Animal Health Research Institute (AHRI).
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Field study on buffaloe oedematous skin disease in Assiut Gavanorate: a model study.

دراسة حقلية لمرض التهاب الجلد الاوديمى فى الجاموس فى محافظة أسيوط : نموذج دراسة.

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الملخص العربي

نظراً لظهور مرض الجلد الأوديمي في الجاموس في صورة متكررة وأصبح متوطن في مصر هدف البحث إلي دراسة حقلية للمرض في منطقة محدودة. واشتملت الدراسة علي ٤٤ جاموسة مصابة بمرض الجلد الأوديمي. لوحظ شكلين للصورة الإكلينيكية للمرض: الأوديمي (٩٥.٥%) والعقدي (٤.٥%) بينما لم تسجل أي حالات للصورة المتقرحة. وقد كانت الأجزاء الأمامية للجسم أكثر عرضة للإصابة (٧٩.٥%). تم عزل ٣٥ عترة من الميكروب المسبب للمرض (كوريني سودوتيبركلوزيس) وقد كان في صورة منفردة في ٣٢ حالة (٨٠ %) وفي صورة مشتركة مع العنقودي إبيدرميدس في ٣ حالات (٧.٥%) .

أظهرت ٢٤ عترة (٦٨.٦%) نتائج إيجابية لاختبار اختزال النيترات و كانت من النوع سيروتيب I بينما الإحدى عشر عترات الأخرى(٣١.٤%) كانت من نوع سيروتيب II . كلا النوعين تم عزلهما من ذبابة الهيبوبوسيكا إكوينا بينما تم عزل سيروتيب I من كل من الشرنقة والجيل الثاني من الذبابة السابق ذكرها.

وقد اثبتت اهذه الدراسة ان ذبابة الهيبوبوسيكا اكوينا هى المسؤل عن نقل هذا المرض حيث تم عزل الميكروب المسبب له من كل من السطح الخارجى للذبابة والمحتويات الداخلية لها. وكذلك من الشرنقة والجيل الثانى للحشرة والذى تم تربيته معمليا بينما لم يتم عزل هذا الميكروب من القمل الماص للدم والموجود على الجاموس المصاب بالمرض . هذا يدل على ان طبيعة التعايش الداخلى لميكروب الكوريني سودوتيبركلوزيس قاصراعلى ذبابة الهيبوبوسيكا اكوينا .

واظهرت نتائج اختبار الحساسية للمضادات الحيوية ان جميع العترات المعزولة اعطت ١٠٠% ايجابية لكل من التوبراميسين والجنتاميسين والسيبروفلوكساسين، يليها الاوكسيتتراسيكلين بنسبة ٨٤%. جميع العترات المعزولة اظهرت مقاومة لكل من البنسيلين و الامبسيلين والكلوكساسيلين.

وبالعلاج باستخدام المضادات الحيوية طبقا لاختبارات الحساسية وحقن مضادات الهستامين فضلا عن مكافحة الطفيليات الخارجية امكن الوصول الى نسب شفاء ٩٧.٧%.

Summary

As the buffaloe oedematous skin disease (OSD) became an endemic disease in Egypt, the present investigation objected to approach the subject in a localized district as a field study. Through a village clinic, 44 buffaloe cows suffering from OSD were included in the study. The disease was observed in two clinical forms, oedematous (95.5 %) and nodular (4.5 %), where ulcerative form was not recorded. Anterior parts of the body were mostly affected (79.5 %). *Corynebacterium pseudotuberculosis* was the causative agent of the disease. It was isolated from

aspirated exudates as single infection from 32 (80.0%) and as mixed infection with *Staphylococcus epidermidi*s from 3 (7.5 %). Twenty four (68.6 %) of these isolates showed nitrate reduction positive reactions (serotype I), while the other 11 strains (31.4 %) were nitrate reduction negative (serotype II). Both serotypes were recovered from adult *Hippobosca equina* flies. Strains of serotype I were isolated from either pupae or laboratory developed fly.

The present study proved the sole role of *H.equina* fly in disease transmission. *C.pseudotuberculosis* was isolated from external body surface, internal body content of the fly, pupae and the second generation. Failure of its isolation from blood sucking lice confirmed that the endosymbiotic nature of *C.pseudotuberculosis* was limited to *H.equina* fly.

