Journal of Cell and Animal Biology

Volume 9 Number 1, January, 2015
ISSN 1996-0867

Academic Journals

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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academicJournals

Vol. 9(1), pp. 1-8, January, 2015
DOI: 10.5897/JCAB2015.0425
Article Number: E57420150105
ISSN 1996-0867

http://www.academicjournals.org/JCAB

Journal of Cell and Animal Biology

Full Length Research Paper

Light and scanning electron microscopy of the small intestine of goat (*Capra hircus*)

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Received 3 January, 2014; Accepted 12 January, 2015

Sections from duodenum, jejunum and ileum of local breeds of goat (*Capra hircus*) were studied histologically using light and scanning electron microscopy. The wall of goat small intestine is composed of typical layers: lamina epithelialis mucosae, lamina propria, lamina muscularis mucosae, tunica submucosa, tunica muscularis and tunica serosa. Small intestine villi were covered by a simple columnar epithelium with goblet cells and simple tubular glands, the crypts of Lieberkühn, containing paneth cells were observed between the villi. The presence of mucus was extensive in the duodenum and the number of goblet cells was highest in the duodenum. The lamina propria consisted of loose connective tissue rich in blood and lymphatic vessels. The lamina muscularis mucosa was presented as a thin layer of circular smooth muscle fibers at the base of the crypts. The submucosa consisted of conjunctive tissue containing blood and lymphatic vessels. The tunica muscularis consisted of the typical inner circular layer and outer longitudinal layer of smooth muscle. The tunica serosa was the thin, outermost small intestine layer that consisted of a very small amount of conjunctive tissue covered by mesothelium. The submucosa was devoid of glands in all three small intestine regions. Scanning electron microscopy showed finger shaped villi in the jujenum, tongue shaped in ileum, leave like in duodenum; the villus has corrugated surface. The corrugations are deep, irregular clefts cutting into the side of the villus. The corrugations are scarce in the duodenum, few in jujenum and numerous in ileum. The surface of villi presents the goblet cell orifices.

Keyword: goat, intestine

INTRODUCTION

Recently, goats became an important component of animal production in Egypt. At the economic level of individual families, small ruminants serve as investment and insurance due to their high fertility, short generation interval, ability to produce under limited feed resources and their adaptability to harsh environments (Sedeke, 2007). Goats are useful to humans when they are living as they serve as a renewable source of milk, manure and fiber, and then they can also provide meat and hides (Abdel Aziz, 2010). Goats produce about 2% of the world's total annual milk supply (FAO, 1997).

Due to their small size and greater efficiency of nutrient processing, goats are easier and cheaper to manage as compared to cattle, but can still be used for transporting items. Goat intestine can be used to make "catgut", which is still in used for internal human surgical sutures and

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strings for musical instruments. The small intestine responsible for digestion and absorbtion is anatomically divided into three parts, the duodenum, the jejunum and the ileum. The wall structures of these three parts are similar except few differences. The duodenum is the shortest while the jejunum is the longest (David, 1987). There is little information about the histologic structure of goat small intestine, so the current study was carried out to provide basic information concerning the goat small intestine for anatomists and nutritionists.

MATERIALS AND METHODS

Randomly taken parts from duodenum, jejunum and ileum of ten healthy freshly slaughtered adult goats (five for each sex, about 3 to 4 years of age) were collected from a local slaughterhouse in the Suez governorate, Egypt. All procedures were carried out in full compliance with the guidelines provided by the Suez Canal University Institutional Animal Use oversight committee and are in accordance with international protocols for biomedical investigations.

For light microscopy, sections of intestine (for light microspy, 2 cm length pieces from the three regions of the small intestine) were immersed in 10% formalin for 24 h then processed routinely for paraffin embedding, sectioned at 4-5 µm, then stained routinely with H&E, periodic acid-Schiff (PAS) or Masson's trichrome stain before being coverslipped (Bancroft and Stevens, 1982). Stained sections were documented for analysis using an Olympus microscope, model BX50.

For scanning electron microscopy, the specimens (1 cm length pieces from the three regions of the small intestine) were placed in 1% paraformaldhyde and 1.5% glutraldhyde in 0.1 M phosphate buffer (pH 7.2) for 2 h at 4°C. These pieces were then cut into smaller pieces (about 5 x 5 mm). The pieces were washed with 0.1 M phosphate buffer (pH 7.2) then put in 0.1% osmium tetraoxide in the same buffer for 2 h. Then, the pieces were dehydrated using a graded ethanolseries from 70 to 100%, critical point dried in a Seevac CO2 Critical Point Dryer, mounted on _inch Zeissaluminum SEM mounts using colloidal silver paste, and sputter coated with gold using a Hummer II Sputter Coater then examined by a JEOL-5400 LV scanning electron microscope. These procedures were as described by Yamauchi et al. (1990) and Maneewan and Yamauchi (2003). Anatomical nomenclature used in this study was based on the Nomina Anatomica Veterinaria (I. C. V. G. A. N., 2005) whenever possible.

RESULTS

Light microscopy

The wall of the goat small intestine is composed of the typical layers found in mammals: lamina epithelialis mucosae, lamina propria, lamina muscularis mucosae, tunicasubmucosa, tunic muscularisand tunica serosa. The mucosal epithelium in all three small intestine segments was simple columnar (Figures 1 to 3). Goblet cells were scattered among the columnar epithelial cells (Figures 1 to 4). Villi consisted of tongue-like elevations of mucosa with a lamina propriacore (Figures 1 to 3). Bifurcated and trifurcated villi were observed (Figures 1, 2 and 3).

The ileum had the shortest villi and the least number of goblet cells (Figure 3). Between individual villi was observed simple tubular glands, the crypts of Lieberkühn, extending down into the lamina propria of the mucosa as deep as the muscularis mucosae (Figures 1 to 3). At higher magnification, the epithelial cells had a flat luminal surface (Figures 1 to 3). The crypts contained paneth cells in all three segments of the small intestine and were distinguished by the presence of large acidophilic granules in the cytoplasm (Figures 1 to 3). The lamina propria consisted of loose connective tissue rich in blood and lymphatic vessels in the core of the villi and between crypts (Figures 1 to 3). Mucus was observed to be extensive in the duodenum (Figure 4) and goblet cells also were densest in duodenum as compared to the ileum and jejunum (Figures 1 to 3). Goblet cells were present in the epithelium lining the crypts as well (Figures 1 to 3).

