قسم الباثولوجيـــــا
كلية الطب البيطرى ـ جامعة أسيوط
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دراسة عن الحصبة الكلبية فى محافظة أسيوط

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أجرى البحث على عدد ثلاثه واربعون كلبا ضالا فى محافظة أسيوط . أخـــذت عينات دم منها وأجريت عليها الفحوص الأكلينيكيه وكذلك افلام دموية علـــــى شرائح زجاجية ثم صبغتها بعديد من الصبغات وفحصت جيدا . اخذت عينات من جميع اعضاء هذه الحيوانات بعد قتلها بالصدمة الكهربائية مباشـــــرة ثم فحصها بعد تمريرها وصبغتها بعديد من الصبغات .

أسفرت النتائج عن وجود اجسام ضمينية سيتوبلازم كرات الدم البيضاء فى عـــدد عشرة كلاب وكذلك وجود هذه الأجسام فى سيتوبلازم الخلايا الطلائية المبطنـــة لجدار المعدة والمثانة البولية فى بعض الكلاب ولقد درست خصائص هـــــذه الأجسام الضمينية وكذلك التغيرات الباثولوجية الموجودة بعد دراسة الأعـــراض الأكلينيكية وقد كانت واضحة على ستة عشرة كلبا .

نوقشت النتائج وأمكن تشخيص مرض الحصبة الكلبية وذلك بالاعتماد علـــــى وجود الأجسام الضمينية وكذلك التغيرات الباثولوجية والاكلينيكية .

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STUDIES ON CANINE DISTEMPER AMONG STRAY DOGS IN ASSIUT GOVERNORATE

(With 2 Tables & 6 Figs.)

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SUMMARY

Canine distemper was diagnosed in stray dogs obtained from diferent localities in Assiut Governorate. Diagnosis of the cases was based on the clinical symptoms observed, confirmed by the demonstration of inclusion bodies in mononuclear and poolymorphnuclear circulating leucocytes, hitopathological and haematological findings.

INTRODUCTION

Canine distemper is one of the serious fatal viral disease even in germfree dog, McCULLOUGH; KRAKOWKA and KOESTNER (1974). GORHAM, (1960) proved that in addition to the virus infection, bacterial and mycotic pathogens contribute significantly to mortality rate.

KRAKOWKA; HIGGINS and KOESTNER (1979) mentioned that the viraemic stage in canine distemper infection is usually associated with a leucocytic changes which mainly lymphopenia of both B and T cells. These may persist in the convalescence stage. Variability in the range and severity of the symptoms of the disease has been emphasized by many laboratory workers and clinicans and were assumed to secondery bacterial infection, CORNWELL et al. (1965). SCHALM (1961) found that absolute lymphopenia was a characterstic feature in the naturally infected cases with the disease. May accounts have been given on the pathologic alterations of natural distemper among canines, LAUDER et al. (1954), CAMPBELL (1957) and PITEL and BINDRICH (1958). CORNWELL et al. (1965), SMITH, JONES and HUNT (1974) stated that in experimental studies of distemper as in the natural diseased cases, the most prominent structural changes occur in the lungs,reticuloendothelial centres, central nervous system, integument and lymphoid tissues. Characterstic intracytoplasmic and intranuclear inclusions were demonstrated in the epithelial cells of various tissues as well as in microglia cells. HUNT et al. (1963) proved that the inclusion bodies could be histochemically differeniated from those of infectious canine hepatitis by their negative results for desoxyribonucleic acid reaction, while CORNWELL et al. (1965) mentioned that inclusion bodies in circulating leucocytes were evolved as a diagnostic technique for the disease. SCHALM and GRIBBLE (1974); SCHALM; JAIN and CORROLL (1975), ARCHER and JEFFCOTT (1977) demonstratedintra-erthrocytic inclusion bodies. The scopy of the present study is ,1, to throw some light upon the incidence of canine distemper among stray dogs at Assiut province. Secondly, to register the most prominent clinical, haematological and morphological findings in natural cases. Moreover the inclusion bodies in the circulating blood cells were studied to determine their possible significance in diagnosis of the disease in natural cases.

