

IMMUNOPATHOLOGIC RELATION BETWEEN Nocardia asteroides AND Brucella abortus GAINED EXPERIMENTALLY

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SUMMARY

In order to demonstrated the pathologic changes and immune response of Brucella abortus strain 19. Vaccinated guinea pigs were experimentally infected with Nocardia asteroides, 24 guinea pigs of both six were divided into four equal groups. The first group was inoculated sub-cutaneously with Brucella abortus strain 19 vaccine. The second group was inoculated intrapulmonary with 1×10^3 C.F.U of viable Nocardia asteroides. Thirty days post-inoculation the first, second and third groups were challenged intrapulmonary with 2.5×10^9 C.F.U. of Nocardia asteroides, the last group served as control. The results showed that mortality was 100 % in non-vaccinated animals, 33.4 % in immunized animals with Nocardia asteroides and zero in Brucella abortus vaccinated animals.

Histopathological examination of dead animals showed pyogranulomatous lesions in examined internal organs.

Immunological studies showed that the skin test was positive in Brucella abortus vaccinated animals and in immunized animals with Nocardia asteroides while gel diffusion test was positive in Brucella abortus strain 19 vaccinated animals only.

INTRODUCTION

Nocardiosis is an acute or chronic contagious disease caused by Nocardia spp. It characterized by mycetoma or systemic form which infect lung, heart, kidney, spleen, liver, bone, joint and brain (1,2). In dairy farm, the disease causes high economic losses due to mastitis and death of infected animals (3). The pathogenic mechanism of nocardial infection have not understood, some workers have reported experiment indicating that cellular immune response plays important role in resistance (4). A rapid diagnosis of Nocardiosis is difficult due to its slow growth and to contamination. Vaccination against Nocardia need to control this zoonotic disease which affects most mammals including human being with worldwide distribution (5). Cross reaction between Nocardia and other bacteria was reported by some workers (6,7). Alwan (3) reported that Br. abortus strain 19 vaccine has significant influence on pathogenesis of Nocardial infection. The purpose of this is to demonstrate cross reaction between Nocardia and Br. abortus.

MATERIALS AND METHODS

Experimental animals:

Twenty four guinea pigs of 350-450 g. body weight of both sex were used. They were kept under daily observation to ensure their fitness for the experiment. They were housed in a previously disinfected cages of 15 x 15 x 40 cm and were allowed two weeks of adaptation before treatment.

Preparation of bacterial inoculum:

An isolate of N. asteroides was obtained from the milk of mastitic cow at Al- Dejala station. The isolate was identified and confirmed as mentioned by Alwan (3). The number of

viable organism in the inoculum was determined to Miles and Misera (8).

Experimental design:

The 24 healthy guinea pigs were randomly divided equally into 4 groups, group one was injected s/c with 0.5 ml of Br. abortus strain 19 vaccine containing 1.5×10^9 C.F.U. Groups two immunized intrapulmonary with 1×10^3 C.F.U. of viable N. asteroides. After 30-days post immunization, the 1st, 2nd, and 3rd groups were challenged intrapulmonary with 0.5 ml of bacterial suspension contain 2.5×10^9 C.F.U. of N. asteroides. The 4th group which served as control and was injected intrapulmonary with 0.5 ml of sterile normal saline.

Clinical signs, bacterial examination as well as pathological changes were recorded on animals.

Preparation of Nocardial antigen:

Ten days old culture of Nocardia asteroides in brain heart infusion broth was centrifuged at 1500 RPM for 15 min. The sediment washed twice and resuspend in 3 ml of sterile normal saline. Concentrated formaline (40 %) was added to this suspension in a ratio of 4 : 100 and then left overnight. The organisms were washed three times with sterile normal saline. The cells were then sonicated at 60 w (20 KGLS) intermittently in asonifier cell disrupter for 3 hr in cold and then centrifuged at 2000 RPM for 15 min. Ammonium sulfate at a concentration of (70 %) was added. The precipitate was the dissolved in sterile saline, then dialysed against normal saline for three days at 4 °C for the removal of ammonium sulfate, this was denoted as crude protein fraction (9). Total protein was then determined with foline phenol respected to serum albumin fraction (9).