The lamina muscularis mucosa consisted of a thin layer of circular smooth muscle fibers at the base of the crypts (Figures 1 to 3). The tunica submucosa was comprised of conjunctive tissue containing blood andlymphatic vessels (Figures 1 to 3). The duodenum did not exhibit any glands of Brunner in the tunica submucosa nor did the tunica submucosa of jejunum contain any glands or lymphoid nodules. On the other hand, aggregations of lymphocytes were observed in the tunica submucosa of the ileum (Figure 3). The tunica muscularis was composed of the typical inner circular layer and an outer longitudinal layer of smooth muscle cells (Figures 1 to 3). The tunica serosa was the thin, outermost layer comprised of a scant amount of conjunctive tissue covered by a mesothelium (Figures 1 to 3).

Scanning electron microscopy

Jejunal villi were finger-shaped with broad bases tapering to a blunt apex, while, tongue shaped villi were found in the ileum and the duodenum presented leave-like villi (Figure 4). A marked feature of the villus was its corrugated surface, which is not easily seen by dissecting microscope. The corrugations consisted of deep, irregular clefts cutting into the sides of each villus and divided the villi into separate islands of tissue (Figure 4). The majority of these corrugations tended to run horizontally (Figure 4). The corrugations ended abruptly and did not form a continuous system of clefts (Figure 4). These corrugations were scarce in duodenum, more frequent in the jejunal villi and most numerous in ileal villi (Figure 4). Circular or oval holes were seen opening into the epithelial surface, which were goblet cell orifices (Figure 4).

Mucus extruding from goblet cells was frequently observed (Figure 4). Individual epithelial cells were sometimes observed (Figure 4) and consisted of flat-topped cellsor cells presented with a gradual outwardly convex surface. The arrangement of the epithelial cells gave a honeycomb appearance on the villous surface (Figure 4).

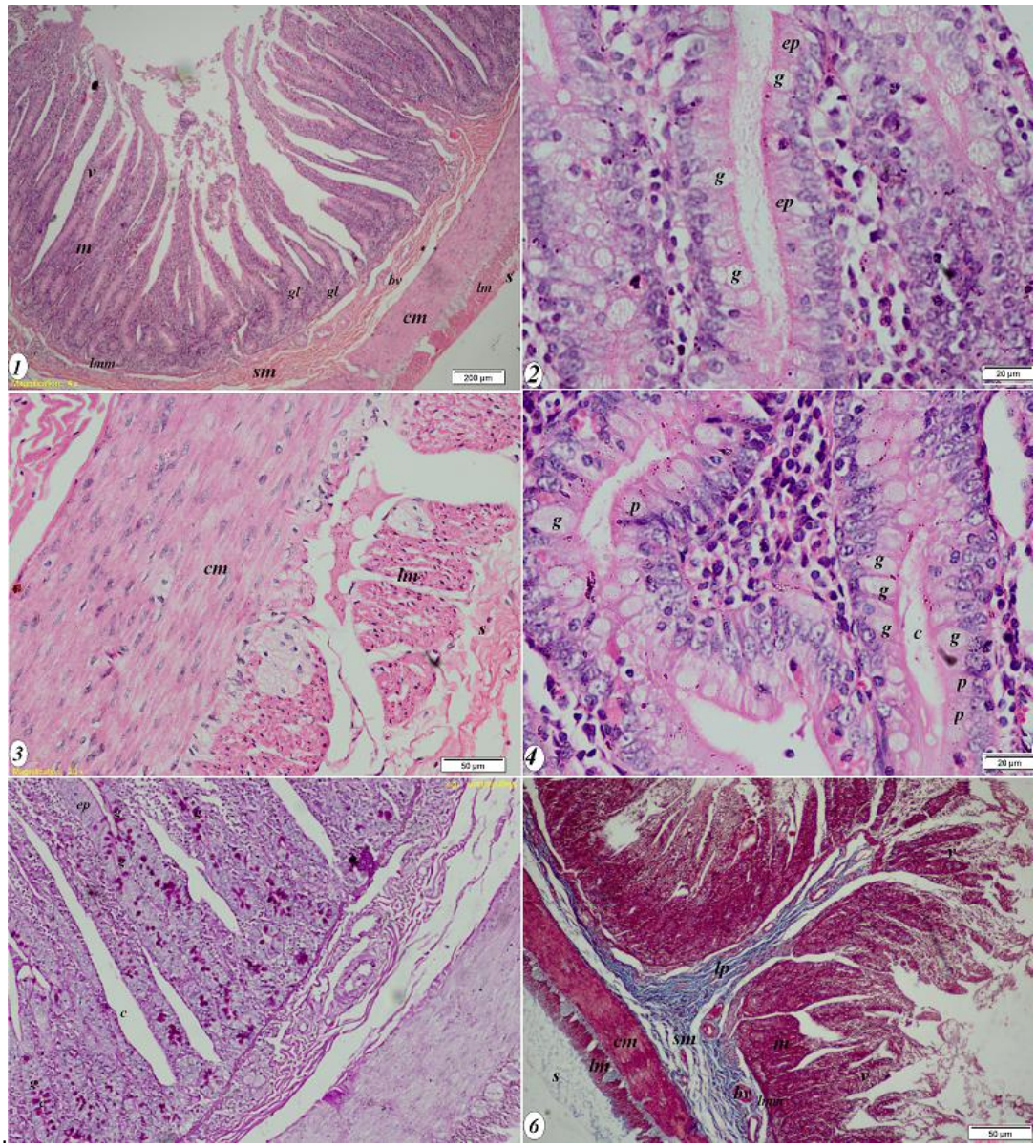

Figure 1. Photomicrograph of duodenum of goat, 1, 2, 3 and 4: H&E; 5: PAS stain; 6: Masson's trichrome stain; v, villus; m, mucosa; lp, lamina propria; gl, mucosal glands; bv, blood capillaries; lmm, lamina muscularis mucosa; sm, Tunica submucosa; cm, Tunica muscularis (*Stratum circulare*); lm, Tunica muscularis (Stratum longitudinal); s, Tunica serosa; se, surface epithelium border; bm, basal lamina of mucosal glands; ep, epithelium; p, paneth cells (contain coarse eosenophilic granules); g, goblet cells; c, crypts.

DISCUSSION

The wall of the goat small intestine is composed of the typical layers: Lamina epithelialis mucosae, lamina propria, lamina muscularis mucosae, tunica submucosa, tunic muscularis and tunica serosa. Villi were present and covered by simple columnar epithelium. Goblet cells were scattered among the simple columnar epithelial cells. These findings are similar to those reported by David (1987) for human small intestine. Among the villi, we observed simple tubular glands, the crypts of Lieberkühn, extending down into the lamina propria to the level of the lamina muscularis mucosae. These findings are similar to that of David (1987) and Leslie and James (2007) in both humans and animals. These crypts contain paneth cells, which have large acidophilic cytoplasmic granules.

