Oedematous skin disease (OSD) transmission among buffaloes.

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Abstract

During buffaloe OSD spread in a village affiliating to Assiut Governorate-Egypt, 44 buffaloe cows hosted and owned sporadically were subjected to the study. From 43 buffaloe cows (had closed lesions either edematous or nodular) and a buffaloe cow (had open ulcerative lesion), *Corynebacterium pseudotuberculosis equi* (*C. ps. equi*) as 72% and *Corynebacterium pseudotuberculosis ovis* (*C. ps. ovis*) as 28% were isolated and identified. Blood sucking insects hosted on the infected buffaloe cows (22) louse fly (*Hippobosca equine*) and 20 *Haematopinus eurysternus* lice were included during the study where both *C. ps. equi* and *C. ps. ovis* were isolated from *Hippobosca equina* (*H equina*) but failed to isolate any biovar of *C. ps.* from *Haematopinus eurysternus* lice (*H. eur.*). Moreover, *C. ps. equi* was isolated from two *H equina* pupae – lab deposited – as well as a *H equina* second generation fly concluding that there is endosymbiosis nature of *C. ps*. limited only to *H. equina* fly which can transmit *C.ps*. vertically.

Key words: **Buffaloes, oedematous skin disease, *Corynebacterium pseudotuberculosis, Hippobosca equina*.**

Introduction

Buffaloe OSD is an endemic disease in Egypt caused by *C. ps.* (Khalel et al 1995, Ali & Zaitoun 1999, Selim 2001) biovar II named *C. ps. equi* (Khater et al., 1983; Barakat et al., 1985, Selim 2001, Soares et al 2013, Selim et al 2016, Almeida et al 2017) which is toxogenic producing diphtheria toxin (Selim et al 2016) characterized mainly by reduction nitrates to nitrites (Selim 2001, Abdel-Latif 2011, Selim et al 2016, Oliviera et al 2016, Almeida et al 2017) ,by the action of nitrate reductase gene (nar G) (Soares et al 2013, Almeida et al 2017). The disease is initiated by intradermal inoculation of *C. ps.* in buffaloe skin through biting of the blood-sucking insects(Khater et al., 1983; Barakat et al., 1985, Selim 2001, Soares et al 2013) especially *H. equina* fly(Ghoneim et al., 2001, Selim 2001, Moussa et al 2016, Viana et al 2017). Many studies on open or ruptured lesions suggested that transmission may be mechanically with contaminated environment (soil & water) with *C. ps*. mainly by *Musca domestica* (Abou-Zaid and Hammam, 1994; Sayed, 2001; Yeruham et al., 2003 and Spier et al., 2004). The work aimed to study the role of *H. equina* (adult fly) and its life cycle stages in OSD transmission.

Material & Methods

Insects sampling: through a private clinic in Fayama village (15 Km. east north from Assiut city, Assiut Governorate) the study conducted on 44 buffaloe cows - owned and hosted sporadically - suffering from OSD ectoparasite infested, where 70 adult

blood sucking insects (40 *H. equina* flies & 30 *H. eur.* lice) were gathered. 22 *H. equina* flies as well as 20 *H. eur*. lice were used for bacteriological examination and the rest were used in parasitological investigation.

The insects were collected directly using sterile forceps from infected buffaloe or donkeys hosted together. They were kept into plastic sacs or wide-naked bottles for laboratory examination where they taxonomically identified (Soulsby, 1982 and Kettle, 1990).

Lab reared *H. equina* flies: 18 flies were still alive for 24 hours where some of them deposited their larvae (Full mature larvae) inside the collected sacs untill larvae pupated. The achieved pupae were incubated at room temperature in plastic sand containers covered with a piece of gauze until giving adult fly (Baraka, 1983). These different stages were photomicrograph.

