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Ultrastructure, Life-Cycle, Survival and Motility of *Demodex bovis* (Acarina: Trombidiformes: Demodicidae) Isolated from Cattle in the Sudan

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Abstract: *The ectoparasite Demodex bovis was isolated from fresh infected purulent material collected from cattle with demodecosis. The life-cycle and ultrastructure of the mite were studied using scanning electron microscope. The mite was tested for survival, motility, and type of motility in tap water, physiological saline, and ringer's solution at increasing temperatures. Furthermore, the survival time of D. bovis in 5% and 10% potassium hydroxide was investigated. The life-cycle of D. bovis passed through several developmental stages from ovum, larva, protonymph, nymph to adult male or female mites. All morphologic characteristics observed under light microscopy were confirmed in electron photomicrographs. The mite showed maximum motility at temperatures simulating the temperature of their habitat in the pilosebaceous units of their host. The longest period of survival was recorded in physiological saline and Ringer's solutions. Examination of the mite during motility testing showed that the movement of the mite was of a crawling nature rather than a fast free progressive movement. The legs of the mite moved forwards in an arc of a small radius and backwards in an arc of maximum radius. This phenomenon allowed the mite to move forwards by pushing its body to the front on the backward swing. The study added a valuable knowledge to the basic biology of D. bovis. The ultrastructure, life cycle, and other biological characteristics of Demodex species isolated from domestic animals in the Sudan should be investigated.*

Keywords: *Mite; ultrastructure; life-cycle; survival; motility*

1. Introduction

Today, over one hundred *Demodex species* (class *Arachnida*, subclass *Acarina*) are known and all are highly specialized, host-specific and are named after their hosts (Baima and Sticherling, 2002; Radostits, *et al.*, 2007; Jarmuda *et al.*, 2012; OIE, 2016). *Demodex* mites can infest different areas of the skin of humans and animals causing cutaneous pathology ranging from asymptomatic to severe and potentially fatal diseases with numerous skin and ocular symptoms (Gortel, 2006; Lacey *et al*., 2009; Lacey *et al*., 2011). The mites that are able to cause severe skin problems are of high medical and veterinary concern (Nutting, 1976; Jarmuda *et al.*, 2012).

Demodex species have undergone several evolutionary changes in a number of morphological features in order to be able to survive in their host tissues. Many differences have been noticed in the shape, tagmata proportions, dimensions, and the position and form of many morphological structures in the hair follicle mites. Such evolutionary changes would make species identification difficult and complicated (Bukva, 1991; Izdebska, 2009; Jarmuda *et al.*, 2012).

The life cycle of *Demodex* mite has five phases of development including mating, deposition of eggs, larval emergence, which develops to protonymphs and nymphs and finally to adult mite. The whole life cycle takes 14 to 18 days (Desch and Nutting, 1977; Lacey *et al*., 2009; Lacey *et al.*, 2011). In the present work, the ultrastructure, life-cycle, survival and motility of *D. bovis* mites isolated from cattle in the Sudan were elaborately studied.

2. Materials And Methods

2.1. Sample Collection

Fresh infected purulent materials were collected from nodules and pustules of 300 cattle investigated by Abu-Samra and Shuaib (2014).

These animals had skin lesions of demodectic mange that are confined to certain parts of the body or generalized. The lesions were in the form of papules, nodules and papules, nodules and few pustules (Fig. 1), pustules and few nodules or pustules and crust-covered lesions (Fig. 2).