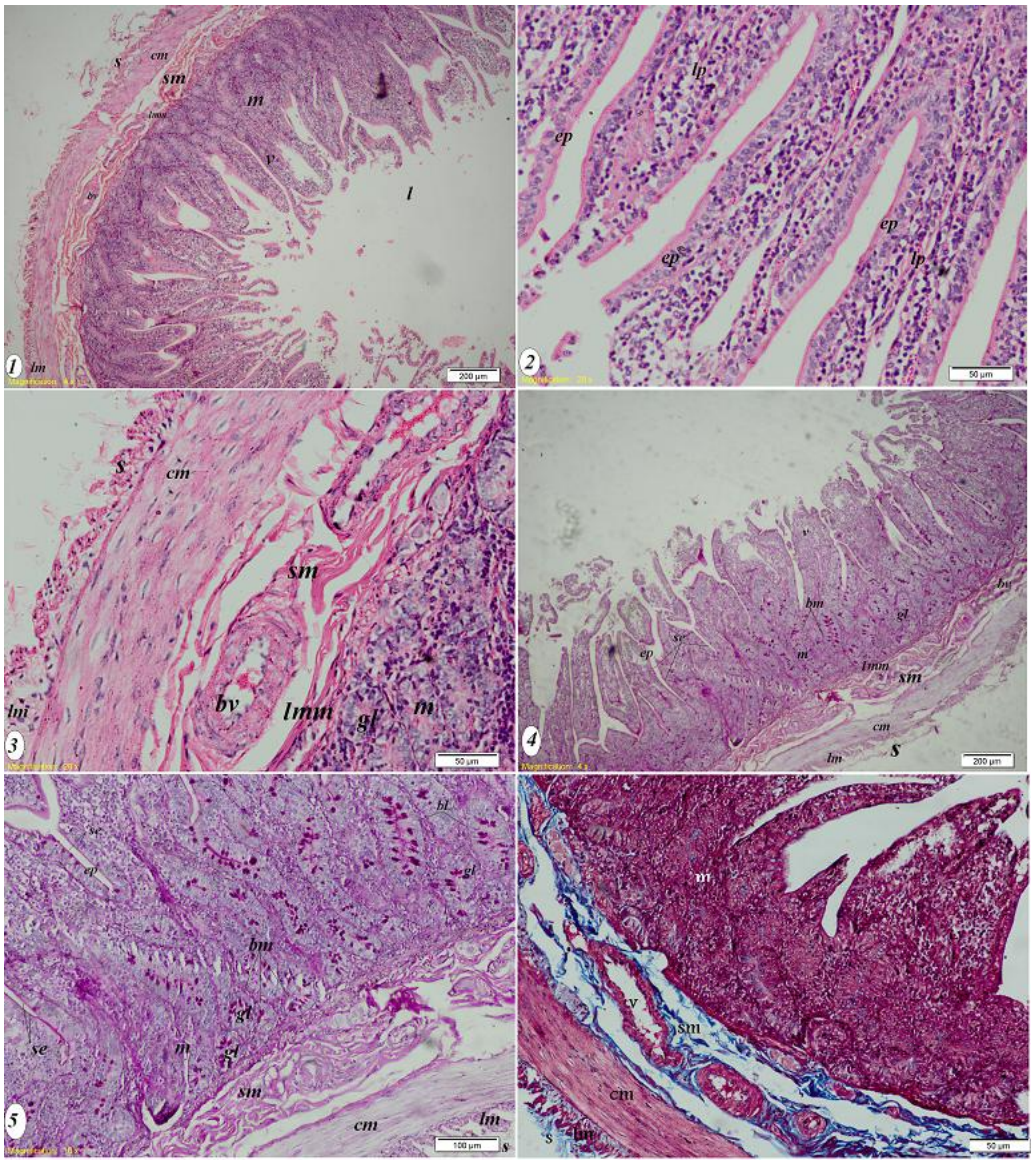

Figure 2. Photomicrograph of jejunum of goat, 1, 2 and 3 H&E; 4 and 5 PAS stain; 6 Masson's strichrom stain; v, villus; m, mucosa; ep, epithelium; lp, lamina propria; gl, mcosal glands; bv, blood capillaries; lmm, lamina muscularis mucosa; sm,Tunicasubmucosa; cm, Tunica muscularis (Stratum circulare); lm, Tunica muscularis (Stratum longitudinal); s, Tunica serosa; se, surface epithelium border; bm, basal lamina of mucosal glands.

Paneth cells have been reported in the crypts of Lieberkühn of small intestine of various mammalian species (Garabedian et al., 1997; Glerean and Castro,1965; Mathanm et al., 1987; Taylor et al., 1964).

Deschner (1967) reported that human paneth cells were not limited to the bases of the crypts but could be observed along the entire length of the crypts and even in the surface epithelium of the villi. No paneth cells have been observed in villi epithelium of sheep (Ergun et al., 2003). Bjerknes and Cheng (1981) and Garabedian et al. (1997) reported that the paneth cells differentiate as such towards the base of the crypts and hence the finding of the young cells at the top and the matured cells at the bottom of the crypts. In the goat, we observed that paneth cells were limited to the base of the crypts.

Goblet cells were also found in the crypts and, as in the villi. These findings are similar to those in humans (David, 1987). Extensive mucus was observed in the duodenum, and goblet cells were the most numberous in the goat duodenum, perhaps due to the absence of Bruner's glands in submucosa of the goat duodenum and also may be due to the rough, dry foods typically consumed

