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CLINICAL AND EXPERIMENTAL STUDIES OF PSEUDOTUBERCULOSIS ON A MULTIPLE-AGES SHEEP AND GOATS FLOCK WITH CONTROL TRIALS VIA TREATMENT AND BCG—VACCINATION.

(With 5 Tables and 15 Figures)

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دراســـــــات إكلينيكية وتجريبية عن مرض الســـل الكاذب في قطيع أغنـــام وماعز متعدد الأعمار مع محاولة السيطرة من خلال العلاج والتحصين بلقاح "بي سي جي"

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اســـتغرق البحث اكثر من ســـنتين تم خلالهما فحص قطيع أغنام وماعز متعدد الأعمار ومختلف الأجناس والتي كانت تربى لغرض إنتاج اللحوم والصـــوف - فحصت جميعها للكشـــف عن مرض الســـل الكاذب. أظهرت الفحوصـــات الإكلينيكية بأن ٩,٢١% من الأغنام والماعز (على التوالي) كانت مصابة بـــالأعراض الظاهرية لمرض الســـل الكاذب (تضخم في الغدد الليمفاوية الســـطحية) وكان التضخم في غدة ليمفاوية سطحية واحدة أو اكثر في الحالات المصابة مع عدم وجـــود أعـــراض مرضية عامة باســـتثناء حالتين من الأغنام أظهرت صعوبة في البلع نتيجة لوجود تضخم شـــديد في الغدد الليمفاوية الفكية وكانت المشـــــاهدة الإكلينيكية الســـائدة. كـــانت ســـقوط الصوف والشـــعر فوق وحول الغدد الليمفاوية المصابة طبيعيا وتجريبيا للحالات المرضية خالية من الصوف والشـــعر وقد تم مناقشـــة الأســـباب المحتملة التـــي أدت إلى هذه الظاهرة. تم عزل ميكروب كوريني الســـل الكاذب في صورة منفردة أو مختلطة مع ميكروبات أخرى. دلت النتائج إلى أن الأغنام كانت اكثر قابلية للإصابة بالمرض من الماعز وان هذا المرض غير مرتبط بالجنس على الرغم من أن التحليـــل الإحصـــائي دل على ذكور الأغنام ظاهريا كانت اكثر قابلية للإصابة بالمرض من غيرهـــا. هـــذا وقد أشارت المشـــــاهدات الحقلية والتحاليل الإحصائية بان هنـــاك علاقـــة بيـــن أعمـــار الحيوانات ونســـبة وجود المرض وتم تفسير هذه العلاقة. أوضح التوزيع التكراري للغدد الليمفاوية السـطحية في الأغنام المصابة أن الغدة الليمفاوية أمام الكتف ثم الغدة الليمفاوية أمام الركبة اكثر إصابة من الغدد النكفية والفكية والثديية. وان تضخم كل من الغدد الليمفاوية أمام الركبة والفكية كانتا الوحيدة المصابة في الماعز. هذه الاختلافات فـــي التوزيـــع التكـــراري

الليمفاوية المصابة في كل من الأغنام والماعز تمت مناقشتها. أوضحت نتائج اختبار الحساسية لبعض العترات المعزولة إلى أن جميعها كانت مقاومة للأستربتوميسين وكانت شديدة الحساسية لمركب السيفرادين ومع هذا كانت محاولات العلاج لبعض الحالات المريضة بهذا العقار غير مجدية. دلت نتائج محاولات التحصين بلقاح "بي سي جي" في الحقل إلى أن هذا اللقاح له دورا فعالا في عدم إصابة الأغنام التي تم تعريضها للعدوى التجريبية وكانت محاولة السيطرة على السل الكاذب في الماعز من خلال التحصين (بي سي جي) غير فعالة.

SUMMARY

A sheep and goats flock of different ages and sexes used for mutton and wool production, was clinically examined for the presence of pseudotuberculosis. The clinical examinations revealed that 20 % and 9.21 % of the examined sheep and goats, respectively, showed the cutaneous lesions (superficial lymphadenitis) of pseudotuberculosis. Enlargement of one or more superficial lymph nodes of the infected cases that showed no marked systemic reactions, with exception of two sheep exhibited difficult swallowing due to severe bilateral mandibular lymphadenitis, were the predominant clinical findings. The woolly and the hairy areas above and surrounds the affected nodes of the naturally and experimentally infected cases were markedly alopecic (peculiar appearance). The probable reasons for such peculiar appearance were discussed. *Corynebacterium pseudotuberculosis* (CP) either in a pure culture or mixed with other bacteria was isolated. Results showed also that and the disease sheep was significantly more susceptible to the disease than goats, was non-sex linked disease, however, statistical analysis indicated that male sheep were apparently more susceptible. Field observation and statistical analysis referred to the presence of a relationship between the ages of animals and the prevalence of the disease. Such relationship was explained. Prescapular followed by prefemoral lymph nodes were significantly more affected than the parotid, mandibular and supra-mammary nodes of the infected sheep. Enlargement of the parotid and the mandibular lymph nodes were the only affected nodes of the diseased goats. Such variation in distribution of the affected nodes in both sheep and goats was monitored. Antibiogram indicated that all CP isolates were streptomycin resistant and were highly sensitive to cephradine. However, treatment with this drug were ineffective. A streptomycin-resistant CP strain was experimentally inoculated (s/c) in a non-previously infected 3 years old

ram inducing severe prescapular lymphadenitis. Experimental infection post field vaccination trials with BCG vaccine in some sheep revealed that this vaccine has a valuable role in control of sheep's pseudotuberculosis. Control of goat's pseudotuberculosis via BCG vaccination was not completely effective.