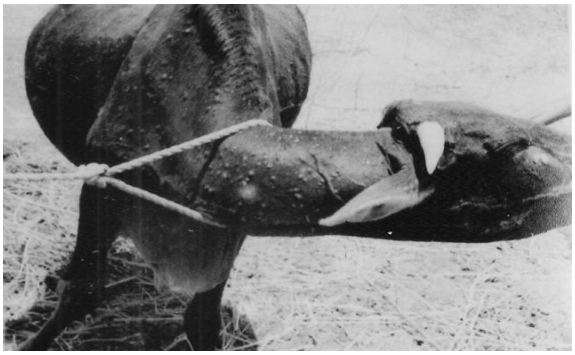

Figure1. *A cow infected with demodectic mange, showing pustules and nodules spread all over the body*

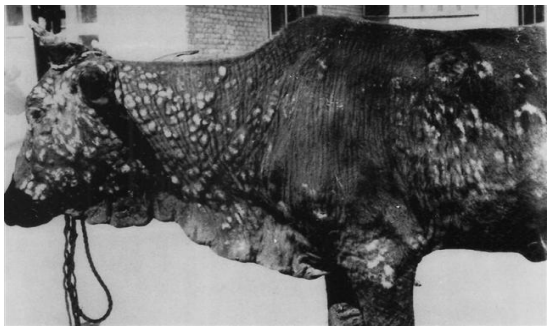

Figure2. *Pustules and crust-covered lesions of demodectic mange involving extensive areas of the body of a heifer. Note marked wrinkling and folding of the skin*

2.2. Laboratory Investigations

Mites were isolated and permanently mounted as previously described by Abu-Samra *et al*., (1984). A small amount from each sample was placed in the middle of a glass slide and 2-3 drops of 10% potassium hydroxide were added. The slide was then heated, covered with a coverslip and examined under a microscope fitted with a camera (BHA Olympus, Japan). Another small amount from each sample was placed in a watch glass flooded with 10% potassium hydroxide and left on the bench at room temperature for 15 minutes, after that, the infected material was teased with a pair of dissection needles until a homogenous material was obtained. Afterward, a drop of the homogenous material was transferred to the middle of a glass slide. The slide was heated, covered with a coverslip and the excess fluid was removed. These preparations and the permanently mounted mites were examined under a microscope fitted with an eyepiece lens micrometer, and the measurements were recorded in µm. The identity of *D. bovis* mites was verified by plotting a graph of the total length (µm) of 100 female mites. The preparations were also mounted on an overhead projector microscope with a screen (Leitz, Wetzlar, Germany). The mite and all its developmental stages were traced on a white sheet of paper and retraced on tracing paper.

The samples were also prepared for examination using a Scanning Electron Microscope (SEM) at Liverpool School of Tropical Medicine. Each sample was fixed in 3% buffered glutaraldhyde at 4ºC for 24 hours. The fixed material was processed by passing it through two changes of isotonic saline for one hour each. The samples were then dehydrated in ascending concentrations of ethanol (25%, 50%, and 70%) for 1-2 hours each, in one change of 95% ethanol for 15 min and finally in three changes of 100% ethanol for 15 min each. The samples were then critically dried in an E 3000 chamber (Polaron Limited, England). A one cm aluminum stubs (Cambridge Steroscan type) were used to mount the specimens using "sticky tabs" as an adhesive, coated in a Polaron E 5100 Series 11 cooled sputter coater to a thickness of between 12 and 45 nm (15 nm/min) and were finally viewed in a Cambridge Steroscan S4-10 SEM.

Survival, motility, and type of motility of *D. bovis* were tested in tap water, physiological saline, and ringer's solution at 5, 10, 15, 20, 25, 30, 40, 45, and 50ºC. The survival time of the mite in 5% and 10% potassium hydroxide was also tested. Examination of the motility was conducted by placing the sample in the middle of clean coverslips with edges smeared with a thin film of petroleum jelly, and immediately before examination, the wells of Kova-slides (ICL Scientific, California) were filled with the test solution at the required temperature and the coverslips with the infected material were carefully inverted over the Kova well containing the test solution and examined under the microscope.

3. RESULTS

Adult *D. bovis* mites and all its developmental stages were found in the crushed specimens of the infected purulent material extracted from skin lesions (Fig. 3). *D. bovis* mites were isolated and identified (Fig. 4).

