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STUDIES ON CUTANEOUS SUPPURATIVE LYMPHANGITIS IN BUFFALOES AT ASSIUT GOVERNORATE-EGYPT

(With 5 Tables and 4 Figures)

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دراسات عن التهاب الجلد التقيحي في الجاموس بمحافظة أسيوط-مصر

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في هذا البحث تم إجراء الفحوصات الإكلينيكية على عدد ٧٥٠ حالة من الجاموس فحصت في مناطق مختلفة في محافظة أسيوط وكان من بينهم ٥٦ حالة (٧,٤٧%) مصابة بعقد جلدية منتشرة على مناطق مختلفة لجسم الحيوان المصاب وعلى طول الأوعية الليمفاوية للحيوانات المريضة. وكان معظم العقد الجلدية من النوع التقيحي كما أظهرت النتائج أيضا أن عدد ٢٧ (٤٨,٢١%) حالة من الحيوانات المصابة كانت تعانى من ورم في الغدد الليمفاوية وخصوصا الغدة الليمفاوية الأمامية للكتف فكانت متورمة. وأظهرت نتائج عينات الفحص البكتريولوجى مدعمة بالفحوصات البيوكيميائية بأن ميكروب الكورينى أوفيز كان منفردا في عدد ٦٧ عينة (٧٨,٨٢%) وكان مشترك مع ميكروب العنقودي الذهبي في عدد ١٣ عينة (١٥,٢٩%). وقد يشير هذا إلي أن ميكروب الكورينى أوفيز هو الميكروب المسئول عن ظهور العقد الجلدية الصديدية على جلد الحيوانات المصابة. وكانت أغلب الحالات المصابة بهذا المرض في الأشهر الحارة من السنة (يونيو حتى أغسطس) وكانت الفروق معنوية جدا بين فصول السنة وتم ذكر الأسباب المحتملة التي أدت إلى ظهور المرض. وبعمل اختبارات الحساسية للميكروبات المعزولة وجد أن المضاد الحيوي سيفالوسبورينز هو أشدهم تأثيرا وفاعلية على أغلب الميكروبات المعزولة (بنسبة حساسية ٩١,٥%)، وقد أجريت محاولات علاجية لبعض الحيوانات المصابة في الحقل باستخدام المضادات الحيوية المختارة مع العلاج الجراحي وكانت النتائج إيجابية وفعالة بعد عشرة أيام في الحالات ذات العقد الجلدية الصغيرة وأيضا العقد الجلدية المغلقة والمفتوحة الصديدية ولم يعطى نتائج إيجابية في حالات الغدد الليمفاوية المتورمة الصديدية.

SUMMARY

Fifty-Six (7.47%) cases of the total clinically examined buffaloes (750) showed cutaneous lesions on the different parts of the infected animal body. Distribution of the skin lesions on different regions of the animal body were described. The predominant clinical findings of the infected buffaloes were various sized cutaneous nodular eruption along the lymphatic vessels particularly in the limbs with and without regional purulent lymphadenitis, and without marked systemic involvement. Most of these cutaneous nodules were filled with pussy material and varied in number from a few to many. Twenty-seven cases (48.21%) of the diseased buffaloes with cutaneous nodular lesions showed enlargement of the regional lymph nodes. Sixty-seven (78.82%) of the bacteriological samples yielded *Corynebacterium pseudotuberculosis* in pure culture and 13 (15.29%) of samples yielded *Corynebacterium pseudotuberculosis* associated with *Staphylococcus aureus*. *Corynebacterium pseudo-tuberculosis* was overwhelmingly prominent bacterial isolates suggesting that this isolated pathogen was responsible for the observed suppurative skin lesions of the diseased buffaloes. Regarding the influence of the seasonal variation on the prevalence of the disease, it was found that the peak of infection ($P < 0.01$) occurred during the hot-months (June, July and August). Results of the antibiotic sensitivity test showed that the cephalosporins had strong inhibitory effect (91.5% sensitivity) on the majority of the isolated strains. Therapeutic trials with the choice antibiotic were achieved and gave successful results particularly in infected buffaloes with closed small nodules. Surgical treatment with systemic suitable antibiotic was also effective in cases had cutaneous opened and closed suppurative nodules while in cases had marked purulent lymphadenitis, the therapeutic trials were not completely successful.

