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## SCREENING PICTURE ON PASTEURELLOSIS IN A FLOCK OF SHEEP AT ASSIUT GOVERNORATE

(With One Table and 10 Figures)

By

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### صورة ايضاحية عن مرض الباستيريليا فى قطيع الأغنام بمحافظة أسيوط

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فى هذا البحث تم تشخيص مرض الباستيريليا فى قطيع من الأغنام ( ١٠٠ رأس ) تتراوح أعمارها من ٦ - ١٢ شهراً تربى على أرض صحراوية بقرية جحدم بمحافظة أسيوط بعد دراسة أعراض الأكلينيكية للحالات المريضة والفحوص البكتريولوجية والبيوكيميائية والحقن فى حيوانات التجارب والفحوص السيولوجية للميكروبات المعزولة من الحيوانات المريضة والنافقه وكذلك بعد عمل الصفات التشريحية والهستوباثولوجية للمعينات التى أخذت من الأغنام النافقه. مرض الباستيريليا فى الأغنام كان يتميز اكلينيكياً بأعراض الالتهاب الرئوى الذى كان من النوع الفيبرينيوجينى التكرزى وأعراض التسمم الدموى الحاد الذى ظهر فى صورة استسقاء تحت الجلد فى منطقة البطن والمدر والأرجل الأمامية للأغنام المصابة والنافقة. تم عزل وتصنيف عدد ٤٣ عترة من الباستيريليا ملتوسيدا من النوع ( أ ) كارتتر من الأغنام المريضة والنافقه.

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### SUMMARY

During the period of September to October, 1992, pneumonic and haemorrhagic septicaemic signs appeared among a flock of sheep (100 heads) aged from 6-12 months old, raised on desert land of Gahdem at Assiut Governorate. The clinical conditions of sheep supported by the achieved result obtained from bacteriological: biochemical: laboratory animal inoculation, post-mortum and histopathological examinations conducted us to the correct diagnosis of the infected and dead sheep and the disease condition was pasteurellosis. The disease characterized by pneumonia (Croupus necrotic type). However, septicaemic forms were also observed, in which pulmonary lesions were minimum. Septicaemic form was characterized by extensive pectoral and abdominal subcutaneous oedema. 43 strains of *pasteurella multocida* type A (Carter) were constantly isolated from dead and infected sheep.

### INTRODUCTION

Pasteurellosis is one of the most important diseases which affects sheep as it cause severe economic losses among sheep in most parts of the world, through both death and depression of body weight gains. MARSH (1953) mentioned that term pasteurellosis is generally used as referring to the condition which for many years was known as haemorrhagic septicaemia.

MEGOWON *et al.* (1957); MISERA *et al.* (1970) and LOTFY *et al.* (1977) could isolate *pasteurella multocida* micro-organisms from lung tissues of sheep suffering from pasteurellosis. CALLAN *et al.* (1991) stated that bighorn sheep were particularly susceptible to *pasteurella multocida* and the virulent agents were not determined to have a role in the development of pneumonia in the bighorn sheep. MORAD *et al.* (1980) proved that *pasteurella multocida* type A strains were highly virulent to sheep and to higher mortalities than other strains.

The aim of this work was to investigate the picture of *pasteurella* infection in sheep by clinical: bacteriological: serotyping and pathological examinations.

### MATERIAL and METHODS

#### I- Material:

a) 20 nasal mucous swabs and 10 aspirated fluid exudates from abdominal, chest and legs swelling were collected under



aseptic conditions from 100 naturally infected sheep and were used for bacteriological examinations.

b) 40 samples of heart blood: epicardial fluids, tracheal frothy discharges: subcutaneous exudates and 30 tissue specimens from lung, heart and liver were collected aseptically from ten fresh dead sheep subjected to bacteriological examinations.

c) Specimens from different organs including lung, liver, heart, trachea, lymphnodes and subcutaneous tissues were taken immediately from dead sheep. These specimens were fixed in 10% neutral buffer formalin and furtherly prepared for histopathological examination by convential method.