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MATERIAL and METHODS

The present study was carried out on forty-three stray dogs collected alive from different localities at Assiut province, EL-Weliedia, Abnub and Assiut, to the departement of surgery, faculty of veterinary medicine, Assiut University. Sex, age and weight of these dogs were recorded. The animals were put under clinical observation daily for a week. The sixteen animals which showed symptoms could be probably related to canine distemper were isolated, blood smear preparations from those suspected dogs were fixed in ethyl alcohol-ether, stained with haematoxylin and eosin Gimesa, Methylene blue and Maximow haematoxylin Azur-II-eosin and examined for inclusion bodies.

Differential leucocytic count was performed. Anticoagulated blood samples were collected from the jugular vein was used for haematological studies. Total erthrocytes, totalleucocytes, packed cell volume were estimated. Smears from the anticoagulated blood samples were stained for bile. haemosedrin and desoxyribonucleic acid, BANCROFT (1967).

Samples of tissues for light microscopy were taken from lung, stomach, intestine, lymph nodes, spleen, liver, kidney, urinary bladder and brain within half hour after killing by electric-shock were collected. The samples were fixed in 10% buffered formalin solution, paraffin blocks were made and 6-8 sections were stained and examined.

RESULTS

I - Clinical findings:

Sixteen out from forty three stray dogs obtained were clinically isolated. Sex, age and weight as well as the most prominent clinical features of suspected distemper infection were shown (table I) Although dyspenea was noticed in all animals, coughing and nasal discharge were observed in ten dogs. Diarrhea was seen in eleven animals. Thirteen dogs showed various degrees of foot pad hyperkeratosis. Nervous manifestations could not be observed in any of the animals. In addition no significan deviation from the normal body tempreature could be detected.

II- Haematological findings:

The findings were shown in table I,II. The findings pointed out that anaemia, leucopenia, neutropenia and lymphopenia, with mean values of 4.2, 3.3,1%, 36.7% and 7.0, were prominent and constant in ten animals. The packed cell volume was under the normal thershold in all the sixteen cases. Leucocytosis was seen in some dogs and lymphocytoss in others. In six animals eosinophilia was pronounced, but the monocytic count in all the animals was flactuating within the normal number of canine monocytes.

III- Microscopic findings:

Intracytoplasmic acidophilic inclusion bodies had been detected in the peripheral blood leucocytes of ten animals from the sixteen clinically suspected cases. The inclusions were rounded ot oval and their sizes ranged from 5.1-9.9 m. The majority of them were found in the peripheral rim of the cytoplasm. They were found in the cytoplasm of the neutrophilic cells (Fig. 1 a,b) as well as in th monocytes (Fig. 2). They were usually homogenous, sharply demarcated, singly located and occasionally surrounded by hallow zone. These inclusion bodies were feulgen reaction negative and showed also negative results when specific stains for bile and haemosedrin were used. But positive results with specific inclusion stain as Giemsa. Methylene blue and Maximow haematoxylin azur-II-eosin were obtained.

The lungs of thirteen clinically suspected cases showed varied degrees of pneumonia (Fig. 3), in addition to that three dogs showed suppurative bronchopneumonia.

Examination of the stomach revealed catarrhal gastritis in five animals, however intr-cytoplasmic acidophilic inclusions could be seen in the glandular epithelium of one case (Fig. 4).

Examination of the intestine revealed catarrhal enteritis in all cases. Six dogs showed parasitic enteritis with prominent submucosal eosinophilic cell infiltrations.

The urinary bladder showed variable degree of inflammatory process in all cases examined. The alterations consisted of congestion, oedema and lympohocytic infiltration. In two cases intraepithelial intracytoplasmic acidophilic inclusion bodies were detected (Fig. 5). They were mostly oval, homogenous and surrounded by clear hallow zone. In addition to that the transitional epithelium showed hydropic degeneration.

In the lymph nodes and spleen, moderate degree of lymphoid exhustion was constant finding. In addition some cases showed prominent plasma cell population.

In the liver, congestion and necrobiotic changes were marked in six dogs. However neither intracytoplasmic nor intranuclear inclusions could be detected.

Examination of the kidney revealed prominent focal interstitial nephritis (Fig. 6) in eight cases.

Microscopical examination of the brain revealed no pathological changes Table II summarize the results of the clinical, haematological and morphological findings for each dog.