Keyword: *Pseudotuberculosis in sheep & goats, clinical, epidemiological and BCG-vaccination.*

INTRODUCTION

Pseudotuberculosis is one of the common diseases of the small ruminants primarily sheep due to infection with *Corynebacterium pseudotuberculosis* causing a considerable level of economic losses among the infected flocks. These losses included infertility, lamb-stillborn, retardation of the growth and poorly wool production, premature culling and mortality of the infected cases (Alonso et al., 1992; Skaika et al., 1998 and Stanford et al., 1998). Moreover, from the economical point of view, the repulsive appearance with the low prices of the infected cases with the cutaneous lesions of this disease in the commercial markets should not be neglected as one of the major losses. In addition to the economic losses. pseudotuberculosis had a zoonotic implication (Laven et al., 1997 and Scott et al., 1997).

Wounds of the skin due to careless shearing were overwhelmingly encountered as a principle way for entrance of *Corynebacterium pseudotuberculosis* infection in sheep, while docking, castration and the umbilicus were of minor importance (Nagy, 1976; Dercksen et al., 1993 and Pepin et al., 1993). Pseudotuberculosis characterized principally by single or multiple superficial suppurative lymphadenitis (Scott et al., 1997). However, internal form of pseudotuberculosis due to systemic spread of infection from the primary site (superficial lymph nodes) to the internal organs through lymphomatogenous or probably hematogenous routes causing serious ailments was also reported by Pepin et al. (1993).

In Egypt, Pseudotuberculosis was one of the major endemic diseases and was previously studied by many authors (Zaki and Abdel-Aal, 1974; Mottelib et al., 1976; Seddik et al., 1983 and Abd-El-Rahim et al., 1998). The authors studied the disease in individual rural flocks of sheep and goats, and they focused principally on the epidemiological characterizations of the causative agent of the disease, clinical, biochemical changes associating with the disease and the

serological diagnosis of the infected cases. Zaki and Abdel-Hamid (1974) reported that mouse protection test was more valuable serological test for diagnosis of pseudotuberculosis than hemolysin-inhibition test particularly in endemic localities. Conversely, Abd-El-Ghani et al. (1998) concluded that hemolysin-inhibition test was the best and easy serological test and could be used for diagnosis of pseudotuberculosis. This may suggest that the role of serological tests in diagnosis of pseudotuberculosis is still questionable. Such suggestion was supported by the opinion of Laven et al. (1997) who reported that isolation of the etiologic agent was the only confirmatory method for the diagnosis of pseudotuberculosis, probably due to the lack of the proper antigen for a serological test. Epidemiological data on the prevalence of pseudotuberculosis in both sheep and goats flocks appears to be still brief in the available literature. Moreover, Laven et al. (1997) concluded that the therapeutic trials of the infected cases with pseudotuberculosis even with high doses of antibiotics has a limited value. This may refer to the control of pseudotubercluosis in both sheep and goats, particularly in endemic areas, is a crucial goal. Consequently, the fundamental goal of the following work was directed to detailed study of the clinical picture of some diseased cases in a multiple-age sheep and goats flock at Assiut Governorate-Upper, Egypt. Medication and vaccination were also attempted in a trial to control the disease.

MATERIAL and METHODS

Animals:

A multiple-age sheep and goats flock located in Banoub village, Assiut Governorate, Upper Egypt was clinically examined. This flock was consisted of 95 sheep and 67 goats in different ages and sexes. Usually the rapid growing yearling sheep and the adults of the respective flock were manually sheared twice yearly whereas goats not sheared. On April 1997, 15 males sheep and 9 goats; of different ages and sexes were purchased, and admitted to the flock. Some of them were suffered from abscessiation in their superficial lymph nodes. Eight months later, the owner noticed the increased rate of the cutaneous lymphadenitis of the animals particularly in sheep without success of the parental therapeutic trials (Pan-terramycin, Pfizer-Egypt) even with massive doses.

From the epidemiological point of view, the incidence rate of the present disease was calculated according to the methods reported by Thrusfield (1986) who concluded that the common application of the

incidence rate involves using the average size of the population during the period of observation, multiplied by the period of observation, as denominator. Data of prevalence of the present disease of various ages groups, sex distribution in both sheep and goats and distribution of the affected lesions (cutaneous lymphadenitis) on the infected cases were monitored and tabulated and thereafter statistically analyzed. Moreover, detailed history were taken and discussed

Bacteriological examinations:

Seven sheep and three goats of the clinically diseased cases were subjected to bacteriological examinations. One of the affected nodes of each selected sheep was surgically excised and opened, and the peripheral caseous material was picked up and platted directly onto 7 % blood agar (Oxoid). Concerning goats, one of the affected nodes was surgically incised and the pus material was swabbed. The obtained swabs were platted onto blood agar plates, then incubated for 24 - 48 hours and the suspected colonies were picked up and purified. These purified isolates were, thereafter, morphologically and biochemically identified according to the protocol described by Quinn et al. (1994).

Experimental Study:

In the view of the above facts it is thought to adopt a preliminary experimental study which include the following steps:

Preparation of the inoculating organism:

One of the isolated *Corynebacterium pseudotuberculosis* strains was inoculated into a 100-ml. flask containing 5 ml. brain heart infusion (BHI, Gibco) broth plus 7 % sterile inactivated horse serum (El-Agouza Institute, Egypt) and incubated at 37°C. On the third day post incubation, a 10 ml. of sterile BHI plus serum broth was aseptically added to the inoculated broth and re-incubated. Three days later post incubation, another 25 ml. of sterile BHI plus serum broth was re-added to the inoculated broth and further incubated for another 3 days. Test of sterility was carried out by platting 0.1 ml. of the tested broth onto blood agar and Sabouraud's dextrose agar plates. Ten fold serial dilutions using serial tubes containing BHI plus serum broth was carried out and the colony forming units for each dilution were counted. The dilution of 10^{-7} was used for experimental infection. The stock broth was kept in the refrigerator.