## II- Methods:

a) Bacteriological examinations: Mucous swabs and tissue specimens were streaked on separate serum dextrose agar plate, while, other samples taken for bacteriological examination were inoculated directly on the same media and incubated at 37°C for 1-3 days. Suspected colonies were selected and subcultured in separate plates. The characters of the growing colonies and isolated organisms were described.

b) Biochemical tests: Suspected pasteurella isolates were tested biochemically according to methods of MADSEN et al. (1985).

c) Laboratory Animal inoculation: 45 white mice, 5 weeks old ranging from 40-50 gm in weight were inoculated intra peritoneally with the broth of each isolated strain of pasteurella multocida, as stated by RIMLER and RHOADES (1987).

d) Serotyping Examinations: All isoalted strains of pasteurella multocida were typed by rapid slide agglutination and indirect haemagglutination tests using known specific antisera of pasteurella multocida type A. B. D. and E (carter's type strains) were obtained from department of poultry diseases. Fac. Vet. Med., Assiut University. These methods were carried out as described by (BROGDEN and PAKER, 1979) and CARTER (1972) respectively.

## RESULTS

### History and clinical findings:

During the period of september to october 1992, a diseased condition appeared in a flock of sheep (6-12 months in age) raised on desert land of Gahdam at Assiut Governorate. Clinical examination showed that infected sheep suffering from signs of respiratory manifestations including bilateral mucopurulent nasal discharges, painful cough and painful grunting abdominal respiratory movement. Temperature ranging from 39.5-40°C anorexia: severe depression: stiffness in gait and gradual loss of weight. By auscultation moist rales could be detected. The



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conjunctiva was congested or bluish in colour (Fig. 1). Oedematous swelling of the breast in between fore-legs. The ventral part of the abdomen and the fore legs were also oedematous and swollen (Fig. 2). The mortality rate was 10%.

#### Postmortum examination:

Gross examination of dead sheep revealed that the most important lesions were localised in the lung and subcutaneous tissues of the ventral parts of the abdomen, breast and fore legs. The lung was severely congested, oedematous and heavy. Sometimes the surface of the lung was cyanosed (Fig. 3), cut section of the lung revealed frothy exudate comes out from the trachea, bronchi and bronchioles (Fig. 4). The lung tissue was firm and hepatized in some areas. The pleura of the lung was thickened and dull. Descetion of the subcutaneous tissue in the area of abdomen and breast revealed severe gelatinous infiltration of this tissue with whitish yellow colouration.

The liver was enlarged and congested and the gall-bladder was distended with bile. The blood vessels of the heart were congested and cyanotic. Petechial and echymotic haemorrhages were observed on coronary fat and at the apex of the heart (Fig. 5). The spleen was more or less unchanged.

#### Bacteriological examinations:

43 strains of *Pasteurella multocida* were isolated from dead and infected sheep. All the growing colonies were as the same characters: growing on the solid serum dextrose agar, but, no growth was noticed on MacConkey's agar. On serum blood agar, colonies were rounded, mucoid, smooth, medium sized, grayish in colour and non-haemolytic. Morphologically the isolated organisms were gram negative, short rods, coccobacilli and non-motile. The characters of colonies and micro-organisms were found as described in Bergey's Manual of systemic bacteriology.

#### Biochemical tests:

The obtained results of the total 43 local isolated strains were tabulated as in table (1).

#### Laboratory animal inoculation:

Our results showed that all inoculated mice were died within 24h. after inoculation, while, the control mice survived. The results revealed that all isolated strains of *Pasteurella multocida* were highly pathogenic to mice. The morphological characters of the growing colonies and re-isolated strains from mice were similar to those obtained by the inoculated strains of the primary cultures. In addition, the re-isolated strains from mice when stained with Loeffler's



methylene blue stains appeared with more clear distinct bipolarity (Fig. 7).

#### Serotyping examination:

By using rapid slide agglutination and indirect hemagglutination tests, each isolated strain was typed using known specific antisera type A.B.D. and E. The results revealed that 43 local strains of *pasteurella multocida* were type A. according to classification of CARTER (1963).