DISCUSSION

In the present work, clinicl symptoms could be probably related to canine distemper were observed in sixteen dogs from forty three dogs examined. The clinical signs included dyspenea, footpad hyperkeratosis, depression, diarrhea,cough, nasal discharge, occular discharge and vomiting. However neither dermal nor nervous manifestations were observed. Table II showed variability in the range and severity of the clinical symptoms observed. Such variations could be contributed to the individual susceptability genetic factors, strain of the virus nd secondery pathogens, CORNWELL et al. (1965). Absence of fever inour animals could be attributed to the fact that the animals may be probably in the post-viraemic stage. In our haematological findings, table I,II, anaemia, leucopenia and lymphopenia were detected inten animals. These haematological findings correspond with those given by SCHALM (1961), SCHALM, JAIN and CORRELL (1975). The lymphopenia observed in our findings is probably explained by the lymphoid exhustion noticed in the lymphoreticular tissues. In six dogs eosinophilia was observed, this could be related to the parasitic entiritis could be diagnosed microscopically in these dogs. The neutrophilia observed in three dogs are blamed on the activities of secondery bacterial infection.

On microscopic examination, intracytoplasmic inclusion bodies could be seen in thirteen animals from the sixteen distemper suspected dogs. The inclusions either seen in the blood cells or the transitional epithelium of the urinary bladder or in the glandular epithelium of the stomach have the same staining properities. All of them were round to oval in shape, intrcytoplasmic in location and surrounded by hallow zones. In our present work and of particular interest, intracytoplasmic leucocytic inclusions were seen in ten abimals. ARCHER and GEFFCOTT (1977), SCHALM and GRIBBLE (1974) and SMITH et al. (1974) stated that such inclusions in leucocytes is a good evidence that the virus is present, but their absence is of little value in determining the absence

of the virus. Taking in consideration the concept of the previous authors, our results indicated that an accurate diagnosis of ten animals from the sixteen suspected cases basing upon the demonstration of intracytoplasmic inclusions in their peripheral blood leucocytes. These intracytoplasmic inclusions in the adult leucocytes suggests its eiral origin and probably related to an leucocytic viraemia as reported by CHEVILLE (1976), KRAKOWKA; HIGGINS and KOESTNER (1979). The appearance of inclusion bodies in the stomach and urinary bladder of three animals probably related to the predilection site for this viscerotropic virus. But the microscopic absence of both blood and tissue inclusions in three dogs may correspond with the previous claim tht their disappearance occurs at any stage of the disease, LAUDER et al. (1954). Neither intracytoplasmic nor intranuclear inclusion bodies could be observed in the hepatocytes of all dogs examined. Moreover the inclusions detected in the leucocytes or epithelium had the same staining characters of distemper inclusiond described by HUNT et al. (1963) and ZHDANOW (1975).

Lung inflammation with or without involvement of the bronchi appeared in all clinically suspected cases. This fact was in harmony with the concept UBB and KENNDY (1970), who stated that, proliferative and or exudative pneumonia, unassociated with canine distemper, seldom occur in dogs. Varied degree of interstitial pneumonia was seen in most of the cases and this could be considered as an universal reaction in viral infections. The bronchopneumonia seen in three dogs could be related to secondery bacterial infections. As reported in our findings varied degrees of catarrhal gastroenteritis, cytitis, focal interstitial nephritis and lymphoid depletion were described by LAUDER et al. (1954), CAMBPELL (1957), PITEL and BINDRICH, 1958, CORNWELL et al. 1965, JUBB and KENNEDY, 1970 and SMITH; JONES and HUNT, 1974).

Gross or hisopathological changes were not observed in the brain. This may be due to the strain of the virus. SMITH et al. (1974) stated that variants of the virus have been suggested as the causative agent in infection of the central nervous system or even nervous manifestation occur at the late stage of the disease.

From our results we can conclude that, the demonstration of inclusions in blood leucocytes could be used for accurate diagnosis of canine distemper in ten dogs form the sixteen suspected cases. In the rest of cases the diagnosis could be based upon the finding of inclusion in tissues, clinical, haematological and histopathological findings. The latter could be used to confirm the diagnosis. WATSON and WRIGHT (1974). The absence of inclusions does not preclude the diagnosis and the demonstration of th virus in tissues and smears in such cases requre the flourscence antibody technique.