Experimental infection:

One milliliter of the selected dilution was inoculated subcutaneously in the middle square of each side of the neck of an apparently healthy, non-previously infected (history) 3 years old ram.

The inoculated ram was separately isolated in a good hygienic barn at the Vet. Hospital of Assiut Univ., and kept for weekly observation for 6 months, where the expected abscess of the regional lymph nodes was monitored. Trial for isolation of the inoculated organism from the affected nodes was carried out as described. A control case was used and subcutaneously inoculated with sterile BHI plus serum.

Field Vaccination trials:

Five sheep, less than 6 months in age, group A) and 5 of one-year old sheep (group B) as well as 3 of six-months old goats (group C) were selected for the vaccination trials. These animals were apparently healthy and free from the cutaneous lesions of pseudotuberculosis. The selected animals were inoculated intradermally (inner side of the tail or the thigh) with 0.1 ml. of BCG* vaccine and were kept (without shearing) under monthly observation. Six months later, 3 sheep of group A and 3 sheep of group B were subcutaneously inoculated with the previously prepared broth of *Corynebacterium pseudotuberculosis* ($1x10^7$ CFU). Two of the three BCG vaccinated—goats were subcutaneously inoculated (1 ml.) with the prepared broth at the base of the left ears. These inoculated sheep and goats were kept under monthly observation for 3 months. Thereafter, the owner allowed the testing sheep to the routine shearing.

Antibiogram and therapeutic trials:

I- Antibiogram:

Eight strains (5 isolated from sheep and 3 isolated from goats) of the identified *Corynebacterium pseudotuberculosis* were, in vitro, tested through disc susceptibility test to 6 different members of antibiotics (Table 4). Technique of antibiotic discs susceptibility test and interpretation of the results of the inhibition zones were carried out according to the criteria of Barry (1980).

II- Therapeutic trials:

A- Antibiotic therapy:

Three sheep and one goat of the clinically diseased cases of the investigated flocks were injected intramuscularly daily with high doses

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*: BCG free-dried vaccine (BCG Pasteur vaccine/France, 42A-84-07-B9) 1 mg. The ampoule reconstituted in institute Pasteur diluant (CP 002) (Austin savant) to form 2 ml. (20 doses).

of cephradine at a dose rate of 30 mg/kg body weight (Velosef, Bristol-Meyer, Squibb-Egypt). The therapeutic course persisted for 7 successive days.

B- Surgical interference with or without antibiotic therapy:

The remained cases of the clinically diseased animals were subjected to surgical interference with antibiotic therapy (Velosef, 10 mg/kg BWt, I/M, for 5 days). The small-sized hard infected nodes were ripened by application of Icthyol ointment and thereafter incised and irrigated with povidone iodine 1%. (Betadine, Nile Co., Egypt), while the softened infected nodes were surgically opened and irrigated. On the other hand, the greatly enlarged nodes of the infected sheep were surgically excised as a whole units without injection of antibiotic.

Statistical analysis:

The obtained data were statistically analyzed according to the methods described by Milton and Tsokos (1983). Chi-square (χ^2) were used to reveal the degree of susceptibility of the disease in both species. Chi-square (χ^2) were also used to clear the sex distribution of the disease in sheep and goats. Simple linear regression with calculation of the coefficient of determination (accuracy) (R^2) was utilized to indicate the relationship between the ages of the examined sheep and goats and the percentage of infection with the disease. For obtaining the maximum value of R^2 (good fitting), the investigated animals were divided according to their ages into two groups. The first group animals, their ages were ranged from <1 months to two years, and the second group animals ranged from >2 years to <6 years. Subsequently the regression lines were divided into two set (1 and 2) for each species.

RESULTS

Clinical findings:

The clinical examinations revealed that twenty two cases (20.00 %) out of the clinically examined sheep (n=110) and 7 (9.21 %) out of 76 of the clinically investigated goats showed the characteristic signs of cutaneous lesions of pseudotuberculosis (Table 1). Enlargement of one or more superficial lymph nodes of the clinically diseased animals were the characteristic observed clinical signs. The prescapular followed by the prefemoral lymph nodes of the diseased sheep were the predominant affected nodes (Fig. A) rather than the parotid (Fig. B) and the mandibular nodes. Three cases of the diseased sheep showed enlargement of the supra-mammary lymph nodes (Fig. C) without udder

involvement. The parotid and the mandibular lymph nodes were the only affected nodes of the diseased goats (Fig. D and E). Two of the enlarged nodes were spontaneously ruptured discharging yellowish green slightly thickened pus material, which covered the surrounding wool, while the other enlarged nodes were either hard or soft. No marked systemic involvement of the diseased cases were noticed with exception of two sheep showed difficult swallowing. These two sheep were suffered from severe bilateral enlargement of the mandibular lymph nodes (Fig. F). The woolly layers adjusted to the enlarged lymph nodes of the diseased sheep and goats were free of wool (alopecic areas) (Fig. A, B, D, E and F).

The opened surgically excised lymph nodes showed the characteristic lesion of pseudotuberculosis (onion appearance); multi-layers of caseous material surrounded by a thick wall (Fig. C_1 and F_3).

Results of the collected data of ages and sex distribution of pseudotuberculosis in both sheep and goats were summarized and tabulated in Table 1 and 2, respectively. The frequent distribution of the affected lymph nodes of the examined diseased sheep and goats were illustrated in Table 3.

