Assiut Vet. Med. J. Vol. 44 No. 87, October 2000

Dept. of Poultry Diseases,
Fac.Vet. Med., Assiut Univ.

PARAMYXOVIRUS INFECTION IN PIGEONS

(With 8 Figures)

By

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عدوى فيروس الباراميكسو في الحمام

رجب سيدابراهيم ، فاطمة عبد المجيد مصطفي ، محمد مبارك

تمت هذه الدراسة على أربعة أبراج للحمام ظهر عليها الأعراض العصبية والاسهال في محافظتي أسيوط وسوهاج. عزلت الفيروسات فى كيس الألنتويس لأجنة البيض عند عمر ١٠ أيام وأحدث تكور لشكل الجنين وتشوهات فى أصابع القدم عند العزل الأولى. أظهرت كل الفيروسات المعزولة قدرتها على إحداث تلازن الدم لخلايا الدم الحمراء الخاصة بالدجاج. وعند الفحص السيرولوجى بإستخدام إختبار تثبيط تلازن الدم إتضحت قدرة الفيروسات المعزولة على التثبيط بواسطة المصل المضاد لفيروس النيوكاسيل وعلى الناحية الأخرى وبنفس الكيفية إستطاعت الأجسام المضادة فى المصل المأخوذ من الحمام المصاب أن تثبط تلازن الدم بواسطة فيروس النيوكاسيل (عترة لاسوتا). وعند فحص الفيروسات التى تم حصدها من السوائل الجنينية (الألنتويس) بواسطة الميكروسكوب الإليكترونى النافذ تشابهت هذه الفيروسات مع فيروسات الباراميكسو. وعند فحص قطاعات من أنسجة المخ للأجنة التى تم حقنها بالفيروس إتضح أن هناك عديد من هذه الفيروسات فى مراحل مختلفة من عملية العدوى الخلوية والتبرعم. عند دراسة الشكل البروتينى للفيروسات المعزولة وفيروس لاسوتا بإستخدام طريقة الفصل الكهربائى فى الأكريلاميد ظهر التشابه بين جميع الفيروسات فى الأوزان الجزيئية ١٨٠و ١١٠و٧٥و٦٠و٥٥و٤٠ كيلو دالتون. البروتين ذو الوزن الجزيئى ٤٠ كيلو دالتون ظهر فى جميع الفيروسات المعزولة ولكن كان شديد الصبغة فى كل الفيروسات عنه فى عترة لاسوتا.

SUMMARY

Nervous signs and diarrhoea were investigated in four pigeon lofts at Assiut and Sohage governorates. Viruses were isolated in allantoic sac of 10-day-old chicken embryos, and produced curling of embryos and

deformity of toe fingures on first passage. The isolated viruses produced haemagglutination (HA) of chicken RBCs. The serological testing by haemagglutination inhibition (HI) revealed that HA activity of isolated viruses was inhibited by antisera raised against Newcastle disease virus (NDV) while in same way, antisera from diseased pigeons inhibited the HA activity of NDV (LaSota). Sedemint prepared from allantoic fluids containing the virus was examined by transmission electron microscope (TEM). Virus morphology was similar to that of paramyxoviruses (PMVs). TEM of brain tissues from infected embryos showed viral particles in different stages of cellular infection and budding. Polypeptide profiles of the isolated viruses and LaSota virus were studied by SDS-PAGE and found to share several protein bands of mol.wts. 180,110,75,60, 55 and 40 kDa. Forty kDa Protein band was expressed in all tested viruses but stained dense in all viral isolates than that of LaSota virus. Basing upon HA and HI activities,virus morphology, and polypeptide profiles, it was concluded that the isolated viruses were related to paramyxoviruses.

Key words: *Pigeon-Paramyxovirus-Polypeptides-Ultrastrutural Pathology.*

INTRODUCTION

Avian paramyxoviruses of serotype-1 (PMV-1) were known to infect racing pigeons and other members of the Columbidae family (Lancaster and Alexander, 1975). PMV-1 of racing pigeons was first reported in Sudan and Egypt by Avian Study Group (1984) and Alexander (1985). Naturally occurring Newcastle disease (ND) in pigeons have been reported in Egypt by Ahmed and Reda (1967) and El-Zanaty et al. (1988). Pigeons were known to be relatively resistant to natural infection with NDV until Vindovogel et al. (1982) reported the recovery of a lentogenic strain from pigeons.