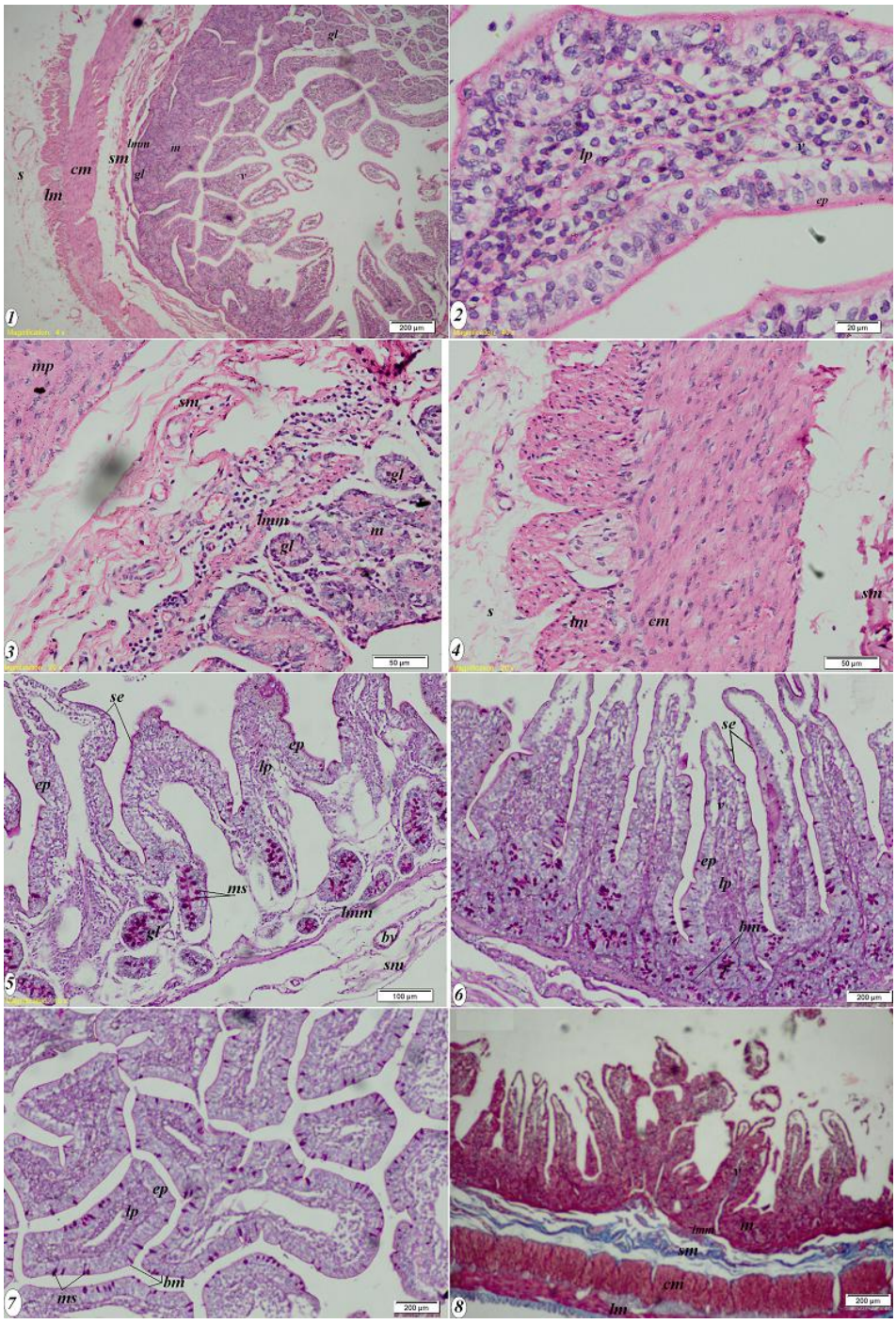

Figure 3. Photomicrograph of ileum of goat, 1, 2, 3 and 4 H&E; 5, 6 and 7 PAS stain; 8 Masson's trichrome stain; v, villus; m, mucosa; lp, lamina propria; gl, mcosal glands; bv, blood capillaries; lmm, lamina muscularis mucosa; sm, Tunicasubmucosa; cm, Tunica muscularis (Stratum circulare); lm, Tunica muscularis (Stratum longitudinal); s, Tunica serosa; se, surface epithelium border; bm, basal lamina of mucosal glands.

by goats. The lamina propria consisted of loose connective tissue rich in blood and lymphatic vessels present in the core of the villi and between crypts. These findings are similar to that of David (1987) and Stevens and Lowe 1992) in both humans and animals. The lamina muscularis mucosa was a very thin layer of circular