Bacteriological examination was conducted with 22 flies gathered in sterile plastic sacs from infested animals (17 affected buffaloes, 4 cattle and a donkey hosted together). In addition to two pupae (lab deposited) and one laboratory developed fly (second generation) as well as 20 *H. eur*. lice for bacterial existence as follows:-

1. Fly was inoculated as it is into a sterile nutrient broth tube for bacterial isolation from body surface, legs and external mouth parts contamination (EBS).
2. The forceps catched fly was washed several times using sterile distilled water to rinse the rest of external contamination.
3. The washed fly was crushed, destructed and macerated into another nutrient broth tube for isolation of gut bacterial content (IBC).
4. The above mentioned three procedures were performed on lice (20) from only affected buffaloes to obtain their external (EBS) and internal (IBC) bacterial contents.
5. From the laboratory deposited pupae, 2 were burst into nutrient broth to isolate their bacterial contents.

Bacteriological examination: the above mentioned test tubes were overnight incubated aerobically at 37ºC, and then were streaked onto 10% sheep blood agar (24 - 48 h). Growing colonies were purified and identified morphologically by Gram's stain. Biochemically tested for motility, glucose and maltose fermentation, catalase activity and nitrate reduction were adopted (Quinn et. al., 2011).

Results

Parasitologically, all diseased buffaloes were infested with dark leathery flies identified as *H. equina*. Adult flies were more abundant on stabled diseased animals. They mainly aggregated under the tail, on the udder, around genitalia and inner aspect of thighs. Flies were dark brown in color measuring 9 x 4.5- 10.5 x 5.0 mm. Their abdominal segmentation was indistinct. Wings were longer than body length while wing veins crowded towards the anterior border. Flies had three pair of feet is provided with strong claws (fig: 1). Five diseased buffaloes were infested also with sucking lice identified as *H. eur.* having a relatively short head and broad thorax and

abdomen measuring about 4 x 2 – 5 x 2.5 mm (fig: 2).





Fig. (1) **Fig. (2)**

Out of 8 full mature *H. equina* larvae creamy in color, oval shape measuring 1.5 x 2.5 mm provided with a small spine posteriorly, 5 pupated (6-10) hours (fig: 3). Pupation period was 30 days where the pupa is broadly oval with two round postero-lateral spiracular lobes producing a full mature fly. Pupa was yellowish in color measuring 4.0 x 2.5- 4.5 x 3 mm. It was soft and covered with sticky layer but at 24 hours later, it became dark red to black in color and quit hard (fig: 4).

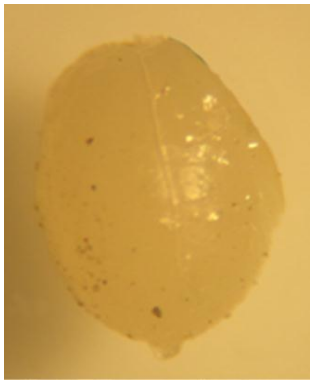




Fig. (3) **Fig. (4)**

Bacteriological investigation results are tabulated in tables (1 & 2).

Table (1): Nitrate reduction (NR) test of *C. ps.* (C. ps.) isolates.

Source of bacterial isolation	No.	*C. ps. equi*		*C. ps. ovis*	
Skin lesion samples	35	24	68.5%	11	31.5%
H. equina flies	13	10	76.9%	3	23.1%
Pupae	2	2	100%	0	0%
Total	50	36	72%	14	28%

Table (2): Bacterial species isolated from buffaloe OSD and hosted blood sucking insects.

Bacterial isolate species	Lesion exudate Samples	*H.equina* buffaloe hosted		*H. eurysternus* buffaloe hosted		Pupae lab. deposited	*H.equina* Lab. Developed	
+ve bacterial isolation	40	EBS	IBS	EBS	IBS		EBS	IBS
C. ps.	32	2	6	-	-	2	-	-
C. ps. + S. epid.	3	-	-	-	-	-	-	-
C. ps. + Anthr.	-	1	2	-	-	-	1	1
S. epid.	5	-	-	-	-	-	-	-
S. sapr.	-	-	2	-	3	-	-	-
Anthr. spp.	-	19	12	9	7	-	-	-
S.sap. + Anthr.	-	-	-	11	10	-	-	-
-ve bacterial isolation	4							
Total	44	22		20		2	1	

Discussion

OSD appears mainly among buffaloes and occasionally cattle in Egypt confined to Lower Egypt as a result of the suitable climatic conditions(Selim 2001, Mohamad and Reda 2015), especially during late spring and early summer(Yeruham et al 1997, Mohamad and Reda 2015, Sokół and Michalski 2015) correlating with *H. equina* breeding season(Barakat et al 1985, Selim 2001, Syame et al 2008, Sokół and Michalski 2015) and lesions are associated with its predilection seats of infestation(Selim 2001,) (hairless areas as axilla and groin(Syame et al 2008), inner aspect of limbs and under tail) (Selim 2001,).