Skin test:

Four weeks after challenge dose, animals of group one, two and four were injected intradermally in the right flank region with a dose of 0.2 ml of Nocardia antigen containing 10 mg/ml protein and in the other side the same dose contained 100 mg/ml protein was injected intradermally. The thickness of skin at the site of injection was measured with diatguge caliper immediately before injection and 24-48 hr after wards. The difference in thickness between the first and second readings was used to calculate the specific reaction.

Gel diffusion tests:

0.36 mg/ml protein and fresh serum collected from animals of first and second as well as the fourth groups. Plates were prepared using 10 ml of 1 % nobel agar containing 0.01 % Sod. azide as preservative. Wells (3 mm in diameter) were cut with a gel punch, four peripheral wells were separated from the central well by 8 mm (center to center). Undiluted serum from the animas was placed in the central well and crude protein fraction of N. asteroides (0.36 mg protein/ml) were placed in peripheral wells, result were read at 24-48 hrs.

RESULTS

Clinical signs:

All inoculated non-vaccinated animals become ill within 24-48 hrs post infection with temperature rising up to 41 °C with depression, anorexia, dyspnea, ataxia, in coordination of movement, paralysis of hind legs was seen during 1-5 days post-infection and all infected animals died during this period. Animals which were vaccinated with Br. abortus strain 19 vaccine showed significant resistance against 2.5×10^9 C.F.U. of N. asteroides cells. Those animals showed mild clinical signs

during the first 3 days post-infection but no one died from this group. Two animals of group two become sick and died at day 5 post infection.

Pathological changes:

Lung:

The macroscopic examination of lung revealed multiple grayish white, firm nodules scattered throughout the right and left lungs. Petechial hemorrhagic spots, congestion and necrotic areas were seen in right apical and diaphragmatic lobes. Microscopical section showed multiple pyogranulomatous lesions in the lung parenchyma. These lesions were formed of center necrotic neutrophils, zone of macrophase, and epitheloid cell as well as lymphocytes.

Heart:

The main lesions in the heart were multiple epitheloid granulomatous lesions together with necrosis of muscle fibers (Fig. 1).

Liver:

The central veins and sinusoids were congested together with hepatocellular swelling. Complex granulomatous lesions were observed in the liver parenchyma. They were consisted of necrotic center, PMNs, mononuclear cell, langerhans giant cells with fibrous connective tissue capsule (Fig. 2). Hepatocytic vacuolar degeneration was seen in midzonal areas.

Kidney:

The kidney had pronounced multiple pyogranulomatous lesion which are scattered throughout the interstitial tissues (Fig. 3). PMNs were aggregated in the renal tubules which are showed cystic dilatation as well as vacuolar changes.

Histological examination also revealed desquamation and sloughing of the epithelial lining of renal tubules.

Brain:

Pyogranulomatous lesions were the most significant lesion in the brain (Fig. 4), composed of caseous necrotic centers and surrounded by neutrophils, histocyte together with glial cell (gliosis). Congestion of blood vessels also seen with perivascular cellular infiltration.

Spleen:

Epitheloid granulomatous lesions are main lesions in the spleen together with destruction of red and white pulp.

Bacteriology:

N. asteroides was isolated from lung, kidney, liver, heart, brain and spleen of dead animals.

Immune Response:

Delayed hypersensitivity:

Animals which were immunized with Br. abortus strain 19 vaccine and N. asteroides showed positive skin reaction 4-weeks after subsequent challenge with N. asteroides. In the first group maximal reaction of 7 mm, and 13 mm was observed at 48 hr after intradermal injection of 10 mg/ml, 100 mg/ml protein respectively. Low, reaction, 6 mm and 10 mm was recorded in the second group after intradermal inoculated of 10 mg/ml and 100 mg/ml protein respectively at the same time.

Gel diffusion test:

The sera which were obtained from vaccinated animals (frist group) formed two precipitation lines with crude protein fracture of N. asteroides. Whereas sera which were taken from the second and four group showed negative reaction (table).