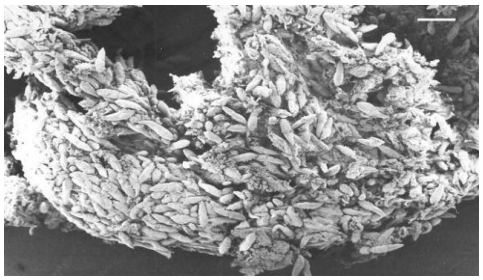

Figure3. *Scanning electron photomicrograph (SEM) of purulent material extracted from skin lesions of bovine demodicosis showing the different developmental stages of Demodex bovis mites. SEM, Scale bar: 240 µm*

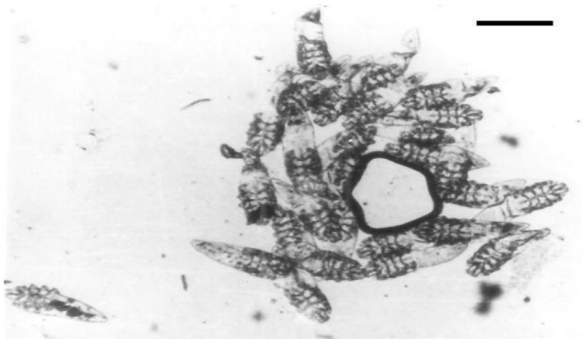

Figure4. *Demodex bovis isolated from purulent material extracted from skin lesions of bovine demodicosis. Light microscopy, Scale bar: 180 µm*

3.1. Ultrastructure and Morphologic Characteristics of the Mite

The gnathosoma of *D. bovis* was roughly trapezoidal in shape and wider at the base (Figs. 5). Dorsal view of the gnathosoma revealed a spatula shaped epistome situated between two roughly triangular-shaped segments of the capitulum. The supracoxal spines were directed medially and bifurcated distally. Ventral view of the capitulum showed that it was roughly conical bearing a rectangular shaped lower jaw plate with the mouth opening at its distal end (Fig. 6). Coxa of palps and palps with two free segments were also seen. The proximal segment was salient, and the distal segment showed palp setae. The dorsum of the podosoma showed fingerprint-like inscriptions. An antero-dorsal view the gnathosoma and frontal view of the capitulum revealed a rectangular epistome hanging over the mouth and covering the chelicerae and labial sheath of chelicerae. The two segments of palp were rounded in outline and compressed antero-posteriorly (Fig. 7). The proximal segment was salient and the distal segment II bears 5-6 distinct finger-like palp setae. The first three pairs of legs were slightly projecting outside the lateral surface of the body, while the fourth pair were shorter and were not projecting from the lateral surface when the mite was viewed from the dorsal surface. However, all four legs can be seen when the mite was viewed from the lateral surface (Fig. 8). The epimeral plates were indistinct meeting smoothly at the midline (Fig. 9). The legs were stumpy with three short segments with the third distal segment bearing a pair of short equal claws. The body of male *D. bovis* was slightly constricted posterior to epimera IV. The male genital organ is situated in an oval-shaped shield on the mid-dorsal surface at the level of epimeral plate II. It had a bulb-like tip and was slightly deviated to the left of the mid-line. The female genital organ is a longitudinal slit opening just behind the arched posterior margin of epimeral plate IV (Fig. 10). The opisthosoma was transversely striated and gradually tapering to a rounded termiunus (Fig. 11). The striations meet at the terminus in an elliptical fashion. The opisthosomal organ was situated mid-ventrally and about 30µm from the opisthosoma terminus. It is a sac-like structure compressed dorso-ventrally. The ova were oval in shape (Fig. 12).