The similaity of PMVs in possessing haemagglutination (HA) and inhibition of this character by specific antisera through haemagglutination inhibition (HI) test requires further tools of differentiation between different serotypes. Consequently, this work was directed to characterize virus isolates from infected pigeons by transmission electron microscopy and furthermore by studying the SDS-PAGE profile of the virus structural polypeptides.

MATERIALS and METHODS

Diseased birds:

Four pigeon lofts, two at Assiut governorate (Mangabad) and two at Sohag provience (Gizerit El-Mabda) were manifested by nervous signs and diarrhoea, as well as variable rates of morbidity and mortality. Clinical data are shown in the following table :

Locality	No. of birds	Age (month)	No. of birds		Morbidity (%)	Mortality (%)
			Diseased	Dead		
Mangabad	150	3	65	45	43.3	30
Mangabad	100	9	30	15	30	15
G.El-Mabda	400	18	80	60	20	15
G.El-Mabda	600	15	220	120	36.7	20

Clinical signs manifested by diseased birds and necropsy findings encountered in dead birds were studied and described.

Microbiological examination:

Viral isolation:

Viral isolation was directed to viruses causing nervous manifestations in pigeons, either Herpes or Paramyxoviruses. Differentiation was done by HA test. In each of the affected lofts, brain samples were collected from the dead birds (pooled sample) and sera from diseased birds were obtained for serological testing. Pooled sample was homogenized to prepare bacteria-free suspension which was inoculated into allantoic sac of 10-day-old embryonating chicken eggs. Three passages were done for viral propagation.

Bacteriological examination:

Culturing was done from brain and parenchymatous organs of affected birds on MacConky's agar and Selenite F broth to exclude *E.coli* and *Salmonella spp.* infections.

Viral identification:

a- Haemagglutination test (HA):

Rapid HA test: The propagated virus was tested by rapid slide HA test using 10% washed chicken RBCs.

Microtiter HA test: It was performed using 1% washed chicken RBCs in ten two-fold dilution.

b- Haemagglutination inhibition (HI) test: This serological test was done twice. One time using antisera from diseased pigeons against embryo propagated -LaSota virus and the other time using antisera

obtained from NDV- vaccinated chickens (antibody titer 1: 128) against embryo propagated- pigeon virus. The test was performed by microplate HI test (ten - two fold dilution) according to Beard and Wilkes (1973).

c- Transmission electron microscopy (TEM) of the isolated virus:

This was done by the procedure of sediment preparation. Embryonic fluids obtained from the inoculated eggs was centrifuged at 15.000 rpm for 1 h. The supernatant was discarded and the sediment pellets were fixed by immersion in 5% buffered glutaraldehyde at 4°C for 15 h. The fixed pelletes were processed for TEM, as done for tissues, and examined for the presence of viral particles.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of viral structural polypeptides:

The four virus isolates were propagated by three passages in allantoic sac of 10-day-old chicken embryos. The allantoic fluids were harvested from dead embryos and filtered through 0.45 micron- filter membrane. Virus was pelleted by centrifugation at 16.000 rpm for 1 hr. The virus pellet was resuspended 1 : 1 weight per volume (W/V) in 2X sample buffer (12.5 mM tris-HCl, pH 6.8; 1% SDS; B-mercaptoethanol; 2% glycerol and bromophenol blue) and the mixture boiled 60-90 seconds at 100°C in boilling water bath. Stacking gel (4%) consisted of 0.5 M Tris HCl (pH 6.8), 1% SDS, 30% N,N-methylene-bis-acrylamide, 10% amonium persulfate (APS) and tetramethylene diamine (TEMED). Separating gel used was 10% concentration. Prestained molecular weight marker (Sigma) was applied. Electrophoresis was done in Protean TM II (Bio-Rad Lab., Richmond, California, USA). Running conditions applied as 30 volts for 30 min in stacking gel followed by 100 volts for 90 min in separating gel. The whole virus polypeptides were detected by staining with coomassie brilliant blue R-250 dissolved in 25% methanol, 10% glacial acetic acid in distilled water for 60 min with gentle shacking and destained for 10-12 h (Laemmli, 1970).

Histopathology:

Representative samples from brain, lung, kidney, heart, liver and spleen in addition to all other organs and tissues, obtained from inoculated chicken embryos were fixed in 10% neutral buffered formalin. The fixed tissue samples were processed routinely for paraffin embedding technique. The embedded tissues were sectioned at 3μm and stained with haematoxylin and eosin (HE) (Bancroft and Stevens, 1982).