Key words: *Cutaneous, Suppurative, Lymphangitis in Buffaloes.*

INTRODUCTION

Buffaloes are most important animals for milk and beef production than cattle in Assiut Governorate (Annual Reports of the Veterinary Authorities 1992 and 1994). In that Governorate, hides production depends also on buffaloes beside cows. Consequently skin diseases of

buffaloes due to infectious and/or non-infectious agents were considered as one of the economic losses. In addition to these losses, the lower prices of the sales buffaloes with skin diseases (peculiar appearance) in the commercial markets should not be neglected. Reports on infectious skin diseases of buffaloes in Assiut Governorate are still apparently brief. Ulcerative lymphangitis of buffaloes caused by *Corynebacterium pseudotuberculosis* was recently reported in different localities of Assiut Governorate by Khalel et al. (1995). However, the authors focused principally on the isolation and identification of the causative agent, resistance and sensitivity of the isolated strains to some different members of antibiotics (in vitro study). They also added that *Streptococcus fecalis* (one isolate) was isolated from a buffalo with closed subcutaneous lesion filled with pussy material. This may refer to the pivotal role of the environmental microbial pollution surrounding the diseased buffaloes.

Corynebacterium pseudotuberculosis (ovis) was well known as a specific infectious agent responsible for caseous lymphadenitis in both sheep and goat (Quinn et al., 1994). This micro-organism was also encountered as a etiological agent of cutaneous ulcerative lymphangitis with and without regional lymph nodes involvement, and with and without marked systemic reactions in cattle (Shpigel et al., 1993 and Abou-Zaid and Hammam, 1994) and in horses (Gillespie and Timoney, 1981). However, Fouad et al. (1972) previously suggested that *Corynebacterium pseudotuberculosis* was secondary agent to the primary parasitic infestation. This may indicate that the affirmed etiologic agent of cutaneous ulcerative lymphangitis is still debatable. From bacteriological point of view, Biberstein et al. (1971) and Barakat et al. (1984) reported that *Corynebacterium pseudotuberculosis* organism was biochemically classified into two biotypes viz., nitrate reducing strain (nitrate positive) and non nitrate reducing strains (nitrate negative). The latter strain was frequently incriminated as a causative agent of ovine and caprine pseudotuberculosis while the former strain causes serious skin diseases in horses and cattle.

The aim of the present work was directed to study a): the clinical picture of buffaloes had grossly suppurative skin lesions, b): the probable etiological agent(s) responsible for such lesions, and c): therapeutic trails with choice drug(s) according to the results of antibiotic sensitivity test.

MATERIAL and METHODS

Animals:

During the period of investigation (Feb., 1997 - Marsh, 1998), a total number of 750 buffaloes at different villages of Assiut Governorate were clinically examined. The cases showed skin lesions were subjected to detailed clinical examinations. Owner's complaint and history of the disease including patient data and prior treatment were also taken and discussed. These buffaloes were at different ages from 6 months to 8 years and from both sexes.

Collection of the samples and culturing procedure:

The unopened cutaneous lesions of the clinically diseased buffaloes were surgically incised under complete aseptic conditions and the included material was swabbed. The collected swabs were directly immersed into sterile centrifuge tubes containing brain heart infusion broth (Difco) supplemented with 7 % sterile inactivated horse serum (Serum and Vaccines Institute, El-Agouza, Cairo). These tubes were incubated for 24 - 48 hours and were centrifuged 3000 rpm., and thereafter the sediments were platted onto brain heart infusion agar plus 10 % sheep blood and incubated for 24-48. The growing colonies, particularly the hemolytic ones, were purified and morphologically and biochemically identified according to the methods described by Quinn et al. (1994). Direct microscopic smears from the opened lesions were made and stained by Gram's stain. On the other side, the collected material from the incised lesions were also directly cultured onto Sabouraud's dextrose agar (Bio-Merieux) plates supplemented with chloramphenicol for mycological analysis.

Antibiotic sensitivity test and therapeutic trials:

The purified isolated strains were tested for the presence of different members of antibiotic discs (Oxoid) (Table 4) using culturing onto Muller and Hinton agar supplemented with 5 % sheep blood, and the inhibition zone was measured and interpreted according to Bauer et al. (1966). The choice antibiotic was used for field treatment of some clinically diseased buffaloes (Table 5). Supportive treatment including dextrose-saline intravenous infusions and vitamins AD_3E intramuscularly (Äderön-Hürön, Germany), were also applied to all treated animals (Table 5).