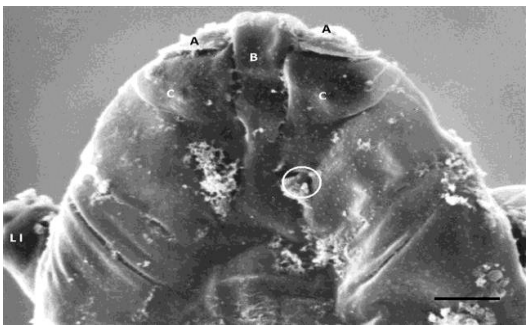

Figure5. *Scanning electron photomicrograph (SEM) of the dorsal surface of gnathosoma of Demodex bovis mite, showing distal most segment of palp (A), hypostome (B), capitulum (C), supra-coxal spine (circle) and the first pair of legs (L I). Scale bar: 5 µm*

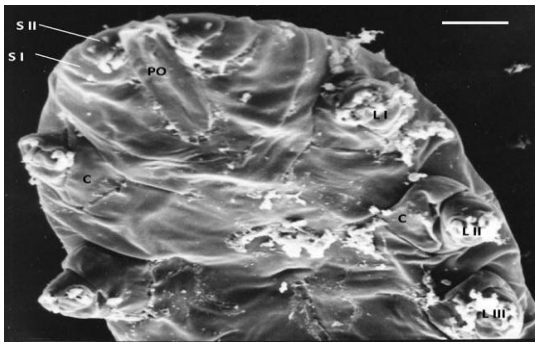

Figure6. *Scanning electron photomicrograph (SEM) of the ventral surface of gnathosoma and ¾ of the podosoma of Demodex bovis mite, showing preoral opening (PO), segment I of palp (SI), segment II of palp (SII), coxa (C) and first three pairs of legs (L I – L III). Scale bar: 10 µm*

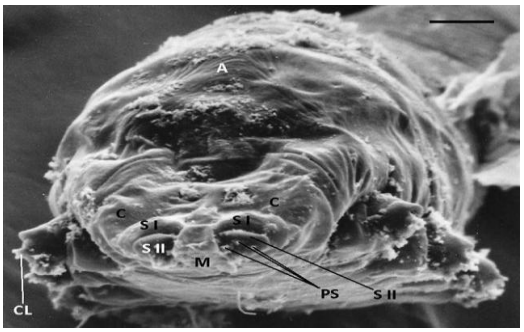

Figure7. *Scanning electron photomicrograph (SEM) of the antero-dorsal and frontal view of female Demodex bovis mite, showing finger-like inscription on the dorsum of podosoma (A), coxa of palp (C), mouth (M), palp setae (PS), segment 1 of palp (S I), segment 2 of palp (S II) and claws (CL). Scale bar: 10 µm*

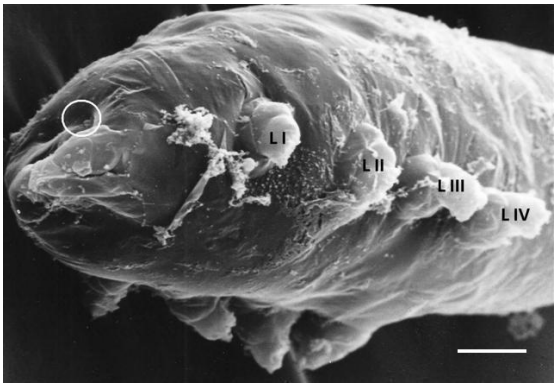

Figure8. *Scanning electron photomicrograph (SEM), lateral view of gnathosoma and podosoma of Demodex bovis mite, showing four pairs of stumpy legs (L I – L IV) and supracoxal spine (circle). Scale bar: 10 µm*

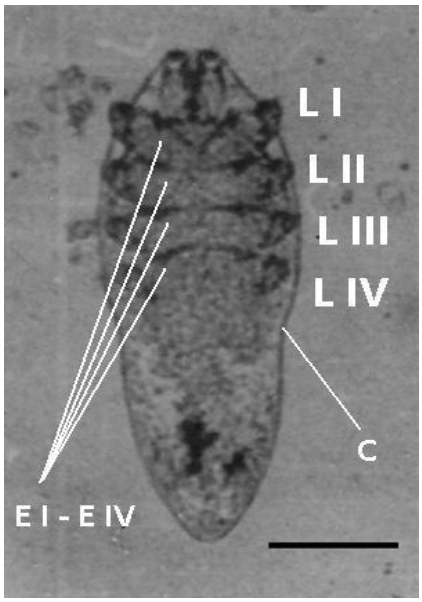

Figure9. *Male Demodex bovis mite isolated from purulent material extracted from skin lesions of bovine demodicosis, showing indistinct epimeral plates (E I – E IV) and four stumpy legs (L I – L IV). Note characteristic constriction posterior to epimra IV (C). Light microscopy, Scale bar: 60 µm*