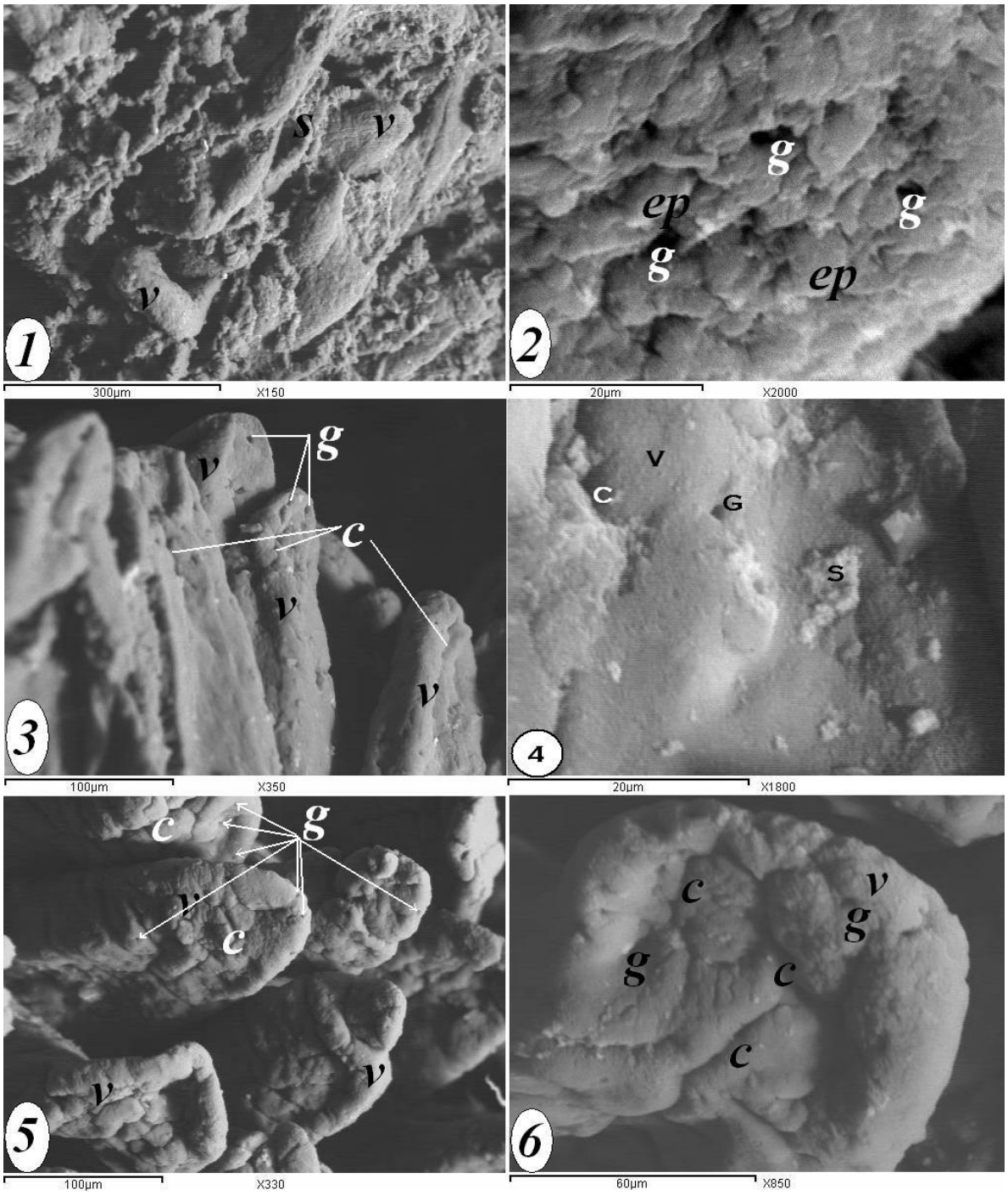

Figure 4. Scanning electron microscopy of the small intestine of goat; 1 and 2, duodenum; 3 and 4, jejunum; 5 and 6, ileum; v, villus; s, mucus secretion; ep, epithelial cells; g, goblet cell orifices; c corrugations.

smooth muscle fibers at the base of the crypts. These findings are similar to that of Lesson and Lesson (1988) in humans and animals. The tunica submucosa, tunica muscularis and tunica serosa were all similar in appearance to these same layers observed in humans and other animals (David, 1987; Fawcett, 1994).

Bifurcated and trifurcated villi were seen. The tunica submucosa of the duodenum lacked mucus secreting Brunner's glands. These findings are in contrast to observations of Carlos et al. (1983) in humans and may

due to the presence of high number of goblet cells in the goat duodenum. The goat jejunum had no gland or lymphoid nodules in the tunica submucosa, similar to observations of Fawcett (1994) in humans. The goat ileum had the shortest villi and least number of goblet cells. These findings are similar to that of David (1987) and Fawcett (1994) in human and animals. We did not observe Peyer's patches in the tunica submucosa of the ileum in contrast to observations of Lesson and Lesson (1988) in humans.

We used scanning electron microscopy to examine the surface structure of the goat small intestine mucosa. Goat ileal villi were broader than those of the jejunum and duodenum in contrast to David (1987) who reported that duodenal villi were the broadest in humans. The shapes of villi in goat small intestine were tongue-shaped, leaf-like and finger-like as observed for humans (David, 1987). As many as 20 crypts may surround each villus (Cocco et al., 1966), but a functional ratio of three crypts to one villus has been proposed (Loehry and Creamer, 1969). We did not observe the crypts with scanning electron microscopy due to villus density and presence of secretions over the epithelial surface but we were able to use light microscope to observe the intestinal crypts. It was possible to see epithelial cell outlines as reported by Granger and Baker (1950).

Marsh et al. (1968), Toner and Carr (1969) and Peter et al. (1970) stated that at high magnification, hexagonal patterns formed by the close-packed apical surfaces of the columnar cells may be seen in the human small intestine, but in our study, the cell outlines seemed to be polygonal. We observed that, the mucus discharge from goblet cell orifices and similar observations were reported by Trier (1963). The state of the goblet cell at the moment of fixation is dependent on numerous factors, eg, mechanical and traumatic (Moe, 1955), degenerative (Padykula, 1962), fasting and drugs used for anaesthesia (Marsh and Swift, 1969). A 'fuzzy coat' layer immediately adjacent to the plasma membrane of the lining cells of the gastrointestinal tract was reported in 1965 (Ito, 1965) and its thickness varied from cell to cell (Ito, 1965; Mukherjee and Williams, 1967). It is resistant to chemical and enzyme activity, suggesting that it is part of the epithelial cell and probably synthesized by it. Despite the most careful cleaning of the specimen, this material is apparent in most specimens. With the scanning electron microscope, it is not possible to say whether the layer we observed is mucus or is superficial layer (Marsh and Swift, 1969). This layer was not detected in our specimens. Overall, most of the LM and SEM results are in line with that recorded before in other animals and human except few differences.

Conflict of interests

The authors declare that there is no conflict of interests

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Vol. 9(1), pp. 9-15 , January, 2015
DOI: 10.5897/JCAB2014.0424
Article Number: 29BCD8A50108
ISSN 1996-0867

http://www.academicjournals.org/JCAB

Journal of Cell and Animal Biology

Full Length Research Paper

Development of new combined method based on reading of ovarian tracheoles and the observation of follicular dilatations for determining the physiological age of *Anopheles gambiae* s.s.

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Received 19 November, 2014; Accepted 5 January, 2015

The conventional dilaceration of ovaries is binding at the beginning of ovary development. However, reading ovarian tracheoles was very easy in stage I-II mean. The combination of both methods can really reduce the number of age indeterminations recorded with the two methods separately. The present study aimed to identify a method capable of determining physiological age of mosquitoes regardless of their ovarian development stage. In the present study, mosquitoes were caught in houses using window traps. After identification of *Anopheles gambiae* s.l species, their ovaries were dissected in distilled water. An ovary was left in distilled water for tracheoles reading and the other in a physiological liquid to search for follicular dilatation after dilaceration of ovary. The other body parts of mosquitoes were used to identify the species of the *A. gambiae* complex by polymerase chain reaction. The ovarian tracheoles reading method was unable to determine physiological age of 25% (n=28) of 112 *A. gambiae* s.s. analyzed. With follicular dilations observation method, the physiological age of 16.96% (n=19) mosquitoes was not determined; but, the age indetermination rates were reduced to 0.89% by combining the two methods. The combination of ovarian tracheoles reading method and follicular dilatation observation method significantly reduced (almost null) the number of physiological age indeterminations recorded using the two methods separately.

Key words: *Anopheles gambiae* s.s, ovary, physiological age, ovarian tracheoles, classical dilacerations, follicular dilatation.