TEM of brain tissues:

Immediately after removal of the brain tissues from the inoculated embryos, 1 mm cubes were chopped and fixed by immersion in 3% buffered glutaraldehyde at 4°C. Tissue samples were then post-fixed in 1% osmium tetroxide, dehydrated in upgraded ethanol series and finally embedded in Epon 812. For orientation and localization, semi-thin sections were made and stained with 1% toluidine blue. Accordingly, ultra-thin sections were prepared, double stained with uranyl acetate and lead citrate and examined under transmission electron microscope (TEM) (JEOL, 100 CX II) operated at 80 Kv.

RESULTS

Clinical examination:

Diseased pigeons of different ages at different localities showed nervous manifestations (tremors, circling and backward movement, torticollis). Moreover, various degrees of greenish watery diarrhea was observed. Morbidity ranged from 20-43.3%, while mortality varied from 15-30%. Higher morbidity and mortality occured in birds aged 3 months. The age susceptibility recorded in this study varied from 3-18 months. Necropsy findings were represented by congestion of brain, liver, spleen and kidney as well as excess catarrh on the intestinal mucosa.

Viral isolation:

Inoculated embryos were found dead 3-6 days postinoculation on the first passage and showed dwarfing and deformity of toe fingers. On third passage, embryos had died within 48 h and lesions were manifested by enlargement and mottling of liver and congestion of kidneys.

Bacterial isolation:

Trials for bacterial isolation from brain, liver, spleen and kidneys of dead pigeons were unsuccessful.

Viral identification:

a- HA test:

Rapid HA test done on allantoic fluids of all inoculated embryos was positive. Variable titers of microplate HA test was demonstrated. On first passage, the titers ranged from 8-16, and increased on the second and third passages to 64 and 256-1024, respectively.

b- HI test:

The isolated virus tested against NDV antisera showed positive HI titer, while antisera from diseased pigeons gave HI titer ranged from 32 to 128 against embryo-propagated LaSota virus.

c- TEM of the isolated virus:

Sediments prepared from the embryonic fluids revealed the presence of mature viral particles (200-350 nm) which had distinct internal helical structures of nucleocapsids (15-17 nm in diameter) and surface spikes (projections) (Fig. 1).

SDS-PAGE of viral structural polypeptides:

Polypeptide profile of the four virus isolates demonstrated complete similarity (Fig. 2). Difference was only at 47 kDa. There was major protein sharing among all isolates at mol. wt. of 110, 75, 60, 55 and 40 kDa. Other polypeptides at 180, 135 and 42 kDa were demonstrated in the four isolated viruses. The PAGE profile of LaSota virus polypeptides shared with the isolated viruses in seven polypeptides at mol. wt. of 180, 135, 110, 75, 60, 55 and 40 kDa. Only there was a structural difference at 40 kDa where it was more dense or stained heavily in case of isolated viruses than that of LaSota virus.

Fig. 1: Viral particle with spikes (surface projections) (small arrow) on its external membrane and internal filamentous coiled nucleocapsid of helical structure (twisting loops) (large arrow). Sediment prepared from embryonic fluid. Transmission electron micrograph. X 150.000.

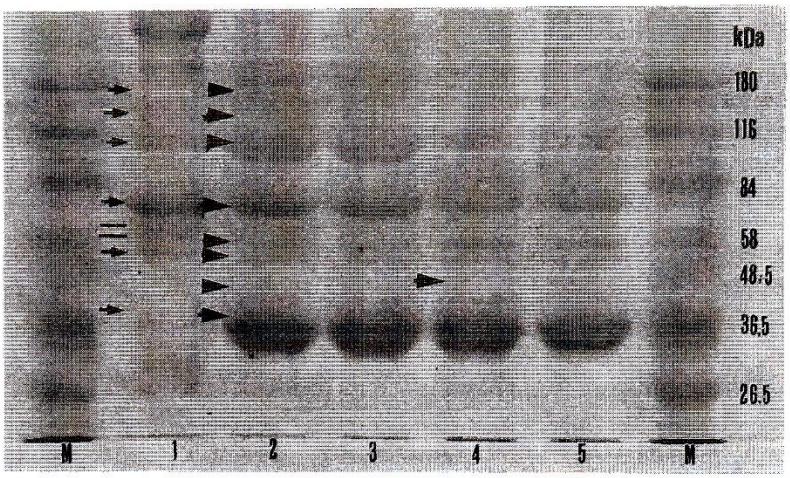


Fig. 2: SDS-PAGE profile of structural polypeptides of LaSota virus (lane1) and isolated viruses (Lanes 2, 3, 4 and 5). Lanes 2 and 3 (viruses isolated from Assiut governorate). Lanes 4 and 5 (viruses isolated from Sohag governorate). M (prestained molecular weight marker). Thin arrows indicate mol. wt. of 180, 135, 110, 75, 60, 55 and 40 kDa. Thick arrows indicate mol.wt. of 180, 135, 110, 75, 60, 55, 42 and 40 kDa in lanes 2, 3, 4 and 5. Single arrow in lane 4 indicate an additional mol. wt. of 47 kDa in lanes 4 and 5.