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DESCRIPTION OF FIGURES

Fig. (1 a,b): Showing leucocytic inclusion. H.E. & X 1250

Fig. (2): Showing mononuclear inclusions. H.E. & X 1250

Fig. (3): Lung showing septal thickening. H.E. & X 322.5

Fig. (4): Showing epithelial inclusions in stomach. H.E. & X 322.5

Fig. (5): Showing epithelial inclusions in urinary bladder. H.E. & X 322.5

Fig. (6): Kidney showing interstitial nephritis. H.E. & X 322.5

Table (1)
Showing the results of th haematological parameters

No.	Total RBCs xlom	PCV %	Total WBCs	Diff. count					
				Lymph.	Neut.	Mono.	Eosi.	Baso.	Band
1	4	34	3.200	9	57	12	12	3	7
2	7	35	19.400	16	61	3	20	-	-
3	6	34	2.600	10	57	11	12	3	7
4	5	25	4.300	10	57	12	12	3	6
5	6	34	19.200	15	78	3	4	-	-
6	7	40	20.600	16	78	3	3	-	-
7	4	33	5.300	8	49	7	36	-	-
8	5	34	2.100	10	56	12	12	3	7
9	5	35	3.400	9	57	12	12	3	7
10	6	41	21.100	15	77	3	5	-	-
11	7	40	21.000	17	62	2	19	-	-
12	5	40	23.600	14	61	4	20	-	1
13	4	35	2.800	10	56	12	12	3	7
14	4	34	4.000	10	57	12	12	3	6
15	5	35	2.300	9	49	9	32	1	-
16	5	31	3.100	8	54	7	30	1	-

Table (II): Showing clinical haematological and histopathological findings

Dog No.	Sex	Age year	Weight Kg.	Clinical findings: Depression	Ocular discharge	Nasal discharge	Cough	Dyspnea	Pad hyper-keratosis	Diarrhea	Vomiting	Skin lesion	Nervous signs	Blood examination: Erythrocytes	P C V	W B CS	Diff. count: Lympho.	Neutro.	Mono.	Eosino.	Inclusion: Blood	U. Bladder	Stomach	Liver	Microscopic finding: Interstit. pneumonia	Broncho. pneumonia	Gastritis	Enteritis	Parasitic Enteritis	Interstit. nephritis	Lymphoid depletion	Brain lesion
1	M	2	8	+	-	++	+	+	++	-	-	-	-	o	o	o	o	o	=	=	+	-	-	-	+	-	-	+	-	+	++	-
2	M	2	8	+	-	+	+	++	-	+	-	-	-	=	o	x	x	=	=	x	-	+	-	-	++	-	-	-	+	-	++	-
3	M	2	7	+	-	-	-	+	-	+++	+	-	-	=	o	o	o	o	=	=	+	-	-	-	-	+	+	+	-	+	+	-
4	F	1	6	-	+	+	+	++	++	+	-	-	-	o	o	o	o	o	=	=	+	-	-	-	+	-	-	++	-	+	++	-
5	F	1	5	+	++	-	-	+	+	-	-	-	-	=	o	x	=	x	=	=	-	-	-	-	+	-	-	++	-	+	+	-
6	F	4	17	+	-	+	+	+	+	++	-	-	-	=	o	x	=	x	=	=	-	-	+	-	++	-	++	+	-	-	++	-
7	F	2	6	+	-	-	-	+	++	+	-	-	-	o	o	o	o	o	=	x	+	-	-	-	+	-	-	-	+	+	++	-
8	M	2	10	+	-	+	++	++	-	+	+	-	-	o	o	o	o	o	=	=	+	-	-	-	+	-	+	+	-	-	++	-
9	M	2	8	-	++	-	-	+	+	-	-	-	-	o	o	o	o	o	=	=	+	-	-	-	-	+	-	+	-	+	++	-
10	F	3	12	-	-	+	+	+	+	+	-	-	-	=	o	x	=	x	=	=	-	-	-	-	-	+	-	+	-	+	+	-
11	F	2	7	+	+	+	+	++	+	-	-	-	-	=	o	x	x	=	=	x	-	-	-	-	+	-	-	-	+	-	++	-
12	F	3	11	+	-	-	-	+	+	++	+	-	-	o	o	x	x	=	=	x	-	+	-	-	+	-	-	-	+	-	++	-
13	F	2	12	+	+	-	-	+	++	-	+	-	-	o	o	o	o	o	=	=	+	-	-	-	+	-	-	+	-	-	++	-
14	M	3	9	+	++	++	+	++	+	+	-	-	-	o	o	o	o	o	=	=	+	-	-	-	+	-	-	++	-	-	++	-
15	M	2	6	-	-	+	+	+	++	+	-	-	-	o	o	o	o	o	=	x	+	-	-	-	+	-	+	-	+	+	++	-
16	M	2	13	+	++	++	+	+	+	+	-	-	-	o	o	o	o	o	=	x	+	-	-	-	++	-	+	-	+	-	+	-