Bacteriological examinations:

The bacteriological examinations indicated that nine samples of the bacteriologically tested samples were *Corynebacterium pseudotuberculosis*—positive. Of these positive samples, five samples yielded *Corynebacterium pseudotuberculosis* in a pure culture while the other yielded *Corynebacterium pseudotuberculosis* mixed with Gram's positive cocci. These cocci was biochemically identified as coagulase negative staphylococci and micrococci. No bacteria could be isolated from one sample.

Experimental infection:

The experimentally inoculated ram with the isolated streptomycin-resistant *Corynebacterium pseudotuberculosis* strain developed bilateral prescapular lymphadenitis after five weeks of inoculation and by increasing the time of observation, the size of the affected nodes was gradually increased (fig. G). The woolly layer above and surrounds the affected nodes of the inoculated ram were markedly alopecic (Fig. G). These greatly enlarged nodes were surgically excised (Fig. G_1) and opened, and the characteristic lesion of pseudotuberculosis (onion appearance) was clearly obvious (Fig. G_2). *Corynebacterium pseudotuberculosis* was successfully re-isolated from these nodes. Body

emperature (Mean, 40 ± 0.3 °C) and the pulse rate (53 ± 4 / min) of the experimentally inoculated animal were increased within the first two weeks post inoculation. Thereafter, the increased temperature and pulse were subsided to the normal level till the end of observation. No infection of the other superficial lymph nodes of the inoculated ram was observed.

Vaccination trials:

The experimentally inoculated sheep, previously vaccinated with BCG vaccine, showed no lesions post *Corynebacterium pseudotuberculosis* inoculation. On the other side, one of the experimentally inoculated goats that previously vaccinated with BCG showed parotid lymphadenitis after 69 days of inoculation, and the inoculated organism could not be re-isolated from the pus material. This case also showed that the hairy layer above the lesion was not sloughed (Fig. H) and the size of that lesion was still approximately fixed during the period of observation.

Antibiogram and treatment:

The antibiogram of the tested *Corynebacterium pseudotuberculosis* isolates revealed that all isolated strains were streptomycin resistant and were sensitive to the other antibiotics particularly cephradine (Table 5).

The therapeutic trials of the diseased cases with the parental antibiotic were unsuccessful to relief the developed lesions, whereas the surgical interference with or without antibiotics were apparently effective.

DISCUSSION

The presence of cutaneous suppurative lymphadenitis with the characteristic onion appearance of the affected lymph nodes, and isolation of *Corynebacterium pseudotuberculosis* refers to the occurrence of pseudotuberculosis in the respective flock. Jubb et al (1993) reported that the internal abscessiation due to *Corynebacterium pseudotuberculosis* infection could be found in animals with no clearl external clinical signs. Moreover, Laven et al. (1997) concluded th isolation of *Corynebacterium pseudotuberculosis* from the affected cas by bacteriological culturing was the only appropriate confirmato method of diagnosis of pseudotuberculosis, and the serological tests h a little of value. This may refer to the insidious nature pseudotuberculosis among the infected sheep and goats. Consequer calculation of the true incidence of pseudotuberculosis is proba

difficult. In the present study, diagnosis of pseudotuberculosis depends basically on observation and palpation of the enlarged superficial lymph nodes of the examined cases in association with microbial culturing. However, calculation of incidence of the cutaneous form of pseudotuberculosis in sheep was 18 cases/ 100 sheep/ year, and in goats it was 12 cases/ 100 goats/ year referring to the spread of the disease throughout the flock.

Enlargement of one or more superficial lymph nodes without marked systemic reactions, with exception of two sheep showed difficult swallowing due to severe bilateral mandibular lymphadenitis that probably induced a pressure on the pharynx, were the characteristic clinical findings of the diseased cases. Similar field observations were previously reported by Laven et al. (1998). The alopecic areas above and surrounds the enlarged lymph nodes of all *Corynebacterium pseudotuberculosis*—positive and of the experimental cases may ascribe to the relative weak dermo-necrotic exotoxin produced by that bacteria (Quinn et al., 1994). The over-sized lymph nodes of the infected cases may induce a pressure atrophy of the overlaying skin led subsequently removal of the wool or hair.

Concerning species susceptibility, the obtained results (Table I) declared that the prevalence of pseudotuberculosis was significantly ($P<0.05$) increased in sheep than goats and may be sheep are more susceptible to the disease. However, according to the history taking, all one-year old sheep and the adults of the investigated flock were subjected to shearing two times per year; at the end of April and at the mid-September, while goats were not sheared. Such history may refer to the pivotal role of cutaneous abrasions or wounds due to improper shearing mechanism in the pathogenesis of pseudotuberculosis. Pseudotuberculosis in sheep were experimentally induced by application of the contaminated pus material with *Corynebacterium pseudotuberculosis* to the freshly shearing wounds or by putting the broth culture of this organism on non-abraded recently shorn skin (Nagy, 1976). This experimental work may prove that the visible or invisible cutaneous abrasions due to careless shearing of sheep or recently shorn skin plays outstanding role in the development of ovine pseudotuberculosis. Consequently the significant ($P<0.05$) increased susceptibility of sheep to pseudotuberculosis than goats is probably ascribe to the shearing mechanism rather than species susceptibility.