Antibiogram of the isolated bacteria revealed their sensitivity 100 % for Tobrarmycin, Gentamycin and Ciprofluxacin followed by Oxytetracycline (84 %). All bacterial isolates showed resistance against Penicillin, Ampicillin and Cloxacillin. Treatment regimen basing on antibiotic, antihistaminic administration and ectoparasitic eradication achieved recovery rate of 97.72 %.

Introduction

Oedematus skin disease (OSD) in buffaloes nowadays becomes an endemic disease in Egypt (Selim, 2001). It was fully studied and discussed through several investigations in different governorates which established that the etiological agent was *Corynebacterium pseudotuberculosis* (Soliman *et.al*.,1963; Fouad *et.al*.,1972; Ibrahim *et.al*.,1983; Barakat *et.al*.,1984; Esmat,1984; Mostafa,1984; Hassan,1988; Zaghawa and El-Gharib,1996; Ghoneim *et.al*.,1999 and Ghoneim *et.al*., 2001 and others). However reports on occurrence of the disease in Assiut governorate still apparently brief (khalel *et.al*.,1995; Ali & Zaitoun, 1999 and Sayed, 2001). Antigenically *C.pseudo*. has two serotypes. Serotype I causes caseous lymphadenitis in sheep, while serotype II causes abscesses in horse and OSD in buffaloes (Barakat *et.al*.,1985). Both serotypes may infect cattle (Yeruham *et.al*.,1997 and Yeruham *et.al*., 2003). Oedematous skin disease has 3 clinical forms; oedematous, nodular and ulcerative (Al-Gaabry & Ammar, 1999).

Several authors suggested that the route of transmission of buffaloe Oedematous Skin Disease is through the mechanical way only either by contamination of external environment (soil & water) with *C. pseudo*. or by external parasites (Khater *et.al*., 1983; Barakat *et.al*., 1984; Khalil *et al*., 1995; Sayed, 2001 and Spier *et.al*, 2004). However, the entry of the host into cattle is not well documented (Yeruham *et.al*., 2004).

The disease appeared in some villages of Assiut governorate as an epidemic every 3-4 years. During May-August 2006 cases were recorded in El-Fayama village 15 km East Assiut city.

The aim of the present study is to record the occurrence, clinical manifestations and the role of ectoparasites in transmission of disease with trials for treatment of affected cases as a field study.

Material and Methods

Animals: Through a private clinic in Fayama village – previously mentioned – the study conducted on 44 buffaloes cows (11 pregnant and 33 non pregnant) aging 3-9 years showing oedematus skin lesions during the period May – August.2006.

Bacteriological sample collection: Lesion form, its body distribution and physical appearance of its aspirated exudates was recorded and tabulated. Forty three closed and one opened – which spontaneously closed – localized or diffuse, cutaneous or lymph node were observed. Every lesion was aspirated aseptically for bacterial isolation. Aspirated exudates specimens were inoculated into nutrient broth media overnight and incubated aerobically at 37ºC, then were streaked onto 10% sheep blood agar (24 - 48 hrs`).

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 according to Quinn *et.al*., (1994).

For the parasitological investigation: Forty adult flies and 30 lice were collected directly by hand or using forceps from infected buffaloes. They were kept into plastic sacs or wide-naked bottles for laboratory examination where they taxonomically

identified under binocular microscope according to their morphological characters (Soulsby, 1982 and Kettle, 1984).

Some collected flies were still alive for 24 hours until deposited their larvae (Full mature larvae) inside the collected sacs, larvae pupated rapidly. The achieved pupae were incubated at room temperature in plastic containers containing sand and covered with a piece of gauze until giving adult fly (Baraka, 1983). Different stages of collected external parasites were measured and photomicrographed.

Bacterialogical examination of Blood sucking ectoparasites: In parallel to samples for bacteriological study twenty two flies and were gathered in sterile test tubes from infested animals (17 affected buffaloes, 4 cattles and a donkey) in addition to one laboratory developed fly for bacterial existence was also examined as follow: fly was inoculated as it is into sterile nutrient broth tube for isolation of body surface, legs and external mouth parts contamination. Then a forceps catched fly was washed several times using sterile distilled water. The washed fly was crushed, destructed and macerated into another nutrient broth tube for isolation of gut bacterial content. Also twenty lice from only affected buffaloes were managed in the same technique to obtain their external and internal bacterial contents.