Statistical analysis:

The obtained data were statistically analyzed according to the methods described by Milton and Tosoku (1983).

RESULTS

Clinical findings:

Fifty-six (7.47 %) cases of the clinically examined buffaloes (750) showed cutaneous nodular lesions on the different parts of the animal body.

The observed skin lesions started as small cutaneous nodules. The arrangement of these nodules were either in a long beading chain along the course of the lymphatic vessels of the limbs particularly on the lower part of the hind-limbs (Fig. 1) or in irregular arrangement on the different parts (wide-spread) of the affected animals (Fig. 2).

The observed nodules were elevated semi-circular in shape similar to nipples, approximately 1 to 2 cm. in diameter, painless and firm. Thereafter, these nodules were gradually increased in sizes (like lemon size) till the point of rupture discharging thick sticky whitish creamy pussy material tinged with blood (Fig. 3) and became painful to touch. The surrounding subcutaneous tissues of the closed or the ruptured suppurative nodules were swollen and edematous. In some cases, such nippled nodules had tendency to coalesce with each other forming irregular suppurative mass. The affected animals refused to walk except under force with unwillingness to rise from their setting positions when they stand.

Twenty-seven cases (48.21%) of the diseased buffaloes with suppurative nodular lesions showed enlargement of the regional lymph nodes (like largest orange in size) (Fig. 4). By incision, these enlarged nodes were filled with odorless caseated pussy material tinged with blood and shreds of clotted blood.

The body temperature of the affected cases were 38.7 ± 0.9 °C, the pulse, and the respiratory rates were within normal range.

Distribution of the skin lesions:

One hundred twenty-six skin lesions were noticed on the skin of 56 clinically infected buffaloes (Table 1). The distribution of these lesions on the various regions of the affected animals were summarized and listed in Table 2.

Seasonal variations:

Results of the influence of the different seasons on distribution of the disease were summarized and tabulated in Table 3.

Bacteriological analysis:

Eighty-five closed cutaneous nodules from the clinically diseased cases (56) were selected, sampled and bacteriologically cultured. Sixty-seven (78.82 %) of the bacteriological samples yielded *Corynebacterium pseudotuberculosis* alone and 13 (15.29 %) samples yielded *Corynebacterium pseudotuberculosis* coupled with *Staphylococcus aureus*. On the other side, *Corynebacterium pseudotuberculosis* could not be isolated from the remained samples. The microscopic slides from the opened nodules showed small Gram's positive rods mixed with other cocci and large bacilli.

Mycological analysis:

The mycological analysis of the collected unopened samples yielded non-pathogenic mycotic agents.

Antibiotic sensitivity tests and therapeutic trials:

Results of the antibiotic sensitivity tests on some different isolated strains were illustrated on Table 4. The therapeutic trials of some diseased buffaloes (Table 5) were not completely effective. The affected cases showed unopened small-sized cutaneous nodules responded well to the applied treatment. The opened nodules were also apparently improved while the large unopened ones were diminished in size and some of them were thereafter inspessiated but not completely disappeared. However, the systemic antibiotic prevented the new spread of nodules.

DISCUSSION

In the present investigation, various sized cutaneous suppurative nodular eruptions along the lymphatic vessels particularly in the limbs, with and/or without regional purulent lymphadenitis and without marked systemic involvement were the predominant clinical findings of the infected buffaloes. Similar clinical findings were previously reported by Soliman et al. (1963) who described the clinical picture of buffaloes' ulcerative lymphangitis and termed this disease in Egypt as "Oedematous skin disease" or "So-called Oedematous skin disease". This opinion was thereafter supported by the study of Awad (1966) who recorded that 15

buffaloes showed various forms of skin suppurative lesions with involvement of the regional lymphadenitis.

The bacteriological analysis of the collected samples adverted that *Corynebaterium pseudotuberculosis* was overwhelmingly prominent bacterial isolates suggesting that this isolated pathogen was responsible for the observed suppurative lesions of the diseased buffaloes. *Corynebaterium pseudotuberculosis* was experimentally inoculated in calves causing localized suppurative inflammation followed by necrosis (Khater et al., 1983 and Torkey et al., 1982). These authors ascribed the resulted macroscopically and the microscopically pathological changes to the leukotoxic nature of the exotoxin produced by *Corynebacterium pseudotuberculosis.* However, Khater et al (1983a) intradermally inoculated ten buffalo-calves with *Corynebacterium pseudotuberculosis* isolated from a buffalo-calve with classical signs of ulcerative lymphangitis and they reported that after several hours the pathological lesions at the site of inoculation were mostly restricted on vascular and lymphatic damage. This may be announced to the presence of the different biological response in buffaloes than cattle under experimental conditions.