Basing on nitrate reduction test, the identification of bacterial isolates all over the study revealed that both *C. ps.* biovars were recovered as *C. ps. Equi* and *C. ps. Ovis* represented 72 & 28 %, while from buffaloe lesions resembled 68.5 & 31.5% respectively (Table 1). Some studies stated that OSD is associated only with *C. ps. Equi*(Selim 2001, Selim et al 2016, Viana et al 2017), while others detected both *C. ps.* biovars as causative agents in cattle ulcerative lymphangitis(Yeruham et.al.,1997 Yeruham et.al., 2003, Yeruham et.al., 2004). *C. ps.* transmission among buffaloe is a conflicting issue since some studies concluded that it is only mechanical by insects(Khalel et al., 1995; Zaghawa and El-Gharib, 1996; Ali and Zaitoun, 1999, Doherr et al., 1999) mainly blood sucking insects - as mentioned above(Khater et al., 1983; Barakat et al., Selim 2001, 1985 Soares et al 2013) - as having a piercing mouthparts that can penetrate the thick buffaloe skin. These conditions coordinates with *H. equina* features which remain for long periods on their hosts and are not easily disturbed in addition it has very long mouthparts are adapted for piercing thick skin (Selim 2001). In the present study, out of 44 affected buffaloe cows, 43 showed closed (edematous or nodular) lesions avoiding suggestion of mechanical transmission unless through piercing the whole skin thickness to contract the pathogens from the infected subcutaneous tissues, the condition presented only related to *H. equine*(Selim 2001) when be with contaminated piercing mouth parts. Even, from the blood sucking *H. eur.* lice with piercing mouth part just a vessel feeder could not reach to the infected subcutaneous tissue(Roberts and Janovy 1996), the present study failed to isolate any *C. ps.* strain from *H. eurysternus* lice infesting the infected buffaloes in different locations (Table 1) suggesting that it cannot act as a mechanical or biological vector

for *C. ps*. These finding concluded that not any blood sucking insect has role in transmission, but among blood sucking insects, it is associated with *H. equina*(Selim 2001, Ghoneim et al 2001). *Musca domestica* (house flies) with mouth part adapted only for a liquid diet not to pierce host skin(Kettle 1990) have been confirmed as potential vectors for *C. ps. equi* among horse(Yeruham et al., 2003 and Spier et al., 2004) or cattle(Abou-Zaid and Hammam, 1994; Sayed, 2001) with ulcerative lesions mechanically. *C. ps. equi* survival inside the fly's gut experimentally - on feeding house flies on *C. ps. equi* broth - revealed that the pathogen presented in fly droppings for only up to 4 h and in saliva up to 3 h post infection(Yeruham et al 1996). Some studies investigated the existence of the *C. ps. equi* inside *Musca domestica* by PCR detection of its phospholipase D (PLD) exotoxin gene(Spier et al 2004, Barba 2015) with great disadvantage that detection of PLD did not inform about the viability of pathogens. In the present study bacterial isolation of *C. ps.* biovar *equi* from all *H. equine* life stages (adult flies, their pupae either gathered or lab deposited as well as the second generation flies) viable up to 30 days post collection ascertained that there is endosymbiosis nature of *C.ps*. limited only to *H. equina* fly which can transmit *C.ps*. vertically.

The study concluded that both *C. ps.* biovars (*equi* & ovis) could be isolated from buffaloe OSD lesions. Its transmission is associated only to *H. equina* fly, the mechanical and biological vector for buffaloe OSD, since it is proved that there is endosymbiosis nature of *C.ps*. limited only to *H. equina* fly which can transmit *C.ps*. vertically.

References

Abdel-Latif, M., M (2011): Studies on some bacterial causes associated with oedematous skin disease in buffaloes in Dakahlia Governorate. Assiut Vet. Med J. vol 57 No 128 , pp 163-172.