Table (1)

| Test                     | Results of reactions |                 |
|--------------------------|----------------------|-----------------|
|                          | No. of positive      | No. of negative |
| <b>Production of:</b>    |                      |                 |
| Oxidase                  | 43                   | 0               |
| Urease                   | 0                    | 43              |
| Catalase                 | 43                   | 0               |
| Indol                    | 43                   | 0               |
| H <sub>2</sub> S         | 0                    | 43              |
| Methyl Red Reaction (MR) | 0                    | 43              |
| Voges-proskauer (VP)     | 0                    | 43              |
| Gas from glucose         | 0                    | 43              |
| <b>Acid from:</b>        |                      |                 |
| Glucose                  | 43                   | 0               |
| Mannose                  | 43                   | 0               |
| Fructose                 | 43                   | 0               |
| Galactose                | 43                   | 0               |
| Maltose                  | 0                    | 43              |
| Rhamnose                 | 0                    | 43              |
| Raffinose                | 0                    | 43              |
| Manitol                  | 43                   | 0               |
| Insitol                  | 41                   | 2               |
| Lactose                  | 0                    | 43              |

#### Histopathological lesions:

The most important pathognomic histopathological lesions were observed in the lung and subcutaneous tissue. The pleura of lung was thickened by oedema congestion, fibrin deposition and mild leucocytic infiltration. The interstitial septa of the lung was also greatly thickened by oedema, fibrin deposition and leucocytic infiltration (Fig. 7). The lymphatic vesseles in the interstitial tissue were dilated and thrombosed (Fig. 8). The bronchial system manifested severe inflammatory reaction. Most of the bronchi and bronchiol were filled with exudate consists of fibrin and leucocytes which in most cases undergone necrotic changes (Fig. 9). The epithelium of bronchi in most cases were



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desquamated and necrosed in few showing degenerative changes. The wall of the large bronchi showed inflammatory reactions manifested by hyperemia and leucocytic infiltration. The interalveolar capillaries of the lung were prominently dilated and filled with blood. The alveoli in focal area were filled with fibrin and leucocytes which in some focal areas undergone necrotic changes (Fig. 10). In some cases examined at an early stage the epithelium of the bronchi were hyperplastic. The interalveolar capillaries were congested. Focal area of mononuclear cells reaction were observed in the alveolar tissue. Examination of the trachea revealed degeneration of the tracheal epithelium. The wall of the trachea showed hyperemia and mononuclear cells infiltration.

Histopathological lesions in the liver revealed focal areas of necrosis and haemorrhage associated with lymphoid and kuffar cells reactions. Most of the hepatic parenchyma suffered degenerative changes. The hepatic sinusoids were dilated and congested. Prominent hypertrophy of the kuffar cells was a constant feature. Sometimes these kuffar cells were proliferating. The epithelium of the large and medium sized bile ducts were desquamated and necrosed. The lumen of the bile ducts were filled with tissue debris consists of necrosed epithelium and leucocytes. Examination of the lymph nodes revealed oedema, haemorrhage and activation of the germinal center. The subcutaneous tissue was infiltrated with oedema and fibrin with few leucocytic infiltration. Blood vessels and capillaries were congested and there was haemorrhage in some areas.

### DISCUSSION

Clinical signs suggesting septicaemic and pneumonic pasteurullosis has been observed in sheep raised on desert land of Gahdam at Assiut Governorate. These signs which indicated involvement of the respiratory and cardiovascular system were equivalent to those observed by BUHR and ZUMPT (1965) and include bilateral mucopurulent nasal discharges, cough and grunting abdominal respiratory movement. Some cases showed oedematous swelling of the abdomen, breast and lower parts of the fore legs.

In this study 43 strains of *Pasteurella multocida* were isolated from dead and infected sheep. They were identified by morphological, cultural, biochemical and serological characteristic to be of type A strain (carter). Our results were not in agreement with those obtained by OCHI (1952) who isolated type B strains from cattle, swine, sheep and Rabbits. While, LOTFY *et al.* (1977) isolated 7 strains of *Pasteurella multocida* type B (carter) from pneumonic sheep. Also NIKIPHOROVA (1958) found type I (Roberts) or type B (carter)



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was a predominant cause of pasteurellosis in sheep, cattle and buffaloes. Moreover, *pasteurella multocida* type III was isolated from sheep with pneumonia in India (PANDE *et al.*, 1961). More recently CALLAN *et al.* (1991) isolated *pasteurella multocida* from lung tissue of dead sheep suffering from bronchopneumonia and stated that viral agents were not determined to have a role in the development of pneumonia in the bighorn sheep. MORAD *et al.* (1980) mentioned that *pasteurella multocida* type A strains were highly virulent to sheep and led to high mortalities while type B produced no mortalities.