From the laboratory deposited pupae, 2 were burst into nutrient broth to isolate their bacterial contents.

In vitro antimicrobial susceptibility testing: of the lesion isolated strains was carried out according to Bauer *et al*, (1966) using disc diffusion technique against: Amekin (30 µg), Ampicillin (10 µg), Cloxacillin (10 µg), Ciprofluxacin (5µg), Ggentamycin (10 µg), Oxytetracycline (30 µg) penicillin (10 IU), Streptomycin (10 µg) and Tobramycin (10 µg). [Bioanalyse]

Treatment regimens were applied as the cases indicated.

Results

The obtained bacteriological results were tabulated in tables 1-5 and plates 1& 2.

Parasitological findings:

In the present study, all diseased buffaloes were infested with dark leathery flies identified as *Hippobosca 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 feets provided with strong claws (plate 3 fig: 1).

Out of 8 full mature larva, 5 pupated (6-10) hours. The larva is creamy in color, oval shape measuring 1.5 x 2.5 mm. It was provided with a small spine posteriorly (plate 3 fig: 2). Pupation period was 30 days where the pupa is broadly oval with two round postero-lateral spiracular lobes. 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 (plate3 fig: 3).

Five diseased animals were infested also with sucking lice identified as *Haematopinus eurysternus*. The sucking louse has a relatively short head and broad thorax and abdomen. Its measuring was 4 x 2 – 5 x 2.5 mm. (plate3 fig: 4).

Discussion

Retrospectively, OSD appeared approximately in epidemic cycle with an interval of three years during which the disease appeared sporadically (Al-Gaebary and Ammar, 1999). Barakat *et.al.,* (1985) recorded five outbreaks of bovine OSD in Egypt mostly in buffaloes. Analysis of previous data revealed that the seasonal pattern of the disease begins as sporadic cases reaching the maximum in summer months (May – July) followed by gradual receding in winter (Fouad *et.al.,* 1974).

As mentioned in the above premise, the disease is caused by *C.pseudotuberculosis*, the dermonecrotic effect is attributed to its phospholipidase exotoxin- vascular toxin- with permeability factor (Afzal *et al.,* 1996).

Oedema begins as an inflammatory oedema up to surrounding tissue necrosis (Selim, 2001). Consequently three clinical forms were recorded; oedematus, nodular and ulcerative (Al- Gaebary and Ammar, 1999).

The present findings revealed that affected buffaloes showed forty two (95.5%) oedematus and two (4.5%) nodular forms (table 1). A lesion in multiple nodular case was ruptured seemed to be ulcerative form (plate 1, fig. d). Oedematus

(diffuse) form may be only skin lesions involved. Associated lesion appeared also in prescapular lymph node (plate 1fig: a& b). Anterior parts of the body were mostly affected (79.5%) more than posterior ones (20.5%) (Table 1). Ali and Zaitoun (1999) achieved that lesions related to the anterior parts of the body resembled 80.7%.

The site of OSD correlated with the feeding habitat of *H. equina* flies. Such flies attach themselves to the inner sides of limbs, belly and neck (Hafez and Hilali, 1978). *C.pseudotuberculosis* was isolated from 35 (87.4%) lesion samples (table2). Abou-Zaid and Hammam (1994), Khalil *et.al.*, (1995), Zaghawa and El-Gharib (1996), Ali and Zaitoun (1999) and Sayed (2001) detected *C. pseudotuberculosis* in 100, 83.3, 88.2, 94.5 and 100% of the infected buffaloes, respectively.

It is well documented that *C. pseudotuberculosis* showed polymorphism of isolates recovered from different animal species allover the world (Brown and Olander, 1987; Claus and Rikisa, 1991 and Sutherland *et al.*, 1996). *C. pseudotuberculosis* was grouped in two different biotypes (serotypes) which differ from each other biochemically, serologically and epidemiologically. The most common feature of variation is the ability of serotype I to reduce nitrate to nitrite and the failure of serotypeII to do that (Shpigel *et al.*, 1993). Barakat *et al.*, (1984) reported that serotype I infects only sheep and goats causing caseous lymphadenitis but not be known to infect any of other animals. Later on, Shpigel *et al.*, (1993) isolated it from cutaneous lesion in cattle. Yeruham *et al.*, (2003) isolated serotype II from cutaneous lesions in cattle. Both serotypes were isolated in the same time from cattle ulcerative dermatitis (Yeruham *et al.*, 2004). So it is obvious that *C.pseudotuberculosis* serotypes are not host specific pathogens. Through the present study-biochemically-both *C.pseudoyuberculosis* serotypes were recovered from OSD lesions and *H. equina* flies (table3).