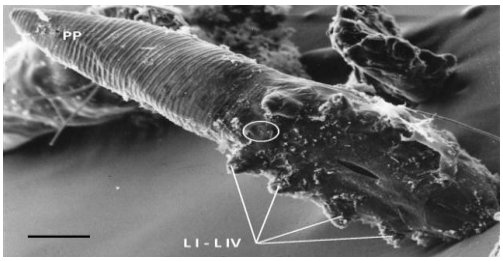

Figure10. *Scanning electron photomicrograph (SEM), ventral surface of female Demodex bovis mite, showing vulva (circle), proctodeal pore (PP) and four pairs of stumpy legs (LI – L IV). Scale bar: 35 µm*

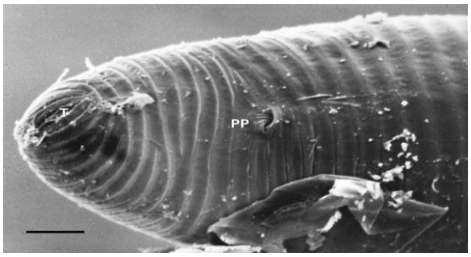

Figure11. *Scanning electron photomicrograph (SEM), ventral surface of opisothoma of Demodex bovis mite, showing proctodeal pore (PP) and terminus (T). Scale bar: 175 µm*

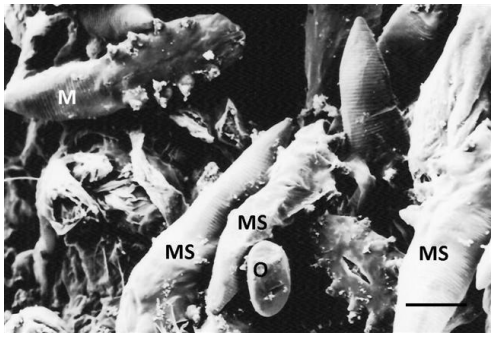

Figure12. *Scanning electron photomicrograph (SEM) of purulent material extracted from skin lesions of bovine demodicosis, showing some developmental stages of Demodex bovis mites. Note adult mite (M), mites in molting sheath (MS) and ovum (O). SEM, Scale bar: 65 µm*

3.2. Life-Cycle of the Mite

Gravid females increased in width, and produced fertilized ova (Fig. 13[1*]). The ova underwent a series of maturation and differentiation processes (Fig. 13[2*-10*]), resulting in the appearance of a thin and regular horse shoe-shaped structure at the cranial pole of the ovum, representing the pharyngeal bulb (Fig. 13[11*]). This structure became larger and thicker, and showed two symmetrical knobs at both ends (Fig. 13[12*]). The knobs fused posteriorly forming two small protuberances surmounting the body of the developing larva within the ovum shell (Fig. 13[13*]). The primary developing larva regressed to the caudal pole of the ovum shell (Fig. 13[14*]), and

ultimately freed itself from the ovum shell, appearing in several stages (Fig. 13[15*-17*]&14) before the final stage, in which it made its appearance possessing three pairs of buds at the ventero-lateral edge of the anterior part of the body (Fig. 13[18*]). This final larval stage had tiny palpal claws, peg supracoxal spines and a horse shoe pharyngeal bulb opening posteriorly. The final larval stage was very brief and moulted undergoing a series of changes in length and width (Fig. 15[1*- 4*]), and developed to protonymph (Fig. 15[5*-6*]). At this stage the protonymph had gnathosomal structures similar to the larva but with three pairs of epimeral scutes. The protonymph (Fig. 15[7*-9*]&16) moulted to a nymph, showing four pairs of legs and four pairs of epimeral scutes. Finally, the nymph moulted to adult mite (Fig. 15[10*]), showing well developed gnathosomal, podosomal and opisthosomal structures, and became sexually differentiated to female (Fig. 15[11a*]) and male (Fig. 15[11b*]) mites.