Abou-Zaid, A.A. and Hammam, H.M. (1994): Studies on some skin affections in cattle, 2- ulcerative lymphangitis. 6th Sci. Cong. 20-22 Nov. 1994, Fact. Vet. Med. Assiut. Egypt.

Ali, H.S. and Zaitoun, A.M. (1999): Studies on cutaneous suppurative lymphangitis in buffaloes at Assiut Governorate. Egypt. Assiut Vet. Med. J.(41) 81:208-222.

Almeida S, Dorneles EMS, Diniz C, Abreu V, Sousa C, Alves J, Carneiro A, Bagano P, Spier S, Barh D, Lage AP, Figueiredo H, Azevedo V (2017): Quadruplex PCR assay for identification of *Corynebacterium pseudotuberculosis* differentiating biovar *Ovis* and *Equi*. BMC Vet Res. 2017 Sep 25;13(1):290. doi: 10.1186/s12917-017-1210-5.

Baraka, A.A.M. (1983): Some morphobiological studies on Gastrophilus species infesting donkeys in Egypt. M. Sc. Faculty, Vet. Med. Cairo, Unive.

Barakat, A.A; Osman, R.M.; Afifi, E.A.; Gad, A.S. and Shouman, M.T. (1985): Occurrence of bovine oedematous skin disease in different provinces. J. Egypt Med. Assiut. 44, 225-231.

Doherr, M.G.; Carpenter, T.E.; Wilson, W.D. and Gaadnter, I.A. (1999): Evaluation of temporal and spatial dustening of horses with *C.pseudotuberculosis* infection.Am. J. Vet. Res. 60 (3): 284-291.

Ghoneim, M.A.; Mousa, W.M.; Ibrahim, A.K.; Amin, A.S.; Khafagy, A and Selim, S.A (2001): Role of *Hippobosca equina* as a transmitter of *C. pseudotuberculosis* among buffaloes by PCR and Dot blot hydridization.

Kettle, D.S. (1990): Muscidae (House flies, Stable flies). In Medical and Veterinary Entomology; 223–240.

Khalel, N. G.; Seddek, S. R. and Nashed, S.M. (1995): Studies on ulcerative lymphangitis in buffaloes in Assiut. Assiut Vet. Med. J. 33 (65): 93-99.

Khater, A. r. ;Deeb,S. ;Bayoumi,A.H. and Salem,H. (1983): Expermintal infection with *Corynebacterium pseudotuberculosis* (ovis). II Pathological changes in cattle. Assiut Vet. Med. J. 11(21): 85- 89.

M. Barba, A.J. Stewart, T. Passler, A.A. Wooldridge, E. van Santen, M.F. Chamorro, R.C. Cattley, T. Hathcock, J.A. Hogsette, and X.P. Hu (2015): Experimental Transmission of *Corynebacterium pseudotuberculosis* Biovar equi in Horses by House Flies. J Vet Intern Med 2015;29:636–643

Mohamed S.and Reda L (2015): Oedematous skin Disease in Buffaloes and Cows. *Egypt. J. Vet. Sci.* Vol. 45-46, pp. 1- 10

Moussa IM, Ali MS, Hessain AM, Kabli SA, Hemeg HA, Selim SA (2016): Vaccination against *Corynebacterium pseudotuberculosis* infections controlling caseous lymphadenitis (CLA) and oedematous skin disease. Saudi J Biol Sci. 2016 Nov;23(6):718-723. Epub 2016 Jun 17.

Oliveira A, Teixeira P, Azevedo M, Jamal SB, Tiwari S, Almeida S, Silva A, Barh D, Dorneles EM, Haas DJ, Heinemann MB, Ghosh P, Lage AP, Figueiredo H, Ferreira RS, Azevedo V (2016): *Corynebacterium pseudotuberculosis* may be under anagenesis and biovar *Equi* forms biovar *Ovis*: a phylogenic inference from sequence and structural analysis. BMC Microbiol. 2016 Jun 2;16:100. doi: 10.1186/s12866-016-0717-4.

Quinn, PJ; M; Markey, BK; Leonard, FC; Patrick, ES; Fanning, S; Hartigan, PJ (2011): Clinical vet. Microbiology, 2nd edition, pp. 548 – 564, published by Wiley-Blackwell, A John & Sores Ltd. publications.