DISCUSSION

The results showed that Br. abortus strain 19 vaccinated animals have significant resistance against subsequently challenge with viable N. asteroides compared with non-vaccinated guinea pigs which died during 1-5 days post infection. Khalifa et al., (10) reported that mixing antigenic components of Br. abortus with N. asteroides stimulated immune response better than using these antigen alone. The results obtained from the present study gave indication that immunization with Br. abortus strain 19 vaccine provide protection against N. asteroides. This finding agrees with those described by Alwan (3). Mirden et al., (11) demonstrated across protection between Listeria monocytogenes, Brucella abortus and mycobacterium tuberculosis which has been attributed to shared antigen. Beaman and Smather (7) revealed that mice infected with N. asteroides had significant resistance against subsequent challenge with Listeria monocytogenes.

The pathological changes which observed in the internal organs of dead animals were in accordance with that reported by auther authors (3,2). Immunological study revealed that both immunized animals with Br. abortus strain 19 vaccine and N. asteroides gave clear positive skin reaction. It was previously reported that when certain pathogens of intracellular infection are introduced into host they activate the macrophage of reticuloendothelial system to engulf their antigen, break them, and excrete the product (12,9). The antigen activated small lymphocyte transform to large blast cell from which in turn propagate smaller specially sensitized lymphocyte which called, T. lymphocyte and these cells involved in cellular immunity or delayed hypersensitivity (4,13). Gregory et al., (13) reported that growth of N. asteroides markedly inhibited in activated macrophage. Krick and Rmington (15) found that immunization

with N. asteroides resulted in protection against subsequent challenge with N. asteroides.

Table 1 demonstrate that Br. abortus strain 19 vaccine gave clear humoral response against subsequent challenge with N. asteroides while immunized with N. asteroides gave negative humoral response.

This finding also may indicate a direct cross protection between N. asteroides and Br. abortus which may emphasize the existence of common antigens. The result of gel diffusion test is in agreement with those reported by Sundraraj and Agarwib (16). They found no humoral immune response in animal immunized with N. asteroides. The mechanism of pathogenesis and specific mechanisms of host immunity against nocardial infection have not been established clearly. Yet Beaman Smathers (7) suggested that cell mediated immunity plays a significant role in certain types of Nocardia infection. Beaman et al., (4) suggested that T. cells are essential for a marked host response against infection with virulent strain of N. asteroides. Immunological study revealed that Br. abortus strain 19 vaccine elicit both cellular and humoral immune response against N. asteroides.

Differences in mortality rate between first and second group indicate that cellular and humoral immune response has a role in protection of host against N. asteroides infection.

Table 1 : The immunological response of guinea pigs vaccinated with Br. abortus and N. asteroides after challenge with N. asteroides.

Animal Groups	Skin hypersensitivity						Gel diffusion reaction
	Protein concentration 10 mg/ml			Protein concentration 100 mg/ml			
	Average thickness before injection	Average thickness at 48 hrs after injection	Skin reaction	Average thickness before injection	Average thickness at 48 hrs after injection	Skin reaction	
1	3 mm	9 mm	7 mm	3 mm	16 mm	13 mm	Two ppt line
2	2.5 mm	8.5 mm	6 mm	2.5 mm	12.5 mm	10 mm	-
4	2 mm	2 mm	0	2 mm	2 mm	0	-