According to the history taken and the frequent field observations of the infected animals, the beginning of the disease was started by small number (2 or 3) of cutaneous nodules as a primary focus and thereafter they increased gradually in number in the neighboring areas forming multiple nodules in the later stages of the disease. This may be refer to the spread of infection within special cutaneous passages, likewise blood vessels or through the lymphatic vessels (metastasis). It is suggested that spreading of the bacteria occurred through the lymphatic vessels rather than the hematogenous route because of, from pathological point of view, Khater et al. (1983a) concluded that the intradermally inoculated bacteria in buffalo-calves induced thrombosis of the blood vessels and this may prevented the migration of the bacteria to another site. This may be advert that either the inoculated strain by Khater et al. (1983) was non metastatic in nature or to the biological different strains of *Corynebacterium pseudotuberculosis* species. *Corynebacterium pseudotuberculosis* organism was biologically classified into two different biotypes (Biberstein et al., 1971 and Barakat et al., 1984).

Concerning the distribution of the cutaneous nodules on the diseased buffaloes, Table 2 showed that the anterior portions of the infected animals particularly the neck and shoulders including prescapular

lymph nodes were predominant affected areas suggesting that rubbing these front portions against the rough objects, which probably contaminated with the infected material (pus), leading to cutaneous abrasions. The latter was considered the major portal of entry of the *Corynebacterium pseudotuberculosis* infection (Quinn et al., 1994).

Regarding the influence of the seasonal variation on the prevalence of the disease Table 3 and Fig. A showed that the rate of infection was highly significantly ($P<0.01$) increased during the summer season. Such results was coincided with the opinion of Shpigel (1993) who concluded that the peak of infection with the disease occurred in June, July and August months. Generally, in Assiut Governorate, there is no line of demarcation between the different seasons, and April, May, June, July, August and often mid-September were considered to be hot-or warm-months (Meteorological station of Faculty of Agriculture, Assiut University). Fig. B indicated that 60.8 % of the diseased cases were noticed during the hot-months. This probably suggested that either the warm or hot climate play a major or minor role in the activation of the causative agent, or facilitate the pathogenesis of the disease, or there is relation between the fly population during the hot months and the disease. It is also probable that wallowing of buffaloes in the muddy channels as a skin-wetting agent during the summer season should not be neglected as enhancing factor for growth of *Corynebacterium pseudotuberculosis*. *Corynebacterium pseudotuberculosis* was soil-borne infection and the wetted soi-medium produced a favorable chance for increasing the survival period of that bacteria (Augustine and Renshaw, 1982).

Results of antibiotic sensitivity tests adverted that cephalosporins had strong inhibitory effect (91.5 % sensitivity) on the majority of strains. The therapeutic trials with this drugs were successful particularly in diseased buffaloes with closed small nodules (early stages of the disease). Surgical interference (drainage) with systemic antibiotic was also effective in cases with little number of the large suppurative nodules. In buffaloes with marked prescapular suppurative lymphadenitis, the therapeutic trials were not completely successful. This may be related to the high amount of pussy material in the enlarged nodes. Pussy material was a protective agent preventing the diffusion of the antibiotic (Quinn et al., 1994).

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Table 1: Nr. of the observed skin lesions/buffalo.

Nr. of the skin lesions	Nr. of the diseased cases	% to all ill cases (n =56)
4	10	17.86
3	12	21.43
2	16	28.57
1	18	32.14

Table 2: Frequent distribution of the skin lesions on different regions of the animal body.

Site of lesion	Nr. Of lesions	% to all lesions
Neck	18	14.29
Prescapular L.Ns	27	21.43
Shoulders	30	23.81
Limbs	12	09.52
Knees	21	16.67
Flanks	18	14.29

Table 3: Seasonal influence on the infection rate of the disease.

