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AN OUTBREAK OF CUTANEOUS CRYPTOCOCCOSIS
 IN FEEDLOT SHEEP FLOCK
 AT ASSIUT GOVERNORATE - UPPER EGYPT
 (With 9 Figures)

By

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انتشار مرض الكريبتوكوكسيس الجلدي
 في قطيع أغنام للتسمين
 بمحافظة أسيوط - صعيد مصر

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كان الهدف من هذا البحث هو توضيح الصورة الأكلينيكية مع معرفة الاسباب المحتملة لقطيع من الاغنام يعانى من عقيدات جلدية بمزرعة منقباد العسكرية بمحافظة أسيوط وكذلك عمل اختبار الحساسية للميكروبات المعزولة واتضح أن ٦٧.٦٠% من الأغنام الناضجة بهذه المزرعة كانت تعانى من هذه العقيدات وقد ذكرت الاعراض الاكلينيكية والصفة التشريحية للأغنام النافقة . كما أظهر الفحص الميكروبى لهذه العقيدات عن عزل خميرة الكريبتوكوكس نيوفورمانس ويعمل اختبار الضراوة فى فتران التجارب اتضح أنها من النوع الممرض . كما أن من أهم الأعراض الأكلينيكية التى ظهرت على الحيوانات المصابة عبارة عن عقيدات جلدية شبه مستديرة الشكل وسهلة النزاع من مكانها فى أماكن متفرقة من جسم الحيوان خاصة على الجانب الداخلى للذيل (البلية) . هذا وقد شوهدت صعوبات تنفسية على معظم الحيوانات المصابة، وربما يلعب الحمام دورا فى حمل ونقل العدوى بهذا النوع من الخمائر، كما أن الإصابة الشديدة بالديدان المعدية والمعوية ربما تهيئ فرصة للإصابة بهذا المرض عن طريق تثبيط الحالة المناعية وبمعمل اختبار الحساسية فى المعمل وجد أن عصارة الثوم ذو تأثير فعال على الخميرة المعزولة يعكس المركبات الأخرى التى أختلفت نتائجها .

SUMMARY

The primary goal of this research was to clear up the clinical observations and probable aetiological agents of cutaneous nodules of a sheep flock belonged to Military farm located in Manqubad village at Assiut Governorate. Source of infection as well as antibiogram of isolated organism was also achieved. Of seventy nine adults sheep, forty eight were found to be suffering from cutaneous nodules. History, clinical and necropsy findings were described. Microbiological examinations and pathogenicity test (mice inoculation test) revealed that pathogenic strains of Cryptococcus (Cr) neoformans yeast cells were recovered from the collected samples. No bacterial nor fungal pathogens were detected. The chief clinical symptoms of infected sheep were semicircular caseated nodules on various parts of the animals' skin particularly on the inner side of the tail. Respiratory disorders were observed in the most infected animals. Pigeons may act as carriers hosts and they may play a pivotal role in spread of Cr. neoformans infection. Apparently, heavy infestation of gastro-intestinal parasitism may produce a chance for cryptococcal infection through minimizing the immunological states of the host. Cr. neoformans was overstrongly sensitive to garlic juice in vitro in comparison with nystatin. Neomycin and polymixin B were found to be infective.

INTRODUCTION

Cr. neoformans is one of the principal pathogenic yeasts responsible for animals cryptococcosis and infections are usually gained by respiratory route as a pulmonary disease with secondary haematogenous spread to other part of the infected host (CARTER, 1986).

Reports on ovine and caprine cryptococcosis are uncommon, however, cryptococcal skin lesion on top of the head of a British Alpine buck was reported by CHAPMAN et al. (1990). In large animals, on the other hand, cryptococcosis was recorded as sporadic cases by RAHMAN et al. (1983), RAMADAN et al. (1989) and ZAITOUN et al. (1991). In Egypt, cutaneous cryptococcosis in sheep is still scanty in the available literature.

Pigeons and their droppings were implicated as harbour hosts for Cr. neoformans infection (STENDERUP et al., 1989 and WEBER & schaffer, 1991).

In the late days of Dec., 1989, seven heads of sheep from Military sheep farm located in Manqubad village near Assiut city, Egypt, were admitted to Veterinary Hospital, Assiut University, with a history of anorexia and raised semicircular nodules on the various parts of the animals' skin accompanied by respiratory disorders.