The results of the post mortem examination showed that the most important lesions were observed in lung. However, in some cases septicemic picture overlap the pulmonary changes. In such cases pneumonic lesions were minimum and sometimes could be only discovered microscopically. It has been also observed that sheep in which the septicemic picture predominating has an extensive pectoral and abdominal subcutaneous oedema. These findings were in agreement with those described by BUHR and ZUMPT (1965) for sheep pasteurellosis. Microscopically the lung from dead sheep showed croupous necrotic pneumonia which could be considered as a pathognomonic lesions for sheep pasteurellosis (JUBB and KENNEDY, 1985). However, histopathological lesions pathognomonic for septicemic forms were also observed (MISERA *et al.*, 1970). In addition severe congestion, degenerative and necrotic changes were observed in the liver, prominent lesions were in the bile duct. These lesions in our opinion may be caused by the toxin of the *pasteurella multocida* organism. Toxins might be also the direct cause of oedema, hemorrhage and congestion of the lymph nodes and subcutaneous tissue, Kupfer cells activation in the liver and increased population of lymphoid cells of the germinal centers of the lymph node might represent an immune reaction to *pasteurella* infections.

From these studies, it has been concluded that pneumonic and septicemic pasteurellosis could be diagnosed histopathologically in sheep at Assiut Governorate. *Pasteurella multocida* type A strains (carter) was constantly isolated from diseased sheep.

## REFERENCES

- Bergey's Manual of systemic bacteriology (1984): Volume 1 page 552. published by Williams and Wilkins co., Baltimore, Md. USA.
- Brogden, K.A. and Packer, R.A. (1979): Comparison of *pasteurella multocida* serotyping systems. Am. J. Vet. Res. Vol. 40: No. 9: 1332-1335.



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- Buhr, W.H. and Zumpt, I.F. (1965): A note on a disease of sheep probably pasteurellosis in male king district. J. South Africa. Med. Asso., Vol. 26: No. 1: p. 1965.
- Callan, R.J.; Bunch, T.D.; Workman, G.W. and Mock, R.E. (1991): Development of pneumonia in desert bighorn sheep after exposure to a flock of exotic wild and domestic sheep. J. Am. Vet. Med. Asso., Vol. 198(6): 1052-1056.
- Carter, G.R. (1963): Immunological differentiation of type B and E strains of *pasteurella multocida*. Canad. Vet. J. ol. 4: 61-63.
- Carter, G.R. (1972): Improved haemagglutination test for identifying type A strains of *pasteurella multocida*. J. App. Microbiol., Vol. 24: 162-163.
- Jubb, K.V.E. and Kennedy, P.C. (1985): Pathology of domestic animals. 3<sup>rd</sup> Edition. Vol. 2. pp. 493.
- Lotfy, O.; Barakat, A.; Fayed, A.A.; Eldin, M.S. and Awad, H.H. (1977): Typing of 14 *pasteurella multocida* strains of animal origin. The 1<sup>st</sup> Arab Biologists congress. Alexandria 26-30 Oct. 1977. Egypt.
- Madsen, E.B.; Bisgaard, M.; Mutters, R. and Pedersen, K.B. (1985): Characterization of *pasteurella* species isolated from lungs of calves with pneumonia. Can. J. Comp. Med., Vol. 49: 63-67.
- Marsh, M. (1953): The role of *pasteurella* in sheep diseases. J.A.V.M. Asso., Vol. 123: 205-208.
- Mcgowon, B.; Maulton, J.E. and Sultz, C.L. (1957): Pneumonia in california Lambs. J. Am. Vet. Med. Asso., Vol. 131: 318-327.
- Misera, H.N.; Mulick, S.G.; Roa, C.C. and Kher, S.S. (1970): Analysis of results of bacteriological examination of morbid materials from cases of sheep pneumonia during period of 1959-1960. Indian Vet. J., Vol. 4: 714-720.
- Morad, M.; El-Amrousi, S.; Barakat, A. and El-Allawy, T. (1980): Experimental studies of some strains of *pasteurella multocida* from different animals in Assiut. Assiut Vet. Med. J., Vol. 7 No. (13&14): 341-346.
- Nikiphorova, N.M. (1958): Pasteurellosis in domestic animals in USSR. Bull. off. int. epiz., 50: 157-188.
- Ochi, Y. (1952): Haemorrhagic septicaemia and its prevention Bull. off. int. epiz., 38: 226-232.
- Pande, P.G.; Sekariak, P.C.; Shukia, R.R. and Baxi, K.K. (1961): A rare serotypes of *pasteurella multocida*. causing pneumonia in sheep in India. Current. Sci. 30: 267-268.
- Rimler, R.B. and Rhoades, K.R. (1987): Serogroup F. A new capsule serogroup of *pasteurella multocida*. J. Clin. Mic., Vol. 25. No. 4: 615-618.



