat (LCG) : length of caecum rabbit (LCR) = 0.58:1
- (d) Length of human vermiform appendix (LHVA): length of rabbit vermiform appendix (LRVA) = 0.87:1. In many mammals, particularly herbivores, caecum and appendix are large and constitute a highly important site of digestion of cellulose by symbiotic bacteria.^[24]

Symbiotic bacteria of these herbivorous mammals secrete cellulase, an enzyme that digests cellulose. So, the size of caecum in herbivores have to much larger for providing proper time for digestion of cellulose and also much space for harbouring symbiotic bacteria. Human caecum is much shorter in length as main bulk of human diet is (boiled) starch; and so, there is no need of having larger caecum. It has been observed that microbial biofilms are more abundant in vermiform appendix, which is a source of symbiotic bacteria to re-inoculate the gut with its normal gut flora, which may have been flushed out during infection with pathogenic bacteria to restore the balance.^[4] Thus, although the human beings are more hygienic in behaviour than other mammals and avail safe drinking water, vermiform appendix is needed by them. Requirement of well formed appendix in the

case of some mammals, particularly ruminant (like goat, sheep and horse etc.), may not be necessary as microbial biofilms may have safe place in the most dependent part of their large caecum.

Histological differences in vermiform appendix / caecum of human, rabbit and goat:

In all the three animals under study, the lining epithelium of the mucosa of the vermiform appendix / caecum was of simple columnar variety, interspersed with mucous secreting goblet cells. Intestinal glands were found in the lamina propria of all three animals under study, but their numbers were variable and their shapes were different. Intestinal glands, in the section of human appendix, were not closely packed and they were short and straight. Intestinal glands, in the section of vermiform appendix of rabbit, were more in number than that of the human vermiform appendix and flask shaped glands were found associated with each tall conoid lymphoid follicles. Intestinal glands, in the section of caecum of goat, were densely packed. They were oval, tortuous and straight. Number of goblet cells in the intestinal glands of human vermiform appendix and in vermiform appendix of rabbit were not numerous, but the same (i.e. goblet cells), in intestinal gland of caecum of goat, were found to be plenty. Number of lymphoid follicles in the lamina propria of the human vermiform appendix are plenty, but not as numerous as they were found in the vermiform appendix of rabbit. Lymphoid follicles in the caecum of goat were occasional. Muscularis mucosae, in human and rabbit, were broken or distorted due to extension of lymphoid follicles into the sub mucosa. Muscularis mucosae were well defined in goat. Sub mucosa, in all the three animals under study, contained blood vessels, lymphatics, collagen fibers and elastic fibers etc. However, sub mucosa of human vermiform appendix and caecum of goat were well defined whereas in rabbit, it was thin and ill defined. Serosus coat invested the structure except at the line of attachment of mesentery (if present) in all the three animals. It has been observed that immunoglobulin A (IgA) and mucin, two of the most abundantly produced molecules by immune system support growth of microbial biofilms in laboratory experiments and they are associated with microbial biofilms in the mammalian gut.^[3] Number of goblet cells were numerous in the caecum of goat, as they required more lubricant to expel rough remnants of herbivorous diet and more secretion of mucin may be helping this animal in its immune system.^[25] Abundant lymphoid follicles in vermiform appendix of rabbit followed by human vermiform appendix may be associated with degrees of functions of the immune system of these mammals as required.

CONCLUSION

Histological sections showed that mucous membrane in all animals were lined by simple columnar epithelium, interspersed with mucous secreting goblet cells. In human vermiform appendix, intestinal glands were short and straight and not closely packed; in rabbit along with intestinal glands, flask shaped glands in association of lymphoid follicles were seen and in goat intestinal glands were numerous and closely packed. Lymphoid follicles, in lamina propria of human vermiform appendix were plenty. In rabbit, lymphoid follicles were numerous and conoid in shape, whereas, in caecum of goat, they were occasional. Morphological and histological differences as observed in vermiform appendix and caecum of three mammals in this study appear to be associated with their different dietary habits.

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