Symptomatic treatment for these admitted cases by wide spectrum antibiotic and antiparasitic drugs were unsuccessful. Another diseased sheep, one week after, were admitted to the same hospital with very similar signs plus additional informations of subsequent spread of the same picture among sheep flock.

A visit to the respective farm was carried and thorough clinical and microbiological investigations were adopted to reach for the probable aetiological agents, where cryptococcosis was suspected to induce such clinical picture. Source of infection as well as antibiogram of isolated microorganism were also achieved.

MATERIAL and METHODS

Animals and farm characteristics:

Immediately, after visting, it was noticed that the general health condition of sheep flock was inferior. Farm's veterinarians declared that this flock had a previous exposures to heavy infestation by gastro-intestinal parasites prior the present outbreak. The investigated flock was consisted of 90 animals: 79 adults (3-5 years in age) and 11 lambs ranging from one week to one month. All sheep were kept at night in a close barn with rice straw litter while at day all were left in open grazing area with exception of lambs. These, after feeding with colostrum, were set apart from their dams and were fed then on other sutiable ration. Sometimes, sheep flock were grazed in cultivated land for soil fertilization. Towers' pigeons were frequently seen in these areas (Manqubad and neighbouring villages).

Samples collection and culturing procedure:

Twenty three of infected animals were selected according to the severity of clinical signs and subjected to clinical, bacteriological and mycological examinations.

Some of cutaneous nodules of each animal were incised and the evacuated contents were inoculated in brain heart infusion (BHI) broth and

Sabouraud's dextrose (SD) broth for bacterial and mycotic growth respectively. The inoculated BHI broths were incubated aerobically at 37°C for 24-96 h. and were daily streaked then on BHI agar and 5% ovine blood agar while SD broths were incubated for 7 days at both room temperature (25°C) and at 37°C and were plated then onto SD agar plates supplemented with chloramphenicol and with and without cycloheximide and incubated at the same both temperature for four weeks with daily observation. Direct microscopic smear were also made from different nodules stained by Gram's stain.

Bacterial and fungal isolates were identified as the methods described by CARTER (1986). Isolated yeast, on the other side, was identified on the basis of morphological feature of colony, encapsulation, growth on corn meal agar medium as well as biochemical reactions (AL-DOORY, 1980). Three pulmonary swabs from three freshly dead animals were taken and subjected to bacteriological and mycological examinations.

Pigeons:

Three of pigeons were captured and isolated in a separate cage for one week and were then slaughtered. Samples of heart, liver, lung, kidney, brain and intestinal contents (large intestine, rectum and cloaca) were collected from each bird and subjected to mycological examinations.

Animal inoculation:

Isolated yeast was cultured in BHI broth for 72h. and then harvested by centrifugation (1000 xg for 15 min). The harvested cells were washed three times in physiological saline and suspended in phosphate buffer saline pH 7 and were then inoculated in white mice (intraperitoneal route) to determine the degree of pathogenicity (CARTER, 1986). Control mice were utilized.

Antibiogram:

Antibiogram of isolated yeast was done by using some drugs namely neomycin 25 ug, polymixin B 10 ug and nystatin 100 ug & 200 ug as well as garlic juice. Garlic (*Allium sativum*) juice was prepared in concentration of 250 ug per disc by the methods described by ZAITOUN *et al.* (1991).

RESULTS

Clinical observation:

Clinical observations of infected sheep flock revealed that 60.76% (48

of 79) of adults sheep were suffered from cutaneous nodules. Lambs appeared clinically devoid of any obvious symptoms. All infected animals had the same clinical picture; a wide spread of easily detached elevated, caseated, semicircular nodules about 1 to 5 mm. in diameter (Fig. 4 & 5) on the various part of the animals' skin particularly on the inner side of the tail (Fig. 1 & 2). No signs of pain were noted during palpation of these nodules. By incision, nodules contain white creamy caseated material similar to clotted milk (Fig. 6). Yeast cells were easily demonstrated from such caseated material by direct microscopic smear stained by Gram's stain. Some of these nodules were spontaneously ruptured discharging a thick grayish honey like matter heavily infected with yeast cells. Another nodules were sloughed out leaving erosions (Fig. 3) and contaminated, thereafter, by pus producing organisms.

