EXPERIMENTAL STUDY OF NOCARDIOSIS IN GUINEA PIGS

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SUMMARY

The pathology of local isolated strain of Nocardia asteroides was study in pigs . 60 animals were divided into two groups .

The Ist group (40 animals) was given a single intrapulmonary of $3.3X10^7$ CFU of N. astoroides . The 2 nd group (20 animals) served as control . Eight animals died during the first 3 weeks post – infection while the remaining were sacrificed at 6, 12, 24, 48 hrs and 4, 8, 16, 24 and 62 days P.I.

Early pathological examination revealed acute suppurative changes which seen turned into disseminated chronic form . Pyogranul matous and epithelioid granuloma have represented the main pathological changes at 8-24 days P.I. The results revealed arisein the level of bacterial counts followed by drop in count within 8-24 days P.I. Excepted kidney in which high bacterial counts persisted toward the end of the experiment.

INTRODUCTION

Necardiosis is an economical and public health disease caused by N.asteroides and it is affects a wild range of domestic and wild animals as well as human (1) .

Experimental induction of the disease was limited , this is probably due te the fact that the pathogenic Nocardia was difficult te propagate at the laboratory environment . Gordon (2) found miliary granulematous in the spleen , liver , intestine, kidney and lung of the guinea pigs during the first two weeks after intraperitonal . AL-Khafajy (3) reported that the guinea pigs inoculated with Nocardia asteroides showed weight loss , irregular pyrexia at 24 hr after infection .

The present investigation aimed to study the pathogeneses of Nocardia asteroides in guinea pigs .

MATERIALS AND METHODS

Experimental design

Sixty healthy guinea pigs are randomly divided into two groups , a group of 40 animals received an intrapulmonary dose of $3.3X10^7$ CFU of N.asteroides and cantrol groups of 20 animals which receive 1 ML of sterile brain heart infusion broth by the same route .

Four animals of treated group and two of control group were sacrificed at 6,12,24,48 hr and 4,8,16,24,42, and 62 days post-infection .

Preparation of bacterial inoculum

An isolate of N. asteroides was obtain from the milk of a mastitic cow at AL-dejiala station . The isolation was identified and confirmed as mention by Alwan (4) . The number of viable organisms in the inocolum was determined according to Miles et. al. , (5) .

RESULTS

Fig: 1 . Showed a numbers of CFU of N. asteroides recovered from internal organs of animals according to time of sacrificing .

Pathological changes

Grossly :

Macroscopic examination showed congestion and hemorrhagic changes in internal organs at 6-48h P.I. Multifocal necrotic areas were seen in liver , kidney at 4 days P.I. Nodular lesions of variable size and number were observed on examined organs at 8-24 days P.I. as well as sinus tract which connect the lung to chest wall . Firm fibrous adhesion between lung and chest wall of most infected animals were seen at 42-62 days P.I.

Microscopic appearance :

6-48h

In all animals , the intraalveolar tissues were distended due to vascular engorgment , odema and PMNs cells infiltration the adjacent are also infiltrated by neutrophils .

In the liver , the central veins and sinusoides were distended and congested together with acute cellular degeneration in addition to multifocal areas of hepatocellular necrosis with PMNs infiltration . In kidney , there was congestion of inter-tubular and glomerular capillaries with swelling of renal tubular epithelium . Inflammatory cells infiltration also reported between muscle fiber of there were hyperplastic changes in white pulp . Gongestion of meningeal blood vessels were seen as perivascular odema .

4 days P.I.

Pathological changes in most of the eximined organs were generally in accordance with previously mention but were more extensive (Fig:) .

8-24 days P.I.

In all infected animals , pyogranulomatous lesions were the most significant lesions in the examined organs (Fig :).

The granuloma were formed of necrotic center contain neutrophils surrounded by a zone macrophages , lymphocyte , plasma cells along with few langhans giant cells . The outer zone was formed of few fibrous connective tissues later on , granulomatous lesions more epithelioid in nature (Fig:).

42-62 days P.I.

In the lung , there were multifocal organized pleuritis as well as multiple lymphoaytic foci which were mostly located around blood vessels and bronchioles (Fig:) . Multiple foci of mononuclear cells were seen in cortex of kidney , no significant lesions were observed in the other examined organs .