- = absent   +++ = severe   o = decreased
+ = slight   M = male   = = normal
++ = Moderate   F = female   x = increased

Fig. (1 a)

Fig. (1 b)

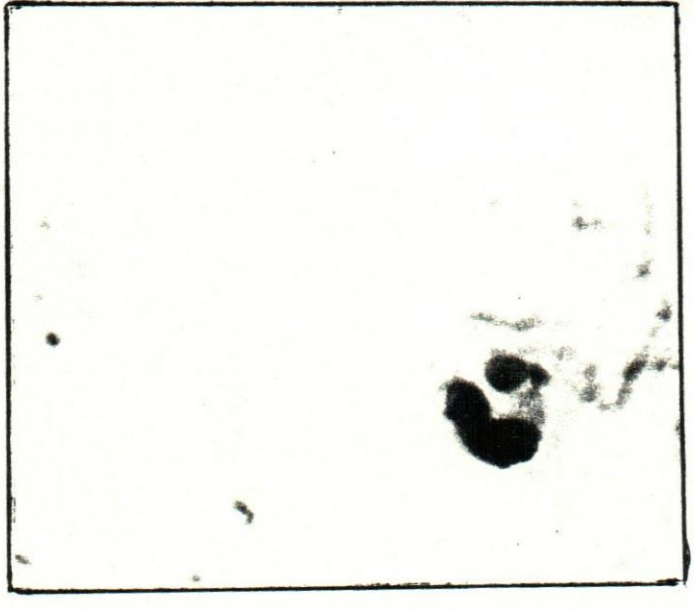

Fig. (2)

Fig. (3)

Fig. (4)

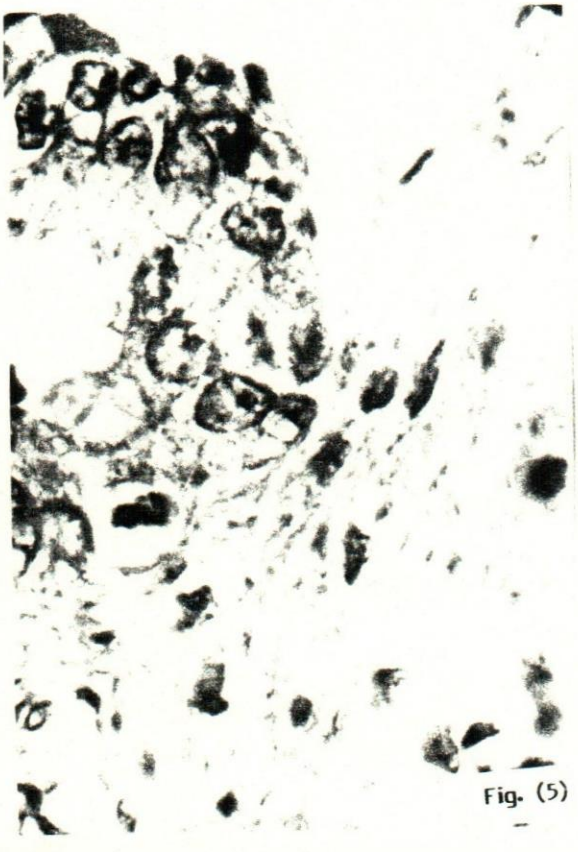

Fig. (5)

Fig. (6)