INTRODUCTION

In 2013, the number of malaria cases was estimated at 198 million in the world with 82% in Africa region and causing 584000 deaths (WHO, 2014). In the same period, the number of deaths due to malaria was

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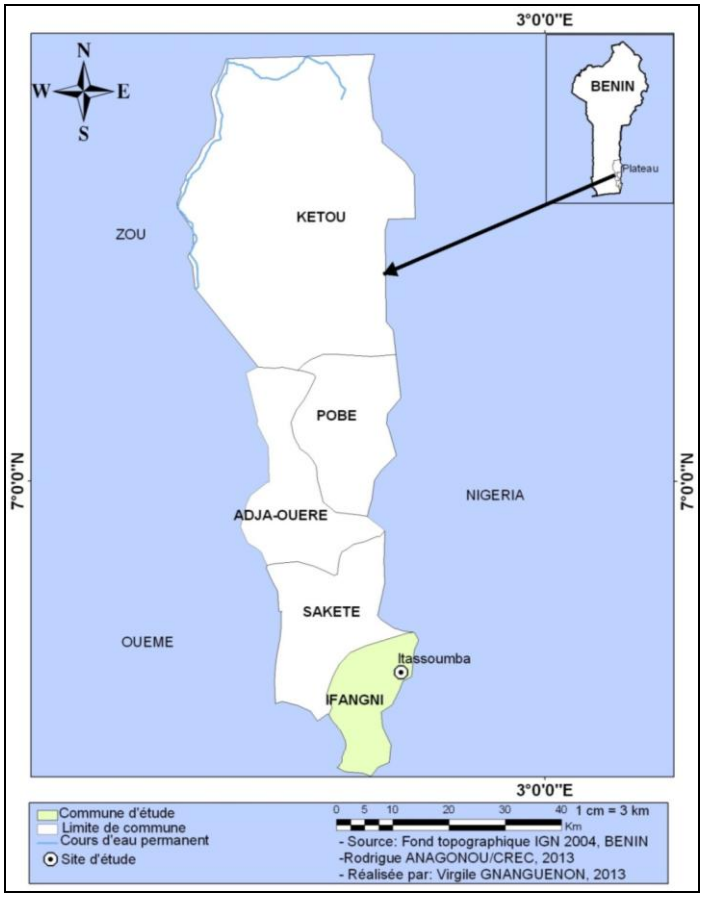

Figure 1. Map showing the village of Itassoumba in the district of Ifangni, Benin.

estimated at 2288 in Benin (OMS, 2014; MS, 2014). Vector control is the main strategy for malaria prevention. The aim of vector control is to reduce the number of infectious vectors (Hamon et al., 1961). Vector longevity is one of the most used indicators for assessing the effectiveness of vector control programs. According to Polovodova method, the number of dilatations observed on ovarioles should be equal to the number of egg-laying (physiological age) in females of mosquitoes (Hugo et al., 2008). Difficulties observed when applying Polovodova method justify the use of Lewis method (Mondet, 1996) to facilitate the identification of nulliparous females which have not laid and those which have laid at least once during their live cycle. With Lewis method, ovarioles are separated in a physiological serum and the absence (nulliparous females) or presence (parous females) of dilatations on ovarioles (follicles) is verified with a microscope (Hamon et al., 1961). At the beginning of ovaries' development, ovarioles are more joined one to others and their isolation is not easy during classical dilaceration of ovaries. In a sample where the number of mosquitoes at beginning of their ovarian development is important, Lewis method becomes difficult and therefore leads to an impossibility to determine physiological age of mosquitoes dissected. However, the usual method of Detinova based on ovarian tracheoles aspects is easily with mosquitoes in stage I-II mean of their ovarian development (Hugo et al., 2008). In a representative sample where about 1/5 of the specimens are not examined, the representativeness of the sample size is weaken. The combination of ovarian tracheoles reading method and follicular dilatations observation method can really reduce the number of mosquitoes whose physiological age cannot be determined when both methods are used separately. The aim of this study was to explore a method capable of determining physiological age of all mosquitoes submitted for examination regardless of their ovarian development stage.

METHODOLOGY

Study area

Entomological surveys were carried out between May and November 2013 at Itassoumba (Figure 1) in Ifangni district (Province of plateau) located 06°38'56''N and 02°43'14''E in South-east of Benin with 71606 inhabitants (INSAE, 2002). Itassoumba

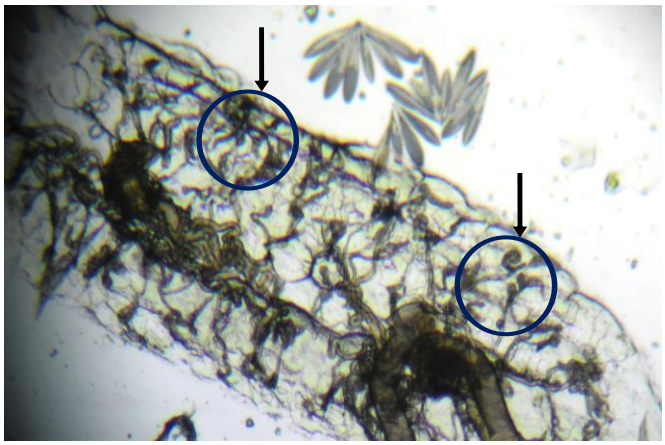

Figure 2. Nulliparous ovary (tracheoles coiled) according to Detinova (CREC, 2013).

has a rugged relief with the presence of some depressions. The climate is Guinean with two dry and rainy seasons: a great rainy season from March to July, a small dry season in August, a small rainy season from September to November and a great dry season from December to February. Itassoumba recorded an annual rainfall between 800 and 1400 mm water. The vegetation includes relics of sacred forests, plantations of oil palms, shrubs and tall grasses. Itassoumba is crossed by swamps. In the dry season, the breeding sites of *Anopheles gambiae* s.l are scarce. They are particularly permanent in Itassoumba due to the presence of fish ponds and marshes for vegetable farming.

Sampling of mosquitoes

Mosquitoes were caught using window traps from 6 to 18 h. Indoor biting and outdoor resting mosquitoes were collected and kept separately in Eppendorf tube until taken to the laboratory.

Identification, dissection of ovaries and determination of physiological age of females of *A. gambiae* s.l

Mosquitoes species collected were morphologically identified (Gillies and Coetzee, 1987; Gillies and De Meillon, 1968). The ovaries of mosquitoes were dissected in distilled water using binocular microscope. One ovary of each mosquito is put on lamella in distilled water and the second on other lamella in physiological liquid (Natrichlorid 0.9% + Neutral red 1/5000-1/3000). After drying, tracheoles of ovaries were examined with a microscope (4-10x) using Detinova method (Hugo et al., 2008). The tracheoles are wound (platoons) in nulliparous mosquitoes but are unwound in parous females. Moreover, the presence of follicular dilatation was verified after classical dilaceration of ovaries transferred in physiological liquid. According to Lewis method (Mondet, 1996), parous mosquitoes have at least one dilatation on their ovarioles but the nulliparous females do not have one.