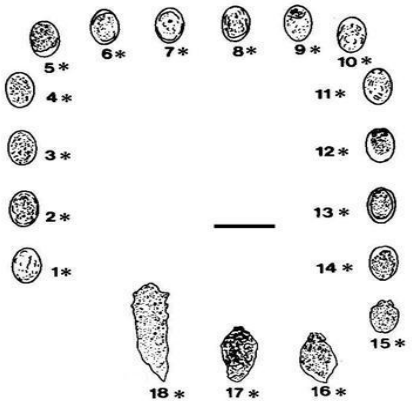

Figure13. *Tracing of the different developmental stages (1* – 18*) of the life-cycle of Demodex bovis mite (ovum – larva) isolated from purulent material extracted from skin lesions of bovine demodicosis. Overhead projector microscope, Scale bar: 70 µm*

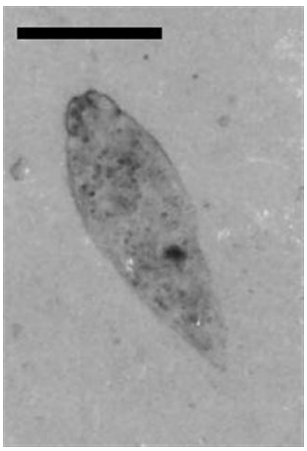

Figure14. *Light microscopy of stage 16* (larva) in the life-cycle of Demodex bovis mite (ovum - larva) isolated from purulent material extracted from skin lesions of bovine demodicosis. Scale bar: 120 µm*

Figure15. *Tracing of the different developmental stages (1* –11a* and 11b*) of the life-cycle of Demodex bovis mite (larva – adult female and male mites) isolated from purulent material extracted from skin lesions of bovine demodicosis. Overhead projector microscope, Scale bar: 140 µm*

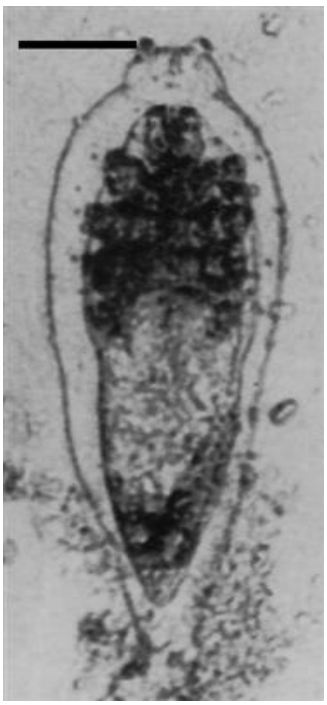

Figure16. *Light microscopy of stage 7* (protonymph) in the life-cycle of Demodex bovis mite (larva – adult female and male mites) isolated from purulent material extracted from skin lesions of bovine demodicosis. Scale bar: 50 µm bar: 50 µm*

3.3. Survival and Motility of the Mite

The mite survived for 3 to 4 days in physiological saline and Ringer's solution. It also survived for 2 days in tap and distilled water and for 1.5-2 and 0.5-1 hours in 5% and 10% potassium hydroxide. It was viable and motile in all solutions but maximum motility

was observed in physiological saline and Ringer's solution. The mite was non-motile in all solutions at 5, 10, 40, 45, and 50ºC. Sluggish motility was observed at 15ºC, improved at 20ºC and 25ºC and was active at 30ºC and 35ºC. The movement of the mite was of a crawling nature rather than a fast free progressive. The legs moved forwards in an arc of a small radius and backwards in an arc of maximum radius. This phenomenon allowed the mite to move only forwards.

4. DISCUSSION

D. bovis is the most common mite of the three species of *Demodex* that are known to infest cattle. This mite infests the hair follicles and has a global geographical distribution although it is more common in tropical areas and is not usually found in temperate parts of the world (Matthes and Bukva, 1993; Radostits, *et al.*, 2007; OIE, 2016). *D. bovis* is host specific and has not been detected in samples taken from humans with skin problems, hence, it has no zoonotic importance (OIE, 2016).