DISCUSSION

Nocardiosis varied in its severity from a self – limiting disease to a fatal disseminating . The recovery of N. asteroides from the lung at 6h and from spleen at 12 hrs P.I. suggested rapid dissemination via the blood to other organs .

This finding was concomitant with Hiramine et.al., (6) whe found that intratracheal inoculation of $5X10^6$ CFU of N. asteroides induced localized lesion in guinea pigs while $5X10^7$ CFU of these organisms led to the dissemination of infection .

Understanding for the development of the pathological changes induced by N. asteroides . These changes include : acute suppurative , subacute, pyogranulomatous and epithelioid granulomatous reaction . These findings agreed with those observed by Floba et. al. ,(7) , jubb et. al. , (8) , kissqne , (9) and AL- kafajy (3) .

Gentral necrosis which accompanied with granulomatous lesion in the present study agree with other reports in tuberculosis and actinomycosis (10) . Hypersensitivity reaction produces necrosis as well as lysosomal enzyme released from dead phagocytic cells (11) .

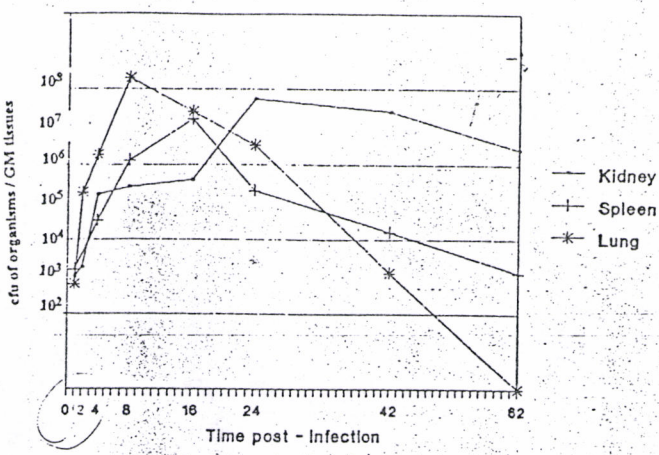
The presence of epithelioid granulomatous may indicate that our isolates of N.asteroides is virulent .

Miller et. al. , (12) reported that cord factor which was isolate from Nocardial cell wall elicited granulomatous lesion in mice after S/C injection .

The clearance and reventitation of alveoli which was associated with lymphoid hyperplasia in the interstial tissue on day 42-62 P.I. give the impression that the lung has started to recover from infection and have began resolution processes . The disappearance lesions at that peroid was not unexpected since Adam (13) reported that the epithelioid granulomas may persist untill the invassive microorganisms are destroyed , then the lesions will slowly resolve as the mononuclear cells die or revert to less mature from and further develop into simple chronic inflammationf .

Results of bacteriological examination showed that the number of viable N. asteroides was recovered from the examined organs correlated well with pathological changes . This data gava the impression that the body resistance against nocardial infection was not effective till after the 3 rd week P.I. when bacterial count started to decline . The peak of bacterial counts which was recovered in the liver was highest at day 8 P.I. in comparison with other organs with exception of that of the lung which was even higher . This might be due to the availability of good growth conditions for N. asteroides in the liver . similar

observation were reported by eaman and Maslan (14) in mice and AL- khafajy (3) in guinea pigs .



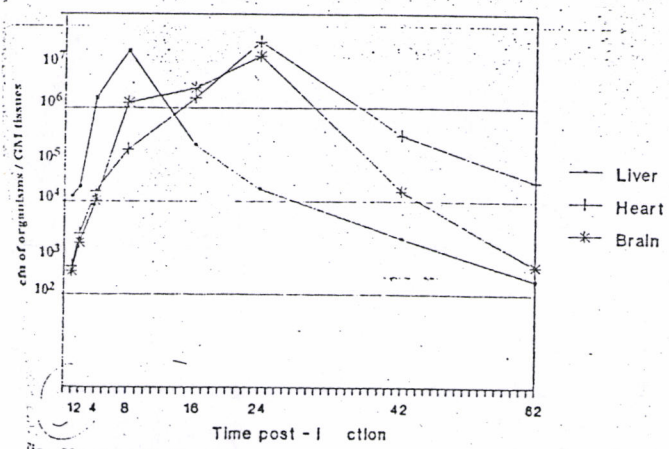


Fig.2. Number of cfu of N.asteroides recovered from some vital organs of guinea pigs . Each point represents the mean (±SEM) of four animals .