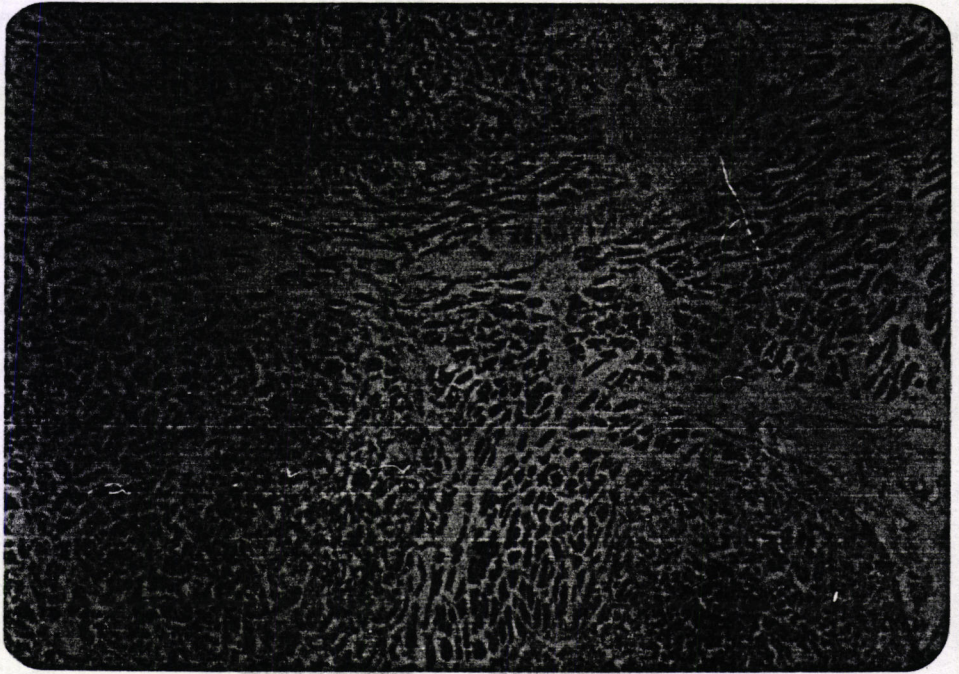


Fig.1- Heart of guinea pig, showing multiple pyogranulomatous lesions
[H and E.X100].

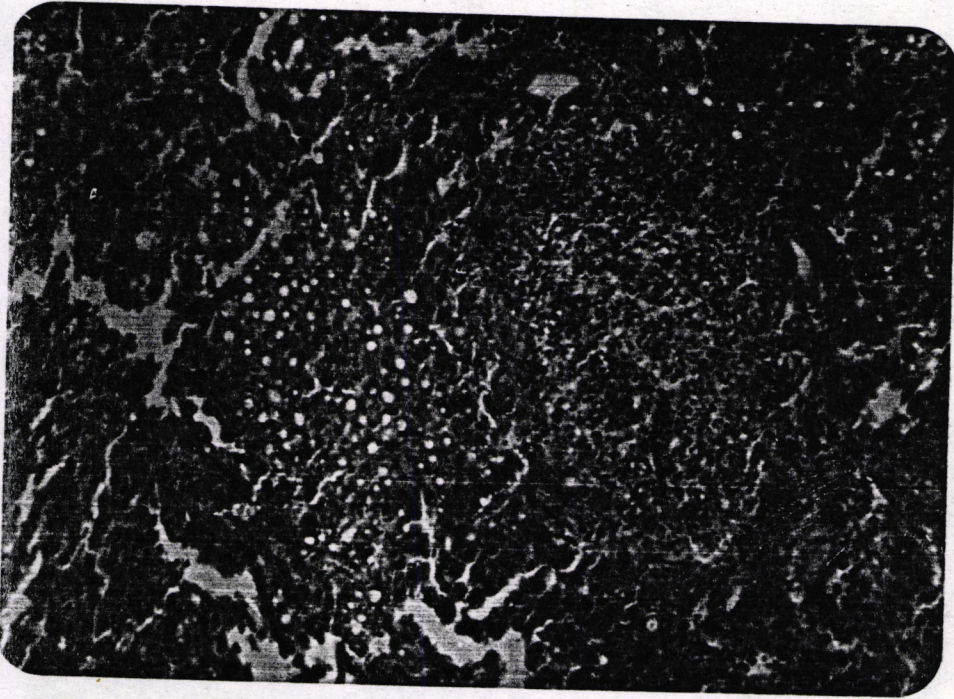


Fig.2: Liver of guinea pig, showing pyogranulomatous lesion with hepatocellular degeneration [H and E.200X].