Most of infected animals had bilateral mucopurulent nasal discharge with signs of dyspnoea accompanied by slightly elevation of body temperature. Two ewes were suffered from painful and hardness mastitis in both quarters yielding a thick yellowish milk with characteristic odour.

Three of adult sheep were succumbed and the predominant necropsy findings were severe pulmonary congestions and tracheal tubes were occluded with purulent discharge and caseated material.

Microbiological examinations:

Microbiological examinations revealed that Cr. neoformans was recovered from all collected samples whereas no significant bacterial nor fungal pathogens were detected.

Pigeons:

The investigated pigeons appeared clinically healthy while necropsy findings revealed that one of three pigeons was suffered from tape worm infestation and brain's capillaries were engorged with blood. From this case, Cr. neoformans was isolated from intestinal contents (large intestine, rectum, cloaca) whilst the other organs (brain, heart, lung, liver and kidney) showed negative isolation.

Mice inoculation test:

The inoculated mice died after two weeks (17 days) and marked capsulated Cr. neoformans cells were easily demonstrated from internal organs particularly brain and lung. On the 13th post inoculation day, Signs of drowsing were markedly observed in inoculated mice. Cr. neoformans were re-isolated from brains of inoculated mice and cultured onto BHI agar and incubated at 37 C for three days. India ink wet mount preparation is greatly helpful in demonstration of capsule.

Antibiogram:

Antibiogram of isolated Cr. neoformans revealed that neomycin and polymixin B were ineffective while nystatin had slight effect (Fig. 7). Cr. neoformans, on the other hand, was overstrongly sensitive to garlic juice in vitro (Fig. 8).

DISCUSSION

The investigated farm was built in the west side of Manqubad village and there are agrarian reform for the surrounding land for vegetable and wheat cultivations. Generally, these areas are characterized by cucurbitaceous cultivation using pigeon droppings as soil fertilizer. On the other side, there are two neighbouring villages (Baheig and El-Ouder) to Manqubad are highly characterized by Pigeons lofts. Pigeons are fly at a wide scale particularly in corn season (September to October). Cr. neoformans was isolated from pigeon droppings in a high percent (STENDERUP et al., 1989). This may appear, overwhelmingly, the origin of infection. Contaminated soil, on the other hand, is probably taken as a source of infection.

Our results indicated that towers' pigeons carried infection without any apparent clinical signs and Cr. neoformans seem to pass to the exterior environment with the excreta where yeast was isolated from the last portion of the large intestine. Pigeons and their droppings may play a pivotal role in animals cryptococcosis (WEBER and SCHAFFER, 1991).

Clinical examinations of infected sheep revealed that all infected animals suffered from cutaneous semicircular, elevated and caseated nodules which exist mostly on the inner side of the tail. Similar picture in sheep could not be traced in the available literature, however, distributed skin nodules over the neck, thorax and abdomen in a domesticated cat due to Cr. neoformans were reported by BERRY et al. (1990).

Generally, heavy infestation with gastro-intestinal parasites results in loss of serum protein through protein losing gastro-entopathy (SOULSBY, 1982). The expected deficiency of immunoglobulines in similar affections was interpreted by WEIR (1977) and could be extended to explain the high incidence of cryptococcosis among investigated sheep.

Results of antibiogram showed that Cr. neoformans has been found highly sensitive to garlic juice in vitro than other drugs. Therapeutic trials should be done to study the effect of garlic in vivo against cryptococcal infection.

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LEGNDES

- Fig. 1 & 2: Intesive cutaneous nodules due to Cryptococcus neoformans infection on the inner of the tail of sheep.
- Fig. 3: Different erosions resulting from spontaneously sloughed nodules on the tail.
- Fig. 4 & 5: Elevated semicircular nodules about 1 to 5 mm. contains white caseated material like clotted milk (Fig. 6).
- Fig. 7: Isolated Cryptococcus neoformans was slighty sensitive in vitro to nystatin 100 ug (A) and 200 ug (B) per disc.
- Fig. 8: Garlic (Allium sativum) juice 250 ug/disc had strongly inhibitory effect on isolated yeast
- Fig. 9: Colonies of Cryptococcus species on SD agar plate (supplemented with chloramphenicol without cycloheximide); circular. in shape whitis creamy in color and has slimy appearance.