Roberts, L. and Janovy. J. (1996): Foundations on Parasitology .Sixth Edition. Boston: McGraw-Hill Higher Education.

Sayed, A.M. (2001): *Corynebacterium pseudotuberculosis* in cattle skin oedematous disease in Assiut Governorate. Assiut Vet. Med. J. 45 (90): 264-273.

Selim SA, Mohamed FH, Hessain AM, Moussa IM (2016): Immunological characterization of diphtheria toxin recovered from *Corynebacterium pseudotuberculosis*. Saudi J Biol Sci. 2016 Mar;23(2):282-7. doi: 10.1016/j.sjbs.2015.11.004. Epub 2015 Nov 23.

Selim, S.A. (2001): Oedematous skin disease of buffaloe in Egypt. J. Vet. Med. B. 48, 241-258.

Soares SC, Silva A, Trost E, Blom J, Ramos R, Carneiro A, et al.(2013): The Pan-Genome of the Animal Pathogen *Corynebacterium pseudotuberculosis* Reveals Differences in Genome Plasticity between the Biovar ovis and equi Strains. PLoS One. 2013; 8.

Sokół R1, Michalski MM (2015): Occurrence of *Hippobosca equina* in Polish primitive horses during the grazing season. Ann Parasitol. 2015;61(2):119-24.

Soulsby, E.J.L .(1982): Helminth, Arthropod and Protozoa of Domesticated Animals. 7th edn., Bailliere. Tindall, London 809p.

Spier, S. J.; Leutenegger, C. M.; Carroll, S. P.; Loye, J.E.; Pusterla, J. B.; Carpenter, T.E.; Miholyi, J.E. and Madigan J.E. (2004): Use of real-time polymerase chain reaction-based fluorogenic 5` nuclease assay to evaluate insect vectors of *C. pseudotuberculosis* infections in horses. Am. J. Vet Res. 65(6): 829-834.

Syame S., M, EL-Hewairy H.M.and Selim S.A (2008).: Protection of Buffaloes Against Oedematous Skin Disease by Recombinent-bacterin and Toxoid-bacterin Vaccines . Global Veterinaria 2 (4): 151-156, 2008, ISSN 1992-6197, IDOSI Publications, 2008.

Viana MVC, Figueiredo H, Ramos R, Guimarães LC, Pereira FL, Dorella FA, Selim SAK, Salaheldean M, Silva A, Wattam AR, Azevedo V (2017): Comparative genomic analysis between *Corynebacterium pseudotuberculosis* strains isolated from buffalo. PLoS One. 2017 Apr 26;12(4):e0176347. doi: 10.1371/journal.pone.0176347. eCollection 2017

Yeruham I, Braverman Y, Shpigel NY, Chizov-Ginzburg A, Saran A, Winkler M. (1996): Mastitis in dairy cattle caused by *Corynebacterium pseudotuberculosis* and the feasibility of transmission by houseflies. Vet Q. 1996 Sep;18(3):87-9.

Yeruham, I.; Elad, D. ;Perl, S. and Ram, A. (2003): A necrotic and ulcerative dermatitis of heels of heifers in a dairy herds infected with *C.pseudotuberculosis*. vet. Record, 152(19): 598-600.

Yeruham, I.; Elad, D.; Van-Ham, M.; Shpigel, N. Y. and Perl, S. (1997): *Corynebacterium pseudotuberculosis* infection in Israeli cattle: clinical and epidemiological studies. Vet. Record, 140, Issue 16, 423-427.

Yeruham, I; Friedman, S; Perl, S; Elad, D.; Barkovich, Y. and Kalgard, Y. (2004): A herd level analysis of *Corynebacterium pseudotuberculosis* outbreak in dairy cattle herd. Vet. Dermatology, 15, 315-320.

Zaghawa, A.A. and El-Gharib, S.A. (1996): An outbreak of oedematous skin disease in Alexandria during 1994: clinical investigations and assessment of epidemiological parameters. 7th sci. cong. 17- 19 Nov. 1996, Fact. Vet. Med., Assiut, Egypt.