Characterization of *A. gambiae* species complex

Abdomen, wings and legs of dissected mosquitoes were used for *A. gambiae* species identification using PCR (Scott et al., 1993).

Statistical analysis

To assess the reliability of method based on the observation of follicular dilatations for the determination of physiological age, we have compared the percentage of parous and nulliparous females between Detinova and Lewis methods by estimating the p-values using Fisher test.

Pairwise comparison of unreadable lamellas following the ovarian development stages was done using pairwise comparison test of multiple proportions (Robert, 1998) with Holm adjustment of p-value (Benjamini and Yekutieli, 2001). The same approach was used for the comparison of indetermination rate obtained by combining Lewis and Detinova methods to that obtained using these methods separately. All statistical analyses were done using R.2.15.2 (Development Core Team, 2011).

RESULTS

Structure of ovaries and ovarioles showing physiological age in *A. gambiae* s.s.

Figures 2 and 3 shows respectively after microscopic observations, the curled aspect of ovarian tracheoles and the absence of follicular dilatation in nulliparous females. However, Figures 4 and 5 show respectively the absence of curled aspect of tracheoles and the presence of a follicular dilatation in parous females.

Comparison of ovarian tracheoles reading method and follicular dilatations observation method for determination of physiological age in *A. gambiae* s.s.

On 112 females of A. gambiae s.s. examined, 20 (17.86%) and 16 (14.28%) nulliparous females were identified respectively by ovarian tracheoles aspect and the observation of follicular dilatations. No significant difference was observed between these two rates (p=0.505) (Table 1). 64 (57.14%) females were parous according to ovarian tracheoles method against 77 (68.75%) for follicular dilatations observation method. No

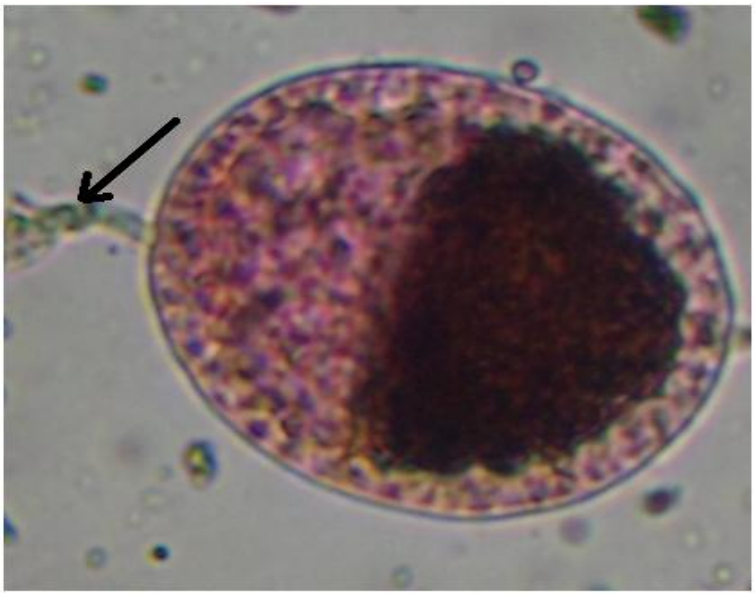

Figure 3. Nulliparous ovary (no dilatation) according to Lewis (CREC, 2013).

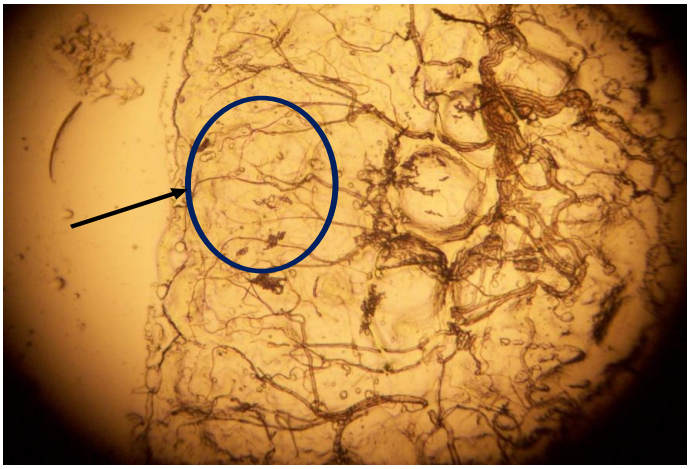

Figure 4. Parous ovary (tracheoles unwound) according to Detinova (CREC, 2013).

significant difference was observed between these two rates (p=0.314) (Table 1). These results confirm that the use of follicular dilatations is a reliable method for A. gambiae s.s physiological age determination.

Influence of ovarian development stage on ovarian tracheoles reading method and the follicular dilatations observation method for determination of *A. gambiae* s.s. physiological age

With 112 mosquitoes examined by ovarian tracheoles reading method, 25% (n=28) were unreadable and this rate increased significantly with ovaries development ($p<0.05$) (Table 2). The unreadable ovaries were 4.94% at stage I-II mean against 85.71% at stage II aged-IV of ovaries development (Table 2). According to the follicular dilatations observation method, the distribution of 16.96% (n =19) of mosquitoes whose age was not determined was homogenous following the ovarian development (Table 2). The majority of indeterminations (84.21%) was recorded in stage I-II mean with the observation of follicular dilatations (Table 2). Overall, the indeterminations of physiological age recorded with ovarian tracheoles reading method were associated with ovaries development. However, with follicular dilatations observa-

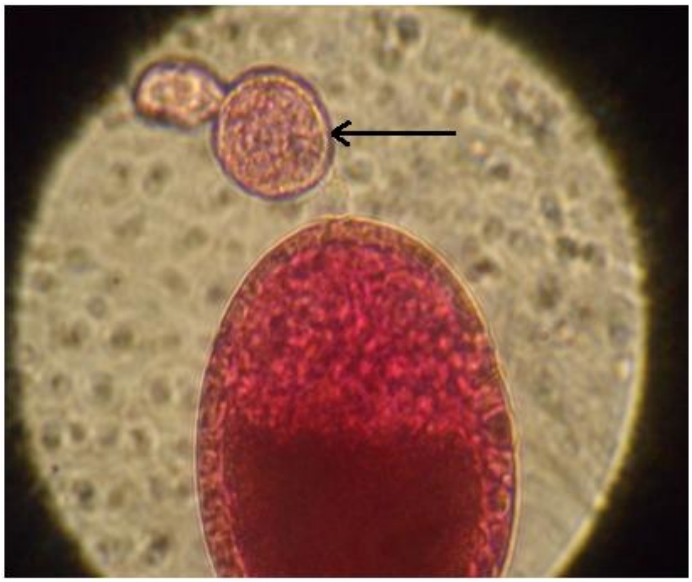

Figure 5. Parous ovariole (having a dilatation) according to Lewis (CREC, 2013).