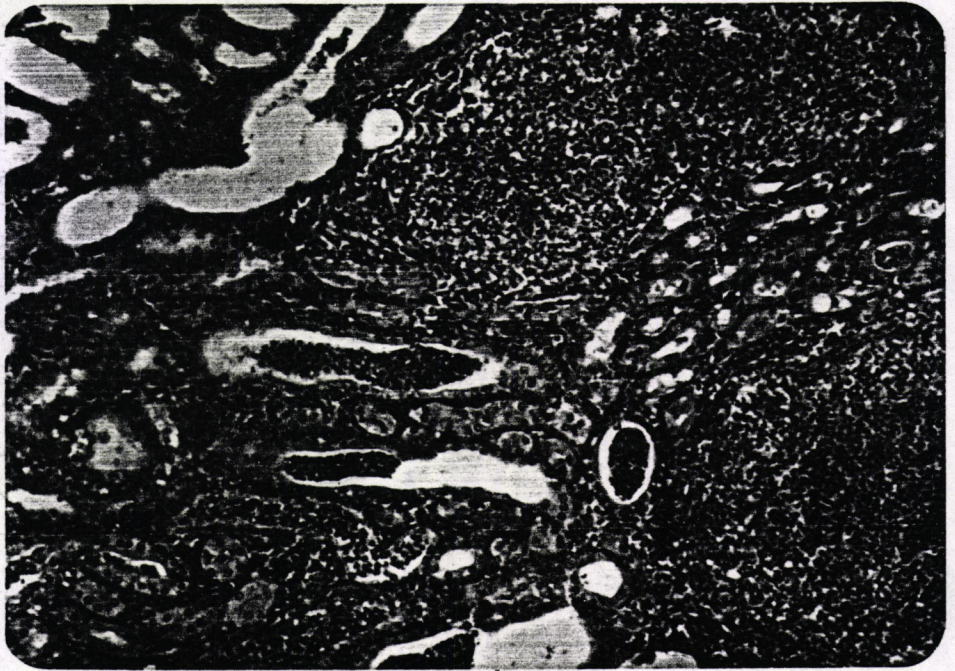


Fig.3: Kidney of guinea pig showing multiple pyogranulomatous lesions.
[H and E.200X].



Fig.4: Brain of gwinea pig, showing multiple pyogranulomatous lesions.
[H and E.100X].

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Br. abortus والعلاقة المرضية المناعية بين جرثيم النوكارديا و

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الخلاصة

لدراسة التغيرات المرضية والاستجابة المناعية لخنازير غينيا الملقحة بـ Br. abortus والممنعة بجرثيم النوكارديا ، استخدم 24 حيوان من كلا الجنسين ووزعت بالتساوي إلى أربعة مجاميع ، المجموعة الأولى تحققت بـ Br. abortus البروسيل تحت الجلد والمجموعة الثانية حقنت مباشرة بالبرئة (1×10^3) من الجرثيم N. asteroides وبعد 30 يوما من التمنيع أعطيت جرعة تحدي (2.5×10^9) من جرثيم N. asteroides عن طريق الرئة إلى المجموعة الأولى والثانية والثالثة أما المجموعة الرابعة فقد استخدمت كحيوانات سيطرة.

أوضحت النتائج أن جميع الحيوانات غير الملقحة هلكت إضافة إلى حيوانين من المجموعة الثانية أما حيوانات المجموعة الملقحة بـ Br. abortus ، لم يهلك أي حيوان منها.

اتسمت التغيرات المرضية في الأعضاء الداخلية التي فحصت للحيوانات المهالكة بالورم الحبيبي القيحي.

أوضحت نتائج الفحص المناعي بأن حيوانات المجموعة الأولى والثانية أعطت تفاعل موجب في اختبار فرط حساسية الجلد إلا ان التفاعل كان أشد في الحيوانات الملقحة بـ Br. abortus بينما اختبار الانتشار المناعي (Gel diffusion) أعطى خطين من الترسيب في تفاعل الضد والمستضد في حيوانات المجموعة الأولى فقط.

لقد أعطت هذه النتائج إشارة إلى ان الاستجابة المناعية الخلوية والخلوية تلعب دورا كبيرا في مقاومة الإصابة بجرثيم النوكارديا في خنازير غينيا.