Season	Examined buffaloes	Diseased Cases	% of infection	Infection Rate
Summer	231	34	14.72**	0.172
Autumn	189	3	1.59	0.016
Winter	105	2	1.90	0.019
Spring	225	17	7.55*	0.082

* significant ($P < 0.05$) ** highly significant ($P < 0.01$)

Table 4: Antibiotic sensitivity tests on some isolated strains (n =10)

Antibiotic	Inhibition zone (IZ)									
	1	2	3	4	5	6	7	8	9	10
Penicillin	2+	2+	-	1+	1+	1+	-	-	-	1+
Ampicillin	1+	3+	-	1+	1+	-	1+	-	-	1+
Cloxacillin	3+	3+	-	3+	2+	2+	2+	3+	3+	3+
Tetracycline	2+	2+	1+	3+	3+	3+	1+	1+	1+	2+
Erythromycin	1+	2+	2+	1+	2+	1+	1+	-	-	2+
Gentamicin	2+	1+	1+	1+	1+	1+	1+	-	-	2+
Cefoperazon*	3+	3+	2+	3+	3+	3+	3+	3+	3+	3+
Cephalexin**	3+	3+	1+	2+	3+	3+	3+	2+	3+	3+

*- Cefobid (Pfizer-Egypt) and ** Velosef (Squibb-Egypt) were disked.
3+ (IZ ≥ 23mm.) 2+ (IZ, 15-18 mm.) 1+ (IZ ≤ 15 mm.)

Table 5: Therapeutic trials of some diseased buffaloes (n = 16).

Cases	Treatment
5 cases showed widespread unopened small—sized nodules	- **Velosef** (15 mg / Kg BW daily for 10 days, I/M route) - supportive therapy
3 cases showed little number of large unopened nodules (4-7 nodules on the shoulder regions)	- large nodules were incised and irrigated with antiseptic solution either **potassium permenganat** (5%) or **povidone iodine** (1%). - **Velosef** (20 mg / Kg BW-I/M) for 10 days - Supportive therapy
6 cases showed multiple number of large unopened nodules on the various parts	- 3 cases were treated by **Velosef** (15 mg / Kg BW twice daily for 1 week, I/M route) and the remainders by **Cefobid** (2.5 ± 0.5 gm / head daily for 1 week, I/M route). - Supportive therapy
2 cases showed suppurative pre-scapular lymphadenitis with and/or cutaneous nodules	- Surgical interference of the enlarged node and thereafter irrigated with 3 % **povidone iodine**. - Velosef (15 mg / Kg BW twice daily - I/M route) for 10 days. - Supportive therapy

Fig. A: The influence of different seasons on the prevalence of the disease.

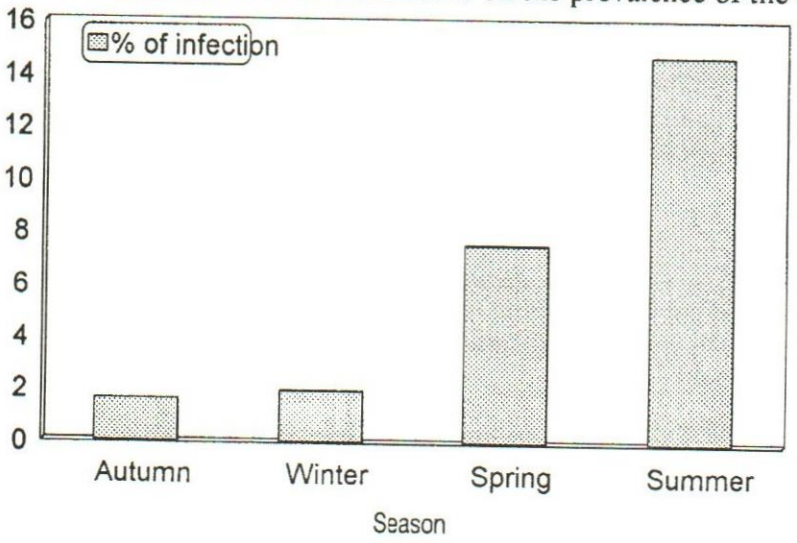

Fig. B: showing that the disease was more prevalent during the hot-months.