Regarding sex susceptibility, the obtained results (Table 2) indicated that there was no significant difference ($P>0.05$) between females and males goats suggesting that caprine pseudotuberculosis is non-sex linked disease. Similar opinion was reported by Ashfaq and Campbell (1979). On the other side, results obtained by Seddik et al. (1983) concluded that there was no significant difference in susceptibility of male and female sheep to pseudotuberculosis. However, Table 2 revealed that the male sheep were apparently more susceptible ($P < 0.05$) to pseudotuberculosis than females. This may ascribe to the unbalanced ratio between females and males sheep of the investigated flock rather than sex-linking susceptibility.

Field observations (Table 1 and Fig. 2) and the statistical analysis (Fig. 3, 4, 5 and 6) referred to the presence of a relationship between the ages of the investigated animals and the percentage of infection with pseudotuberculosis. This percentage was gradually increased by increasing the age of the animal till 30±6 months (coefficient of determination, R^2, was 0.96 and 0.93 in sheep and goats, respectively). Thereafter, the percentage of infection with pseudotuberculosis was gradually decreased by further increasing in age of the animal (R^2 was 0.85 and 0.76 in sheep and goats, respectively). Similar opinion was fairly reported by Ashfaq and Campbell (1979) and Jubb et al. (1993) who concluded that the prevalence of pseudotuberculosis of sheep and goats increased by increasing the age. The decreased prevalence of the disease after the animals reach to four to five years may be ascribed to a build up of acquired immunity. Experimentally, Pepin et al. (1993) inoculated a streptomycin sensitive *Corynebacterium pseudotuberculosis* (less virulent) strain in serologically negative ewes without development of any abnormal lesions, and thereafter these inoculated animals were re-inoculated with streptomycin resistant *Corynebacterium pseudotuberculosis* (19R) (virulent strain). They found that the tested ewes did not develop lesions as a results of challenge exposure, whereas the non-immunized ewes (non-primary inoculated ewes) developed numerous pyogranulomas lesions at the site of inoculation and in the regional lymph nodes as well as in the lungs. Such experimental work may refer to the occurrence of strong protection (acquired immunity) after primary infection with *Corynebacterium pseudotuberculosis*. On the other hand, the decreased prevalence of pseudotuberculosis in young sheep and goats (less than one year) of the investigated flock may refer to the role of maternal immunity. Laak et al.

(1992) concluded that young kids and lambs, which live in the infected flock with pseudotuberculosis had maternal antibodies and when kids and lambs were separated from their dams immediately after birth, maternal antibodies would not be available. Such conclusion may point to the valuable role of the colostral immunity. Consequently, it is suggested that the probabilities of infection with pseudotuberculosis in the lambs and the kids that born from the non-previously infected dams, non-immunized or immune-naive dams appears to be not low. Such suggestion may interpret the results obtained by Seddik et al. (1983) who reported that 20 % of the examined suckling lambs around six months of age (rural cases) in different localities of Assiut Governorate were clinically positive to pseudotuberculosis.

The obtained results (Table 3 and Fig. 6) revealed that the prescapular followed by the prefemoral lymph nodes were the predominant affected nodes of the clinically diseased sheep. This result was coincided with the opinion of Abd-El-Ghani et al. (1998) who reported that the prescapular lymph nodes were the prominent affected nodes of the clinically diseased sheep. Another view was offered by Seddik et al. (1983) who reported that parotid lymphadenitis was clearly remarkable rather than other superficial nodes of the infected sheep. From the pathological point of view, Khater et al. (1984) and Jubb et al. (1993) concluded that *Corynebacterium pseudotuberculosis* was specific infectious agent of the lymphatic vessels and lymph nodes primarily of sheep and goats, and this micro-organism invades the afferent lymphatic vessels of the affected parts through skin injuries and then journey on to the regional lymph nodes under the influence of its exotoxin inducing the characteristic lesion (laminated caseous lymphadenitis) of that nodes. The afferent lymphatic vessels of the wool-shearing areas (lateral sides of the neck beginning from the caudal part of the head including the external ears, shoulder region, lateral sides of the thorax, dorsal back and the lumber region) were drained in both prescapular and prefemoral lymph nodes (Saar and Getty, 1975). Such conclusions may explain the highest percentage of the affected prescapular and prefemoral nodes of the diseased sheep. Furthermore, results of the experimental work in a ram may prove that the inoculated strain in the middle square of the neck of the tested animal was traveled to the regional lymph nodes (prescapular) inducing lymphadenitis.

Results of Table 3 and Fig. 7 showed that the parotid and the mandibular lymph nodes were the only affected superficial nodes of the clinically diseased goats. Both parotid and mandibular lymph nodes

received approximately all afferent lymphatic vessels of the head of goat (Tanudimadja and Ghoshal, 1975). According to the previous history, goats of the investigated flock never been sheared Consequently, it is fairly suggest that oral infection may play a role in the pathogenesis of caprine pseudotuberculosis. Ashfaq and Campbell (1979) suggested that goats contracted the infection not only through skin abrasions or wounds of their heads but also through wounds of their oral mucosae (ingestion route). Wounds of oral mucosae in goats may occur through ingestion of rough diet (chewing of hard fibrous food). However, abrasions of skin of heads of goats due to fighting between kids particularly during the growing and maturity stages should not be neglected as a portal of entry of infection.

Antibiogram of the tested isolates revealed that cephradine had strong inhibitory effect on all isolates (Table 4). However, the therapeutic trials of some diseased cases with this drug was ineffective This may ascribed to the following reasons; the protective nature (non-diffusable medium) of the pussy material (Quinn et al., 1994), the impermeability of the pyogenic capsule (Jubb et al., 1993), and the ability of the micro-organism to habitats the macrophages i.e facultative intracellular bacteria (Tashjian and Campbell, 1983).