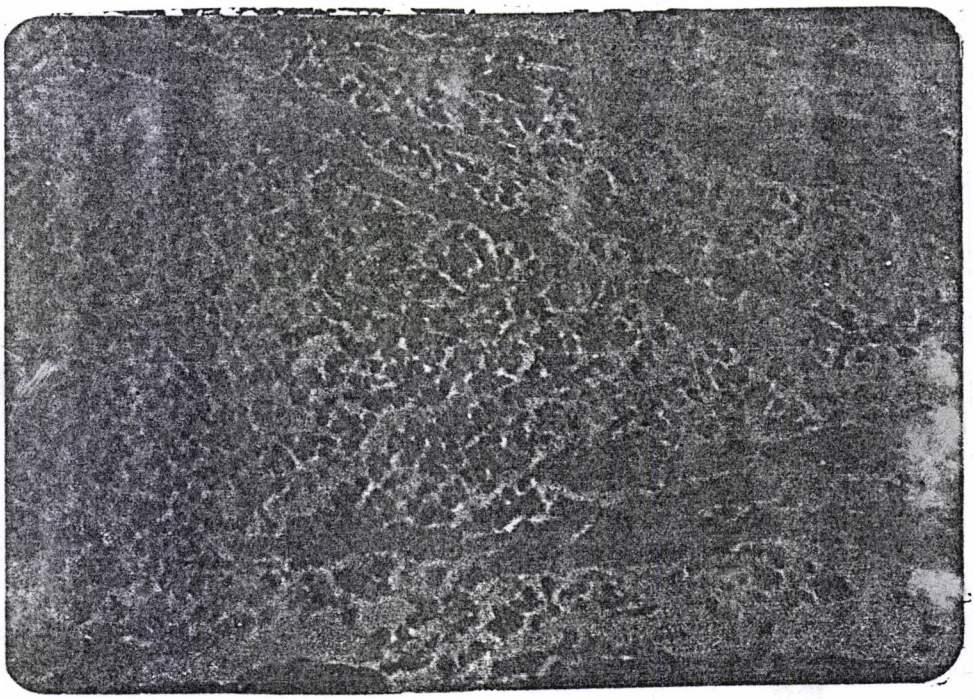


Fig: 2 :Heart of guinea pig sacrificed on day 4 showing cardiac muscle necrosis with aggregate-on of PMNs and mononuclear cells . (H & E . X 200)

Fig . 3 : Lung of animal sacrificed on day 8 P.I. There is a large granulomatous lesion which consisted of liquefactive necrctic center surrounded by a thick zone of epitheliold , mononuclear cells and enveloped [H & E. x 200]