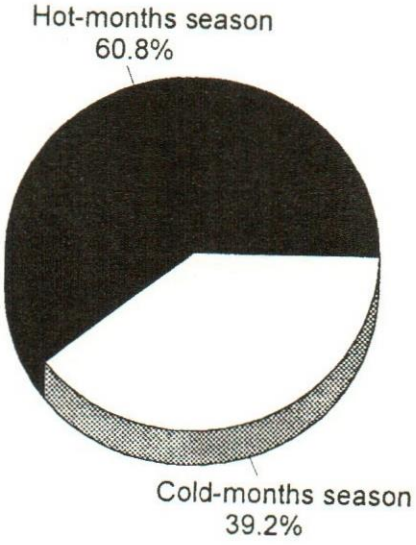

Fig. 1: Skin nodules arranged as long beading chain in the lower part of the left hind limb.

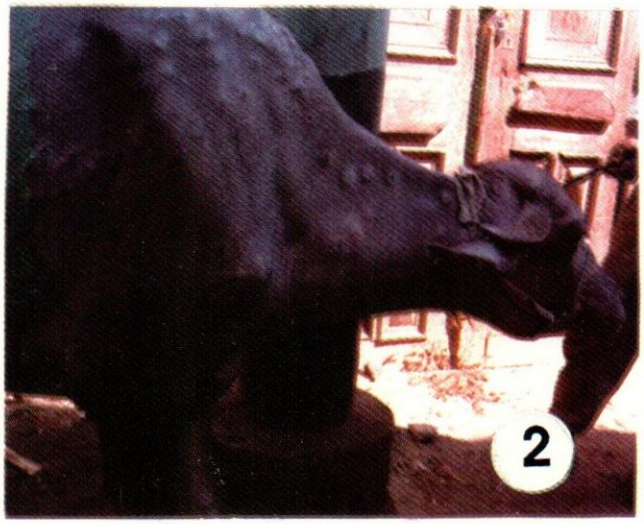

Fig. 2: Skin nodules on different parts of buffalow's body.

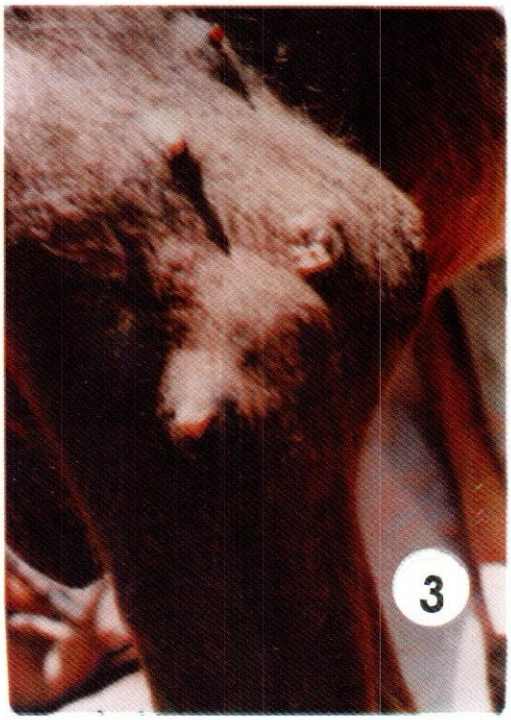

Fig. 3: Opened skin nodules With creamy whitish Pus tinged with blood In the shoulder region.

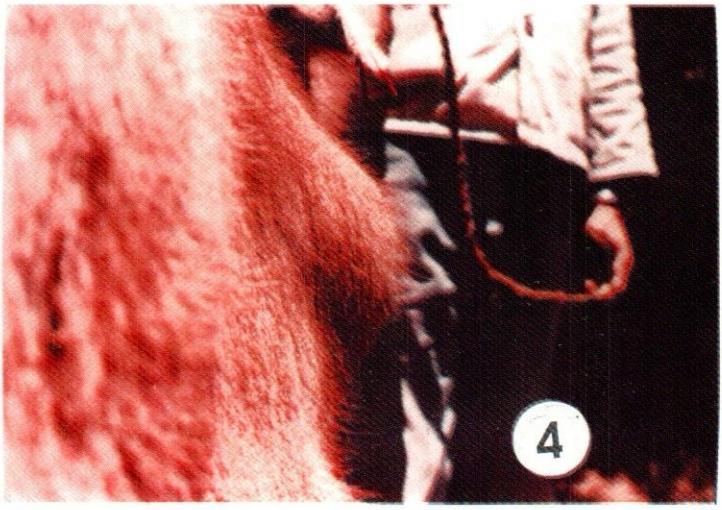

Fig. 4: Enlargement and suppuration of the Prescapular L.n$_s$