Results of the induced vaccination trail with BCG vaccine and according to the history taking, the BCG-vaccinated sheep were still free of the cutaneous lesions of pseudotuberculosis (Aug., 1999), although the persistence of shearing. This may overwhelmingly refer to the valuable role of BCG vaccine in control of ovine pseudotuberculosis. The outstanding role of the attenuated bovine tubercle bacillus of Calmotte and Guerine in control of pseudotuberculosis of sheep was previously studied by Barakat (1980) who concluded that BCG vaccine greatly declined the prevalence of pseudotuberculosis in sheep flock particularly in small ages. On the other side, the obtained results of the vaccination trail with BCG in control of caprine pseudotuberculosis revealed that the role of this vaccine appears to be not completely effective. However, the fixation size of the affected node of the tested goat without appearance of the alopecic lesion may explain that BCG vaccine may propably interfere inhibit the action of the dermo-necrotic toxin produced by the inoculated micro-organism, and may also inhibit the over-growth of the bacteria. In our view, further investigation should urgently be carried out on control of caprine pseudotuberculosis by BCG or other biological products.

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Table 1 : Prevalence (% affected) of pseudotuberculosis in the investigated goats and sheep of different age groups

Age	Goat			Sheep			Total		
	Nr.	D	(%)	Nr.	D	(%)	Nr.	D	(%)
< 1 months	3	0	-	4	0	-	18	0	-
> 1-3 months	5	0	-	6	0	-	11	0	-
> 3-6 months	9	0	-	11	1	9.09	20	1	5.00
> 6-12 months	12	1	8.33	15	2	13.33	27	3	11.11
> 1-2 years	10	3	30.00	20	5	25.00	30	8	26.67
> 2-3 years	10	2	20.00	21	9	42.86	31	11	35.48
> 3-4 years	12	1	8.33	18	4	22.22	30	5	16.67
> 4-5 years	7	0	-	11	1	9.09	18	1	5.55
> 5-6 years	6	0	-	2	0	-	8	0	-
> 6 years	2	0	-	2	0	-	4	0	-
Total	76	7	9.21	110	22*	20.00	186	29	15.59

Nr. = Number of the examined cases. D = Diseased cases (cutaneous lesion).
* $\chi 2$ Sheep • goats = 3.97, significant increase ($P < 0.05$).

Table 2: Sex susceptibility of the examined sheep and goats to pseudotuberculosis.

Species	Sex	Examined animals Nr.	%	Ratio	Diseased cases Nr.	%
Sheep (n = 110)	Females	81	(73.67)	2.79	12	(14.81)
	Males	26	(16.36)		10	(34.48)
Goats (n = 76)	Females	69	(90.79)	9.86	6	(8.96)
	Males	7	(9.21)		1	(14.28)

χ^2 Females • Males sheep = 5.16 - significant difference ($P < 0.01$).
χ^2 Females • Males goats = 0.24 - non-significant difference ($P > 0.05$).
χ^2 Females sheep • Females goats = 1.32 - non-significant difference ($P > 0.05$).
χ^2 Males sheep • Males goats = 1.45 - non-significant difference ($P > 0.05$).

Table 3: Distribution of the affected lymph nodes of the examined diseased sheep (n = 22) and goats (n = 7) with pseudotuberculosis.

Lymph node (affected)	Diseased sheep		Diseased goats	
	Nr. of cases@	Nr of affected lymph nodes	Nr. of cases	Nr. of affected lymph nodes
Parotid	6 (27.27 %)*	6 (9.09 %)**	6 (85.71 %)*	9 (81.82 %)**
Mandibular	2 (9.09 %)	4 (6.06 %)	1 (14.26 %)	2 (18.18 %)
Prescapular	19 (86.36 %)	31 (46.97 %)	-	-
Prefemoral	13 (59.09 %)	19 (28.79 %)	-	-
Supra-mammary	3 (13.64 %)	6 (9.09 %)	-	-

@ : Some diseased cases showed more than one lymph node affected.
* : Percent to the diseased cases.
** : Percent to all affected lymph nodes of the diseased cases of each species.

Table 4 : Leukogram of the diseased sheep and goats with cutaneous lesions of pseudotuberculosis.

Parameter	Diseased sheep (n = 10)	Control sheep* (n = 5)	Diseased goats (n=7)	Control Goats* (n = 5)
Total leukocytes (x10³ cm)	15.93 ± 2.51**	9.3 ± 2.62	19.86 ± 3.5**	8.9 ± 3.2
Neutrophils (%)	37.41 ± 2.81*	34.95 ± 1.41	37.04 ± 1.21*	34.8 ± 2.8
Eosinophils (%)	1.30 ± 0.50	4.86 ± 1.32	1.10 ± 1.65	4.6 ± 1.02
Basophils (%)	0	1.50 ± 0.21	0	1.5 ± 1.10
Lymphocytes (%)	59.23 ± 2.41**	53.62 ± 1.20	60.31 ± 3.1**	53.4 ± 1.7
Monocytes (%)	2.32 ± 1.12	3.81 ± 0.56	1.20 ± 1.12	2.7 ± 0.31

* : The control cases were animals that had no cutaneous lesions
* : Significant increase ($P < 0.05$) ** : Highly significant increase ($P < 0.01$)

Table 5 : Antibiogram of the isolated *Corynebacterium pseudotuberculosis* (n = 8 isolates).

Antibiotic (Disc potency)	Mean S.E. of the inhibition zone	Interpretation
Penicillin (10 IU)	28.56 ± 1.51	Sensitive
Ampicilline (10µg)	30.15 ± 1.23	Sensitive
Streptomycin (10µg)	6.02 ± 0.10	Resistant
Oxytetracycline (30µg)	29.62 ± 1.35	Sensitive
Erythromycin (15µg)	26.31 ± 1.42	Sensitive
Cephradine (30µg)*	31.21 ± 2.41	Highly sensitive

* Velosef (Squibb- Egypt) were disked

Fig 1: Age susceptibility of the investigated sheep and goats to pseudotuberculosis.