In this study, male mites were less in occurrence than female mites in all of the investigated specimens. Such observations have been made since 1960s (Nemeseri and Szeky, 1961; and Spickett, 1961). This could probably be attributed to the fact that fertilization takes place on the skin surface or in the follicle opening, and male mites die few days after copulation, while female mites move back to deposit eggs in the hair follicles where the environment supports the development of the parasite (Rather and Hassan, 2014).

Examination of *D. bovis* isolated from cattle in the Sudan using the scanning electron microscope proved quite fruitful and added valuable knowledge on the basic biology of the mite which is probably not recorded in the available literature. All morphologic characteristics observed under light microscopy were confirmed in electron photomicrographs but the minute structures in the different tagmata were demonstrated with a better resolution and clearer form in the electron photomicrographs. Tracing the stages of development of the mite enabled the assembly of its life cycle, which was found to pass through ovum, larva, protonymph, nymph and adult male or female mites. This finding was typifying the findings of Desch and Nutting (1972), Rather and Hassan (2014), and Mehlhorn (2015) who stated that the development of six-legged larva I and eight-legged protonymph I and II takes up to 10 days after laying of eggs in the hair follicles. After that, adult mites emerge and reach maturity in about 30 days in total. The different developmental stages of the mite and the stages of differentiation, transformation and maturation were clearly observed and studied. It was noticed that each stage underwent several developmental, differentiation and transformation processes before the succeeding stage appeared. Several developmental stages of the larva were seen in most of the specimens examined, but larvae possessing three pairs of legs were few in number. This was probably because the final larval stage was very brief and/or on being relatively small in size, they were capable of moving deep in the pilosebaceous canal and continuously feed on fresh sebum and degenerate small particles of damaged tissue that gravitated to the depth of the of the pilosebaceous canal, before moulting to protonymphs as elaborated by Spickett (1961). Interestingly, the sequence of events from the final larval stage to the final adult stage was gradual and by no means an abrupt one.

The mite *D. bovis* showed maximum motility at temperatures simulating the temperature of their habitat in the pilosebaceous units of their host. Survival of the mites in physiological saline and Ringer's solutions for the longest period of time was probably due to the fact that those solutions were more nearly related to the conditions found in their habitat. In contrast, the mites survived for 1.5 days in a dry atmosphere and for 3 days in a moist atmosphere and were destroyed at 41ºC. Nutting (1976) reported that for several species of *Demodex* mites transfer must occur quickly once they were free on the skin surface because they were rapidly killed by desiccation in ¾-1 hours at 20ºC and 40% relative humidity. Nonetheless, Soulsby (1982) indicated that the mites were fairly resistant and could survive for several days off the host when the surroundings were moist. Confirming the findings of Nutting (1976) and Rufli and Mumcuoglu (1981), the motility of the mite was noticed to be of a crawling nature rather than a substantial progressive movement. This might be due to the structure of the legs of the mite was well adapted for a crawling type of motility within the limited confines of the pilosebaceous units

resisting the highly viscous sebum that was constantly secreted by the sebaceous glands.

In summary, this study added a valuable knowledge to the basic biology of *D. bovis*. Morphologic characteristics of *D. bovis* isolated from cattle in the Sudan was revealed using light microscopy and electron photo micrographs. Moreover, the stages of development of the mite were seen. The mite showed maximum motility at temperatures simulating the temperature of their habitat in their host. And the longest period of time of survival was in physiological saline and Ringer's solutions. The ultrastructure, life cycle, and other biological characteristics of *Demodex* species such as *D. ghanensis*, *D. felis*, *D. caprae*, *D. equi*, *D. ovis*, and *D. canis* isolated from cattle with ocular demodicosis and cats, goats, sheep, equines, and dogs with skin demodicosis in the Sudan should be investigated.