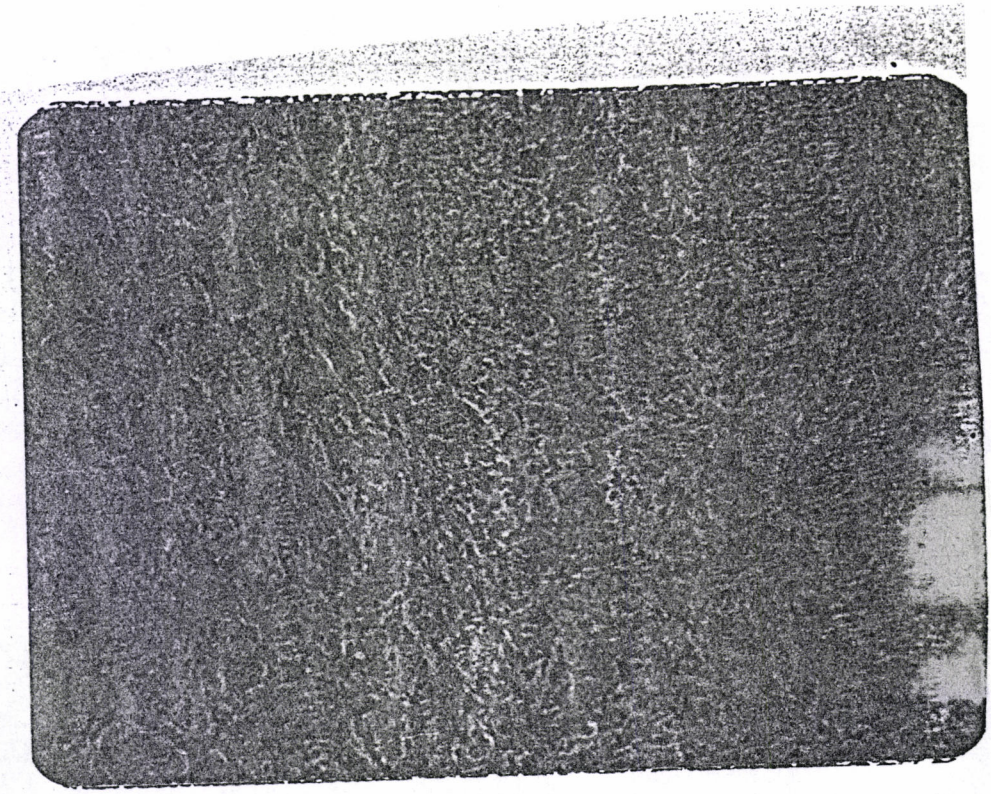


Fig . 4 : kidney of guinea pig sacrificed on day 8 P.I. showing a pyogranulomatous lesion (H & E . X100) .

Fig . 5 : Brain of guinea pig sacrificed at day 8 P.I. Notice multiple pyogranulomatous lesions (H & E . X100) .

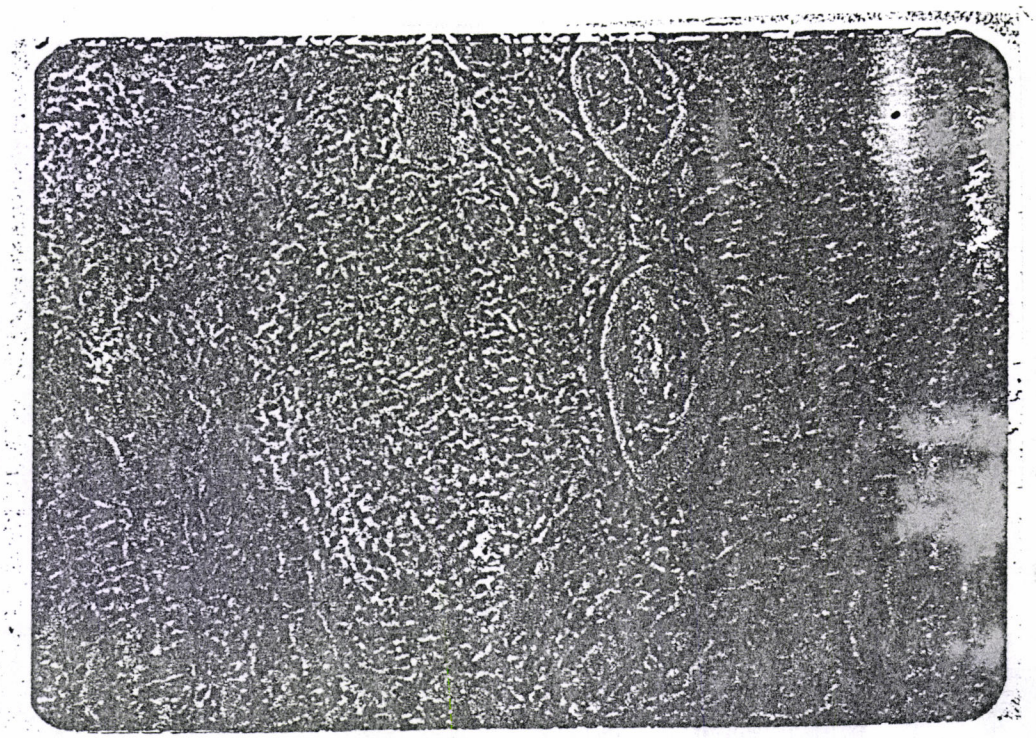


Fig . 6 : kidney of guinea pig sacrificed on day 24 P.I. Showing epitheloid granulomas with obvious necrotic center (H & E . 100 X) .