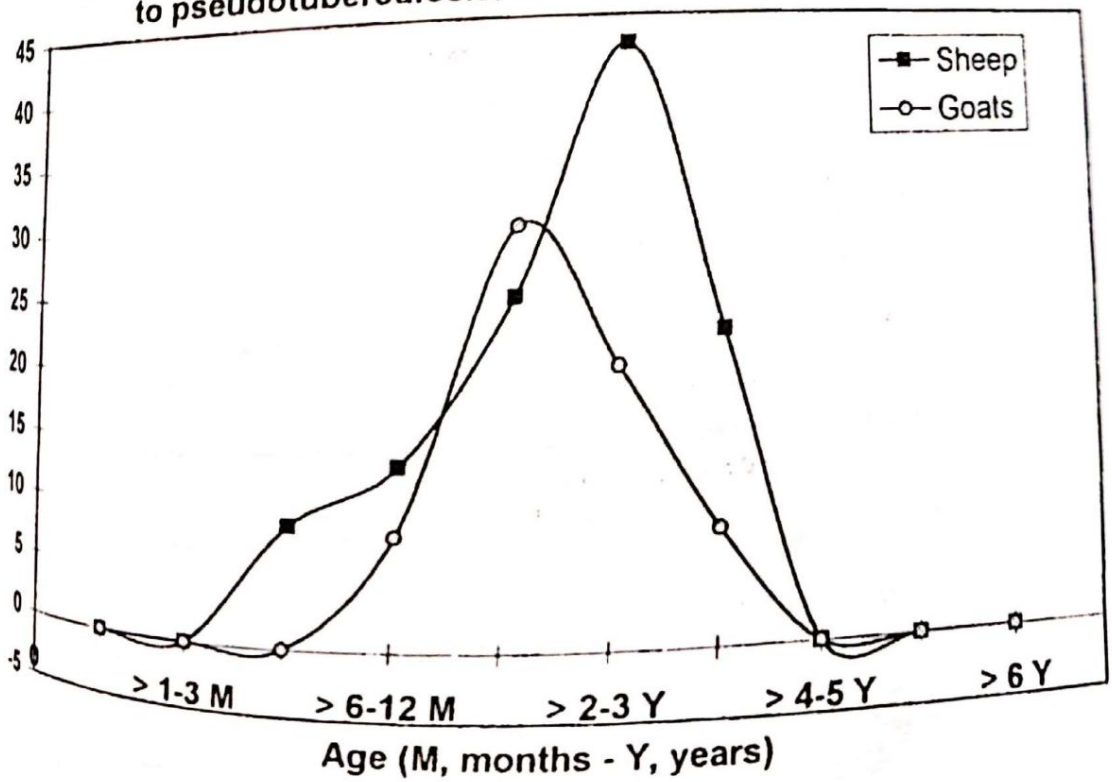

Fig 2: Regression line (set 1) of age upon percent of the diseased sheep with pseudotuberculosis.

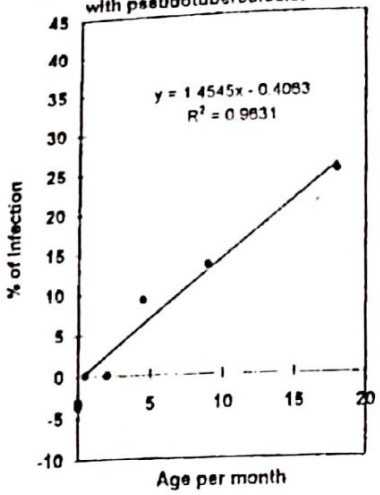

Fig.3: Regression line (set 2) of age upon percent of the diseased sheep with pseudotuberculosis.

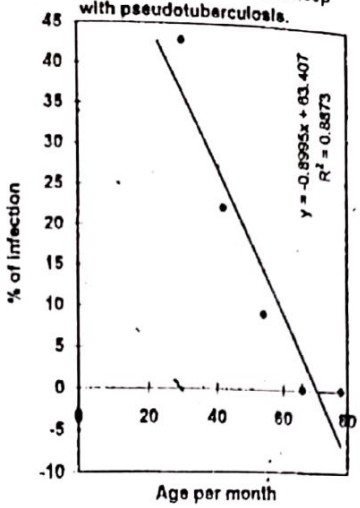

Fig. 4: Regression line (set 1) of age upon percent of the diseased goats with pseudotuberculosis.

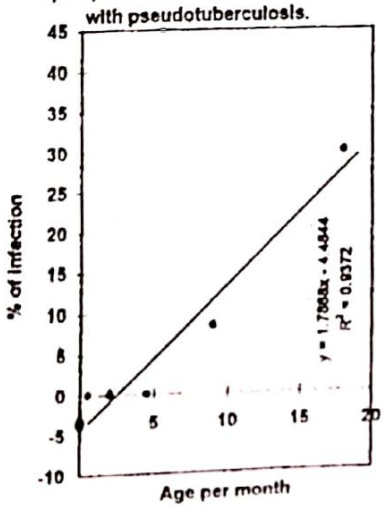

Fig.5: Regression line (set 2)of age upon percent of the diseased goats with pseudotuberculosis.