Table 1. Reliability of the observation of follicular dilatations for the determination of physiological age of *A. gambiae s.s*.

Parity	Tracheoles	Dilatation	p-value
Nulliparous	20 (17.86%)	16 (14.28%)	0.505
Parous	64 (57.14%)	77 (68.75%)	0.314
Unreadable	28 (25.00%)	19 (16.96%)	
Total	112 (100.00%)	112 (100.00%)	-

tion method, no association was observed between the indeterminations of physiological age and the development stage of ovaries.

Efficacy of combination ovarian tracheoles reading method and follicular dilatations observation method for the determination of physiological age of *A. gambiae* s.s.

The two methods based on the reading of ovarian tracheoles and the observation of follicular dilatations were applied simultaneously on 112 females *A. gambiae* s.s (Table 3). The ovarian tracheoles reading method was not able to determine the physiological age of 25% (n=28) *A. gambiae* s.s. while follicular dilatations observation method was not able to determine 16.96% (n=19). The indetermination rate of the physiological age was almost null (00.89%) with the combination of the two methods on the same sample (Table 3). A significant difference was observed between the indeterminations obtained with the two methods separately and those obtained with their combination ($p<0.001$).

Overall, the combination ovarian tracheoles reading method and follicular dilatations observation method significantly reduced (almost null) the number of mosquito whose physiological age could not be determined using these methods separately.

DISCUSSION

The results of this study confirm the inefficacy of Detinova method (1962) observed from stage II age of ovarian development. Several authors (Germain et al., 1974; Cornet et al., 1978) showed that from stage II age, the reserves of vitellus become enormous and recover completely tracheoles network making ovaries unreadable after drying. Reading of ovarian tracheoles is the basic method used for the determination of physiological age of mosquitoes. This method is rapid, cheaper and easy to apply once the ovaries are extracted (Beklemishev et al., 1959). In addition, mosquitoes collected using human landing catch are more indicated for the application of Detinova method based on the ovarian tracheoles aspect. Human landing catch provides overall starve and pre-gravid mosquitoes which are in

Table 2. Variation of indetermination rate of physiological age following the development of ovarian stages after the reading of ovarian tracheoles and the observation of follicular dilatations.

Stage	Total Mstq	Tracheoles		Dilatation	
		N (i)	% (i)	N (i)	% (i)
I	12	0	0.00[b]	7	58.33[a]
IId	50	2	4.00[b]	5	10.00[b]
IIm	19	2	10.53[b]	4	21.05[ab]
IIf	14	9	64.29[a]	0	0.00[b]
III	11	9	81.82[a]	2	18.18[ab]
IV	6	6	100.00[a]	1	16.67[ab]
Total	112	28	25.00	19	16.96

d: Beginning, m: mean, f: aged, N: number, Mstq: mosquito, i: indetermination. Percentages which carry same letters in exposant were not significantly different (p>0.05)

Table 3. Results of the combination of the reading of ovarian tracheoles and the observation of follicular dilatations for the determination of the physiological age of *A. gambiae s.s.*

Methods age	N mstq	N (i)	% (i)
Dilatations	112	19	16.96a
Tracheoles	112	28	25a
Trac & dil	112	1	0.89b

Trac: Tracheole, dil: dilatation, N: number, mstq: mosquito, i: indetermination. Percentages which carry same letters in exposant were not significantly different (p>0.05).

stage I-II mean of their ovarian development. However, window trap catch and indoor residual morning spray catch have the advantage to provide not only, starve and pre-gravid mosquitoes, but also blood fed mosquitoes whose ovaries are over stage II mean.

Classical dilaceration of ovaries requests technical delicacy for physiological age determination of vectors (Mondet, 1996). Ovarioles isolation is not easy at stage I and II, beginning of ovaries development. At the beginning of ovaries' development, the ovarioles are joined one to another and their separation requests manual dexterity; this is not the case for mosquitoes at stage II aged-IV. This phenomenon explains the high number of indetermination age (16/19) recorded in mosquitoes at stage I-II mean of their ovarian development.

The fragility of pedicle of ovarioles was demonstrated (Mondet, 1993; Hoc and Wilkes, 1995b). In nulliparous mosquitoes, the pedicle of ovarioles is very short; this facilitates maintaining ovarioles intact during their isolation. However, in parous females, the pedicle of ovarioles elongates with successive egg-laying (Mondet, 1996). This phenomenon generates and extreme fragility of pedicle susceptible to breakage on the smallest needle moves during isolation of ovarioles (Mondet, 1993; Giglioli, 1965b). After ovarioles isolation, we observed at least a follicular dilatation in parous females during dissections.

This study shows that, the combination of ovarian tracheoles reading method and the follicular dilatations observation was very efficient for determination of *A. gambiae* s.s. physiological age. On 112 mosquitoes examined with the combination of the two methods, the physiological age of only one mosquito (0.89%) was not determined. These results show that the combination of Detinova and Lewis methods is able to determine the physiological age of all mosquitoes submitted for examination. This efficiency confirms the reciprocal complementarily of efficacy that exists between ovarian tracheoles reading method and follicular dilatations observation method. In addition, the combined application of the two methods is constraining when it is not implemented in team. Even for an experimented researcher, the combination of both methods on a

representative sample requests more time. For a systematic use in vector control evaluation, an easy and very rapid physiological age estimation method must be encouraged (Hamon et al., 1961).

Conclusion

The combination of ovarian tracheoles reading method and follicular dilatations observation method is very efficient for *A. gambiae* s.s. physiological age determination. It can be used over stage II age without physiological age indetermination. However, the combination of both methods requires technical expertise and delicacy. Also, the application seems difficult when it is not realized in team.

Conflict of interests

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to the Bill & Melinda GATES Foundation which supported financially this study. Rodrigue ANAGONOU obtained financial support for his doctoral training from Ministry of the Higher Education and of Scientific Research.

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