Regarding disease transmission, all available literature suggested only mechanical way either by direct contact between uninfected animals with infected ones or contaminated soil or water sources (Zaghawa and El-Gharib, 1994; Khalil *et al.*, 1995; Ali and Zaitoun, 1999 and Doherr *et al.*, 1999).

Direct contact of the uninfected animals with contaminated external parasites (mechanically) either by *Musca domestica* or *H. equina* was stated by many authers (Hafez and Hilali, 1978; Addo, 1983; Abou-Zaid and Hammam, 1994; Sayed, 2001; Yeruham *et al.*, 2003 and Spier *et al.*, 2004).

These suggestions could be accepted when lesions (source of infection) were opened in case of ulcerative form, but these contaminations cannot invade intact skin unless the insects were blood suckling.

In the present work, *C. pseudotuberculosis* strains were isolated from 11(50 %) animal hosted *H. equina* flies (table 2) after 24 hours post gathering, also from two pupae after 24-48 hours and finally from the lab developed fly 30 day post pupa incubation (vertical transmission).

The successful positive isolation from all *H. equina* developmental stages, while it could not be isolated from all lice already infested the diseased buffaloes - in spite of both insects have mouth parts adapted for sucking blood - explained the symbiotic association between *C. pseudotuberculosis* and *H .equina*. Achieved results declared the endosymbiotic nature of *C. pseudotuberculosis* is limited to *H. equina* fly only. On other hand all infected cases were detected in summer season which correlated with the peak of population of *H. equina*(Hafez, *et al*., 1979) in addition to failure of isolation of *C. pseudotuberculosis* from lice. All these factors are confirmed the sole role of *H. equina* as a main vector of transmission of OSD.

Sensitivity tests of isolated strains:

The present antimicrobial susceptibility testing showed that all tested *C.pseudotuberculosis* strains were highly sensitive to tobramycin, gentamycin and ciprofloxacin (100%) then oxytetracyclin with 80% (table 4). High sensitivity of gentamycin followed by oxytetracyclin was recorded by Abou-Zaid and Hamam (1994), Khalil *et al*., (1995), Ali and Zaitoun (1999) and Sayed (2001).

Treatment in the present investigation was designed on three bases; the firstly was the onset use of the antibiotic of choice based on antimicrobial susceptibility testing for five successive days. The secondly was suppression and oedema inhibition through I/M administration of corticosteroids. Finally *Hippobosca equina* control

through S/C injection of all infected animals with ivermectin (table 5). All in parallel ways, all stabled animals were atomized externally with diazinone (1%) three successive times with ten days intervals to fight all parasite developmental stages. Stables walls particularly their cracks were atomized with 1% diazinone also three times as mentioned above.

Through the present study, treatment of OSD was carried out as shown in (table 5). It was beneficial in case it act as enlarged suprascapular lymph node to be surgically excised in order to reduce the infection and their toxins which have dermonecrotic effect (plate2, fig a-c). Surgical interference was previously recommended by Khalil *et al.,* (1995), but it be prohibited in early stages except in case of secondary invasion with pyogenic micro-organisms (Selim, 2001).

Owining to application of this treatment regimen, recovery rate achieved was 97.72 % (table 5) at three weeks post administration. Great success in treatment would be obtained when be designed to remove *C.pseudotuberculosis* from tissues using antibiotics and inhibit oedema by hydrocortisone (Sahar, 1998 and Selim, 2001). Al-Gaabary and Ammar (1999) concluded recovery rate of 80 & 90% for the two treated groups three weeks but later reached 100 % for both by the fifth week.

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(Table 1) body distribution and forms of skin lesions in buffaloes OSD.