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Fig. 1: Showed congestion and cyanosis of the conjunctiva.



Fig. 2: Showed oedematous swelling of abdomen, breast and fore legs.



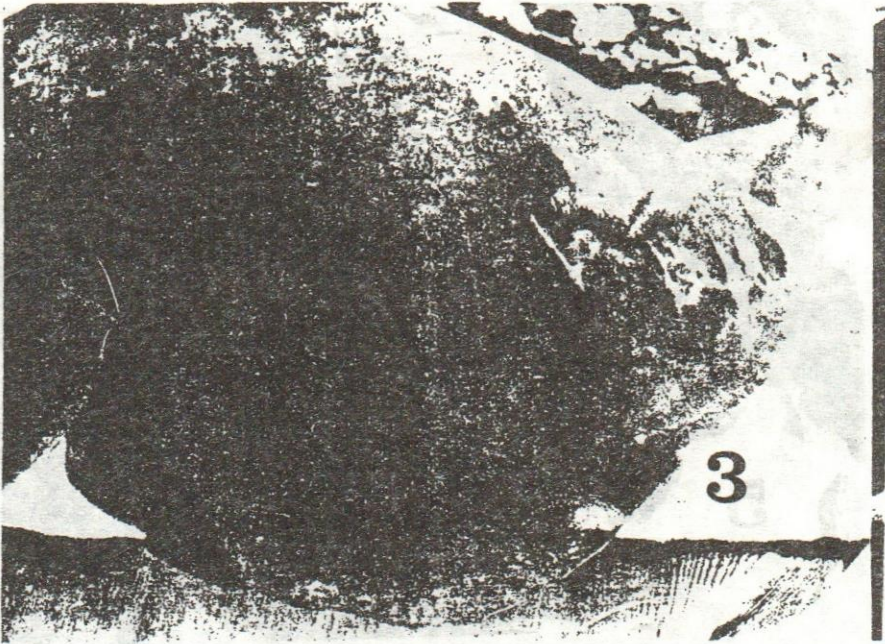


Fig. 3: Cyanosis of the surface of the lung.



Fig. 4: Frothy exudate filling the trachea.



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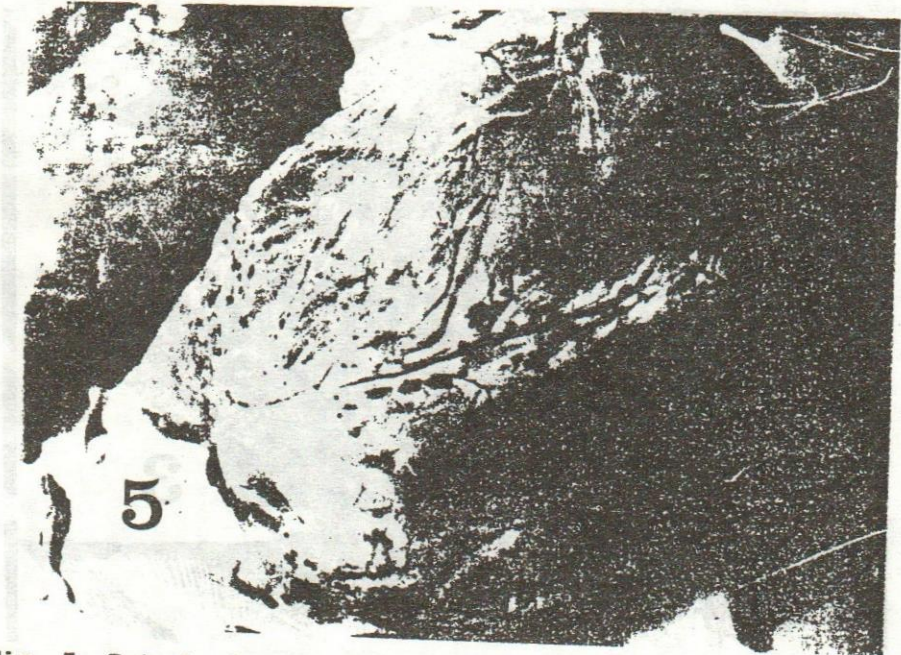


Fig. 5: Petechial and echymotic hemorrhage on the coronary fat of the heart.