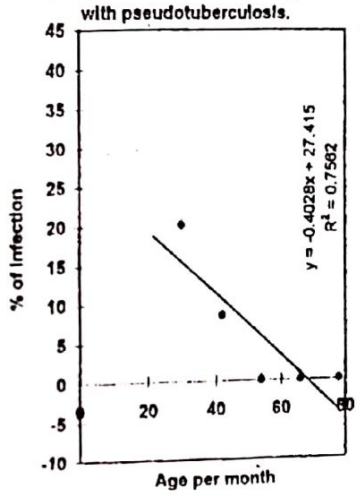

Fig. 6 : Frequent distribution of the affected superficial lymph nodes in 22 diseased sheep with pseudotuberculosis.

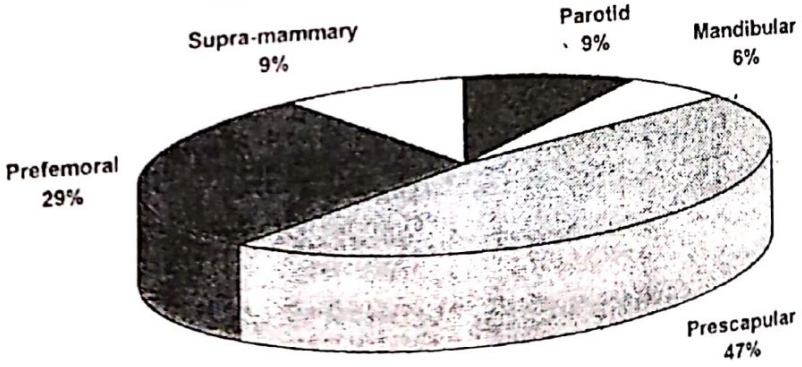

Fig. 7: Frequent distribution of the affected superficial lymph nodes in 7 diseased goats with pseudotuberculosis.

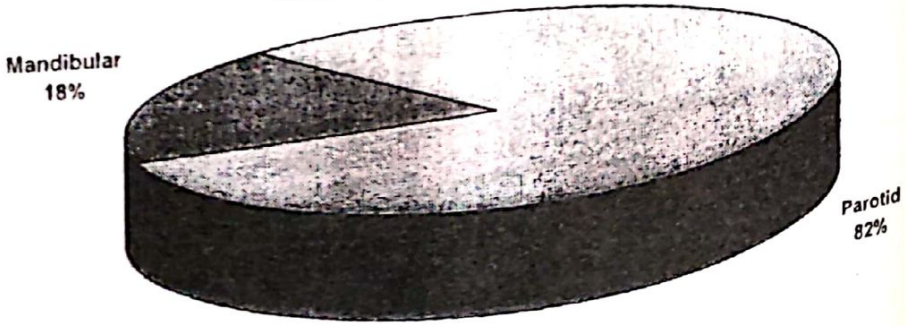

Fig. A,B & C: Showing enlargement of the prescapular (A), the parotid (B) and the supra-mammary (C) lymph nodes of the diseased sheep with cutaneous lesions of pseudotuberculosis. Note: The alopecic woolly areas above the enlarged nodes.

Fig. C1: Showing the characteristic lesions of pseudotuberculosis of the affected node (supra-mammary); multi-layers of caseated material surrounded by a thick wall (onion appearance).

Fig. D & E: Showing enlargement of the parotid (D) and the mandibular (E) lymph nodes of the diseased goats with pseudotuberculosis. Note: The alopecic hairy areas above the lesions.

Fig. F, F1 & F2: Showing severe bilateral enlargement of the mandibular lymph nodes (F) in a sheep with dismorhpogenesis in the shape and size (F1 and F2) of the affected nodes. Note the partial alopecic woolly areas above the enlarged nodes.

Fig. 3: Showing the characteristic onion appearance of the surgically excised node (mandibular node).

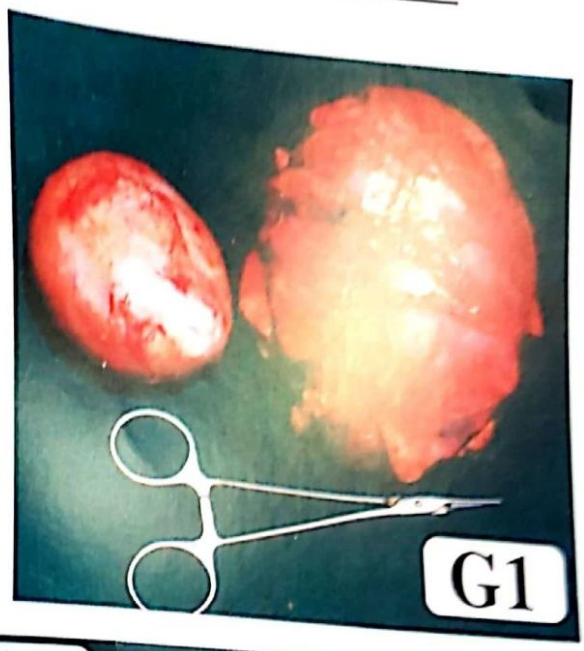

Fig. G, G1 & G2: Severe bilateral enlargement of the prescapular nodes (G) of a 3 year old ram experimentally inoculated with streptomycin-resistant *Corynebacterium pseudotuberculosis*. The time interval between inoculation and the development of such lesions was 143 days. Note: The marked alopecic woolly areas above and surrounds the enlarged nodes (G), the dismorphogenesis in the shape and size of the surgically excised nodes (G1) and the characteristic laminated caseous necrosis (onion appearance).

Fig. H: BCG vaccinated goat with abscess formation in the parotid lymph node as a result of experimental inoculation with streptomycin-resistant of *Corynebacterium pseudotuberculosis*. Note: There is no area of alopecic above the lesion.