Clinical findings of OSD lesions			no	%
Site of body lesions	Anterior part of the body no. 35 (79.5%)	Neck ----------	3	6.8
		Chest ----------	8	18.2
		Sternum ---------	4	9.1
		Shoulder --------	4	9.1
		Shoulder + LN.	2	4.5
		Prescapular LN.	1	2.2
		Fore limbs ------	13	29.5
	Posterior part of the body no. 9 (20.5%)	Back ----------	3	6.8
		Hind limbs ------	6	13.6
Skin lesion forms	Oedematus ----------		42	95.5
	Nodular ----------		2	4.5
	Ulcerative ----------		0	0
Physical appearance of aspirated samples	Serous fluid ----------		1	2.2
	Bloody up to pus tinged w blood pure		35	79.5
	creamy pus ----------		8	18.2

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

Bacterial isolate species	Lesion aspirated samples no.(44)		H .e animal hosted no. (22)				Pupa lab. deposited no. (2)		H .e lab. developed no.(1)				Lice buffalo hosted no.(20)			
			B .S		I.B.C				B .S		I.B.C		B .S		I.B.C	
	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
Positive bacterial isolation	40	90.9														
C. pseudo.----------	32	80.0	2	9.1	6	27.3	2	100	0	0	-	0	-	0	-	0
C. pseudo. + st. epid -----	3	7.5	-	0	-	0	-	0	0	0	-	0	-	0	-	0
C. pseudo. + Anthr.------	-	0	1	4.5	2	9.1	-	0	0	100	1	100	-	0	-	0
Staph. epid.----------	5	12.5	-	0	0		-	0	1	0	-	0	-	0	-	0
Staph. sap.----------	-	0	-	0	2	9.1	-	0	0	0	-	0	-	0	3	15
Anthracoids. Sp.----------	-	0	19	86.4	12	54.5	-	0	0	0	-	0	9	45	7	35
Staph.Sap.+ Anthracoids	-	0	-	0	-	0	-	0	0	0	-	0	11	55	10	50
Negative Bacterial isolation	4	9.1	-	0	-	0	-	0	0	0	-	0	-	0	-	0
Total	44		22				2		1				20			

H .e: *Hippobosca equina* B.S: External body surface I.B.C.: Internal body content

(Table 3): Nitrate reduction (NR) test of *C.pseudotuberculosis* isolates.

Source of isolation	No.	NR +ve	NR -ve
Skin lesion samples	35	24	11
H. equina flies	13	10	3
pupae	2	2	0
Total	50	36	14

(Table 4): Antimicrobial susceptibility testing of bacterial isolates recovered from OSD in buffaloes.

Bacterial isolated species	Tob. 10 µg.		Amk. 30 µg.		GN. 10 µg		CIP. 5 µg.		S. 10 µg.		CX. 1 µg.		Amp. 10 µg.		P. 10 µg.		T. 30 µg.	
	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
C. pseudotuberculosis (25)	25	100	20	80	25	100	25	100	13	52	2	8	2	8	5	20	21	84
Staph. epidermidis (5)	5	100	4	80	4	80	5	100	4	80	-	0	-	0	-	0	5	100

Tob: Tobramycin CIP: Ciprofluxacin Amp: Ampicillin

Amk: Amekin S: Streptomycin P: Penicillin

GN: Ggentamycin CX: Cloxacillin T: Oxytetracycline

(Table 5): Treatment of buffaloes oedematous skin disease and recovery rate.

Clinical findings	No.	treatment	Rec. rate
Sever oedematus lesions (advanced)	6	Ciprofloxacin, flunixin and ivermectin	100%
Moderate diffuse oedema (plate 1 fig: a)	36	Oxytetracycline, phenylbutazone and ivermectin	100%
Single closed enlarged firm prescapular lymph node (plate 2 fig: a)	1	Surgically excised & administration of ciprofloxacin, diphenohydramine HCL and ivermectin.	100%
Advanced oedematous wide lesions associated with haemoglobinurea	1	Fluid therapy, oxytetracycline, pheylbutazone and ivermectin	0% (dead)
Total	44		97.72%

Ciprofloxacin: Cipro. I/M. 1cc/ 40 kg b.wt. 3-5 days.

Oxytetracycline: L.A terramycin deep I/M. 1cc /10 kg b.wt/ 48 hrs 2 doses..

Flunixine: finadyne, I/V 2 ml/50kg b. wt. (Schering-plough veterinarie- france).

Phenylbutazone: buta-fenil slow I/V, 1-2 gm / animal. (laboratories jornel, S.A.- Mixico).

Diphenohydramine HCL: allergamine I/V 1mg/kg b.wt. (Aveco-jordan).

Fluid therapy: sodium hydroxide 0.9% slow I/V infusion (ADWIC-Egypt).

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