Fig. 6: Showed bipolar pasteurella organis.





Fig. 7: Showed thickning of the interstitial septa of the lung.



Fig. 8: Showed dilatation and thrombosis of the lymphatics.



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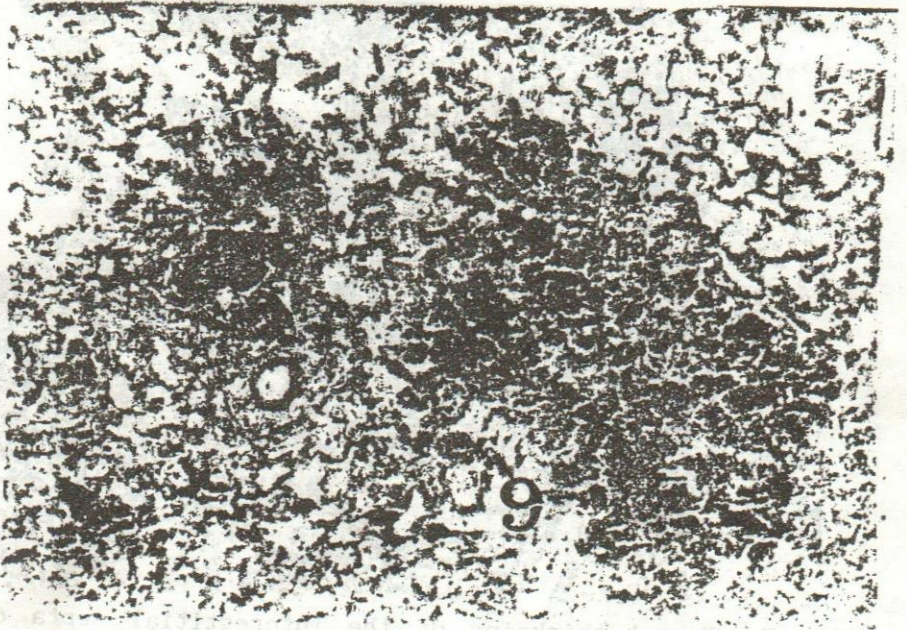


Fig. 9: Showed focal area of necrosis and bronchi filled with exudate.

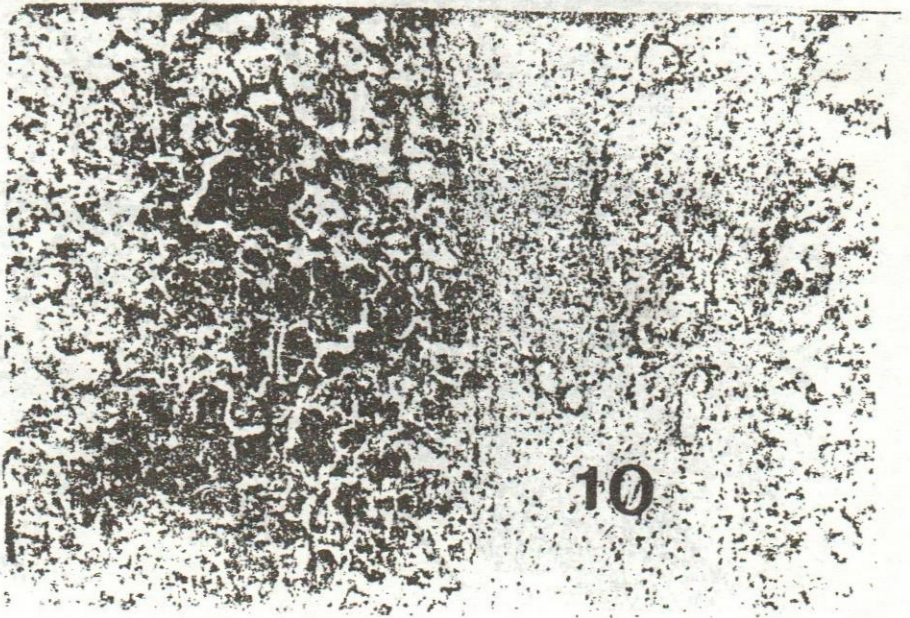


Fig. 10: Showed necrosis of the affected areas in the lung.