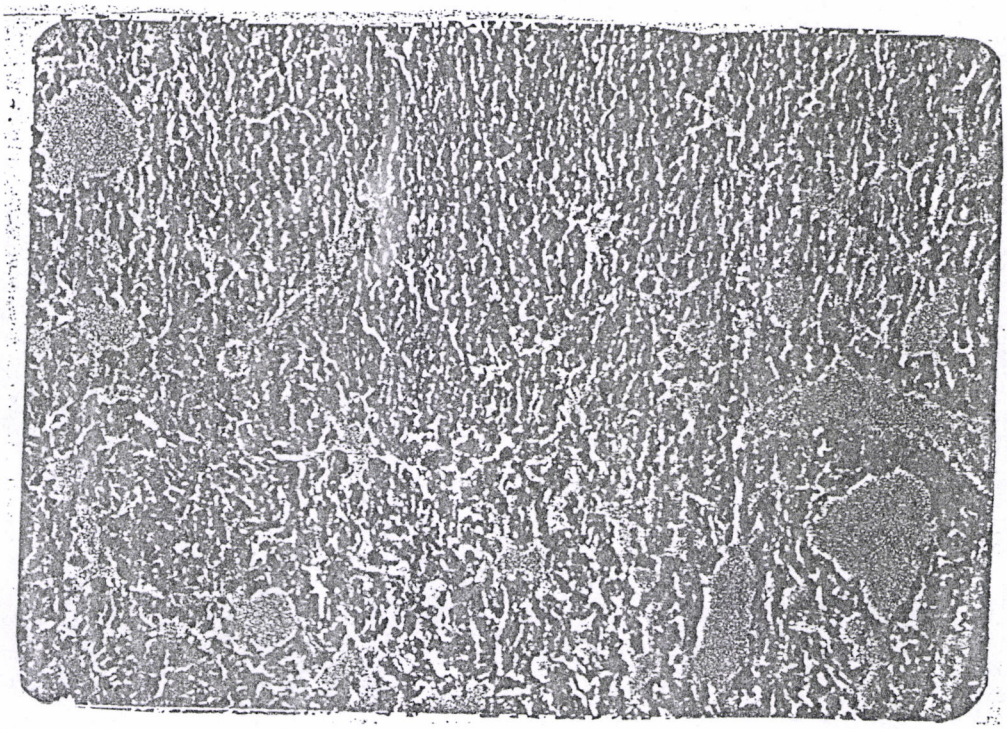


Fig . 7: Lung of guinea pig sacrifices on day 42 (P.I.) . Notice multible lymphocytic foci which were located around blood vessels (H & H. 100 X) .

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دراسة مرضية جراثيم Nocardia asteroides في خنازير غينيا

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الخلاصة

استهدفت هذه الدراسة امراضية جرثومية N. asteroides في خنازير غينيا ولتحقيق هذا الهدف أستخدم 60 من خنازير غينيا قسمت عشوائيا الى مجموعتين ، المجموعة الأولى (40) حيوان حقنت عن طريق الرئة مباشرة بجرعة تعادل $33X100^7$ CFU of N. asteroides أما المجموعة الثانية (20) حيوان استخدمت كحيوانات سيطرة حيث حقنت ب (1) مل من الماء المقطر عن طريق الرئة ايضا .

تميزت التغيرات المرضية بالتهابات قيحية حادة في الأيام الأولى للإصابة وتحولت إلى الطور المزمن ، انتشرت الإصابة إلى معظم أعضاء الجسم الداخلية وتحولت الإصابة إلى التهابات حبيبيه قيحية وحبيبيه ظهرا نية أبتدأ من اليوم الثامن ولغاية الرابع والعشرون بعد الحقن كان مستوى العد الجرثومي في معظم الأعضاء الداخلية على توافق مع شدة التغيرات المرضية حيث وصل إلى أعلى مستوى له في الأيام 8-24 بعد الحقن ثم بدأ بالانخفاض بعد اليوم الرابع والعشرين في الأعضاء التي تم فحصها باستثناء الكلية حيث أستمر العمل الجرثومي إلى نهاية التجربة .