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REFERENCES

[1] Abu-Samra M. T. and Shuaib Y. A. (2014): Bovine Demodicosis: Prevalence, Clinico-pathological and Diagnostic Study. J. Vet. Adv., 4: 381–397.

[2] Abu-Samra M. T., Abdel Aziz M. A., and Salih A. K. (1984): A new technique for the isolation of *Demodex bovis* from preserved infected material. Ann. Trop. Med. Parasititol., 78: 319–321.

[3] Baima B. and Sticherling M. (2002): Demodicidosis revisited. Acta Dermato-Venereologica., 82: 3–6.

[4] Bukva V. (1991): Structural reduction and topological retrieval: problems in taxonomy of Demodecidae. Modern Acarology, 1: 293–300.

[5] Desch, C.E. and Nutting W. B. (1977). Morphology and functional anatomy of *Demodex folliculorum*. Acarologia., 19: 422–462.

[6] Desch, C.E. and Nutting, W. B. (1972). Demodicids of medical and veterinary importance. Proc. 3rd. Internatl. Congress, Acarology, Prauge. P. 499–505.

[7] Gortel K. (2006). Update on canine demodicosis. Vet. Clin. North Am. Small Anim. Pract., 36, 229–241

[8] Izdebska J. (2009). Selected aspects of adaptations to the parasitism of hair follicle mites from hoofed mammals. European Bison Conservation Newsletter, 2: 80-88.

[9] Jarmuda S., O'Reilly N., Zaba R., Jakubowicz O., Szkaradkiewicz A., and Kavanagh K. (2012):Potential role of Demodex mites and bacteria in the induction of rosacea. J Med Microbiol., 61(Pt 11):1504-10. doi: 10.1099/jmm.0.048090-0

[10] Lacey N., Kavanagh K., and Tseng S. C. (2009). Under the lash: Demodex mites in human diseases. Biochem (Lond), 31, 2–6

[11] Lacey N., Ní Raghallaigh S., and Powell F. C. (2011). Demodex mites – commensals, parasites or mutualistic organisms? Dermatology, 222, 128–130.

[12] Matthes H. F. and Bukva V. (1993): Features of Bovine Demodecosis (*Demodex Bovis* Stiles, 1892) In Mongolia: Preliminary Observations. Folia Parasitologica, 154–155.

[13] Mehlhorn H. (2015): *Demodex Species*: in Encyclopedia of Parasitology. Online ISBN: 978-3-642-27769-6. Publisher: Springer Berlin Heidelberg, Germany. pp 1-4.

[14] Nemeseri L. and Szeky A. (1961). Demodicosis in cattle. Acta Vet. Acad. Sci. Hung., 11: 209–221.

[15] Nutting W.B. (1976). Hair follicle mites of medical and veterinary concern. Cornell Vet., 66: 214–231.

[16] OIE (2016): Mange. OIE terrestrial manual, Chapter: 2.7.9. World Organization for Animal Health, Paris, France, pp: 1-12.

[17] Radostits M., C. Gay, K. Hinchcliff and P. Constable (2007): Diseases associated with arthropod parasites, Chapter: 28. In: Veterinary Medicine, A textbook of the diseases of cattle, horses, sheep, pigs and goats. (10th edition). UK, Saunders Elsevier, pp: 1608.

[18] Rather P. A. and Hassan I. (2014): Human *Demodex* Mite: The Versatile Mite of Dermatological Importance. Indian J. Dermatol., 59(1), 60–66. doi: 10.4103/0019-5154.123498

[19] Rufli T. and Mumcuoglu Y. (1981). The hair follicle mite *Demodex folliculorum* and *Demodex brevis*: Biology and medical importance – A review. Dermatologica., 162: 1–11.

[20] Soulsby E.J. (Ed.) (1982). *Helminths, Arthropods and Protozoa of Domesticated Animals.* 7 th Edn., London: Bailliere Tindall. P. 476 – 479.

[21] Spickett S.G. (1961). Studies on *Demodex folliculorum* life history. Parasitol., 51: 181–192.

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