Histopathology:

The most pronounced histological changes were seen in the brain of the inoculated chicken embryos. Microscopical examination of brain tissues of the inoculated embryos revealed obvious neuronal degenerative changes (mainly in mid brain and brain stem) which involved cell swelling, chromatolysis and fading of the nuclear chromatin (Fig. 3) In addition to the degenerative changes, evidence of neuronal necrosis was also detected. The necrotizing changes were in the form of nuclear condensation (pyknosis), increased staining affinity of cytoplasm and even shrinkage of the whole cell. As a reaction of the brain tissue against the degenerated and necrosed neurons, the microglial elements were discerned gathering around the affected cells (Sattelitosis) (Fig. 4). Also, neuronophagia was detected. Spongy status, due to demyelination, was noticed in the brain stem. Perivascular lymphoid cell cuffing was also observed. Pericellular and perivascular edema was

apparent. In the cerebellum, Purkine cells were swollen, disorganized and showed various degrees of chromatolysis. Mild lymphoid cell infiltration was also noticed in the parenchyma of the examined liver, kidneys and pancreas. No comparable histological changes were observed in other organs and tissues.

TEM of the brain tissues:

Both mature and immature free viral particles were detected in the examined brain tissues of the inoculated embryos. The viral particles were mainly found in the neurons of the cerebral gray matter. Mature viral particles were pleomorphic measuring 110-150 nm and composed of nucleic core and external membranes. The nucleic core was bulit-up from central fine filamentous material (helical structure) and peripherally arranged nucleo-capsids (capsomeres). The latter appeared as electron-dense rounded structures (5-7 nm) which were arranged peripherally just beneath the internal membrane of the viral particle. External membrane of the viral particles had fringed appearance made by the surface projections(spikes) (8-10 nm in length) which appeared as radiating fine processes. Degenerated viral particles were also found and recognized by their incomplete enveloping membranes and the released nucleic material. Immature viral particles had no enveloping membranes and only composed of electron-dense core. Budding viral particles from cell membranes were frequently observed (Figs. 5, 6). The particles seemed to acquire their enveloping membranes during process of budding. The budding particles also had spikes (Fig. 7). Electron-dense rounded or ovoid nucleocapsids aligned just beneath the internal membrane of viral particles were obviously noticed (Fig. 8). Neurons harbouring the viral particles had numerous free ribosomes and dilated RER. Mitochondria were swollen and their cristae were deteriorated.

DISCUSSION

Recently, a problem of nervous signs in pigeons is frequently occurring in Upper Egypt. Pigeons have been considered rather than resistant to Newcastle disease and only velogenic strains can evoke CNS symptoms. PMVs with strong serologic relations to NDV were isolated in Europe from domesticated pigeons (Richter, 1983). In the present work, four pigeon lofts suffered from nervous signs and diarrhoea, together with high morbidity and mortality rates. The dead birds did not convey any pathological lesions except that related to congestion of

brain, liver, spleen and kidneys as well as severe enteritis. Several researchers (Hamson et al., 1989; Shakal, 1989; Pennycott, 1994; Yang-ChengYao et al., 1997) dscribed the disease occurrence in pigeons as due to PMV-1 or NDV which belong to the same group. This came in agreement with the present findings. Currently, viral isolation was tried from brains of dead pigeons in embryonating chicken eggs via allantoic sac route by several consequent passages. Roy and Venugopalan (1998) in India characterized three isolates from pigeons closer to LaSota virus. Also, Gurkirpal-Singh (1993) recovered PMV from racing pigeons during Severe outbreak in India and the isolated virus had a mesogenic character and related antigenically to NDV. Kaleta and Baldouf (1988) described the occurrence of lentogenic NDV in free living birds. The virus HA titer on first passge was low, but increased by second and third passages. Furthermore, the isolated viruses tested by HI against anti-NDV antisera and gave positive results, while antisera from diseased pigeons could inhibit the HA of vaccinal strain (LaSota virus) and the recorded titer ranged from (32-128). Such firdings regarding antigenic and serologic relation between PMV-1 and NDV were published by several authors (Biancifiori & Fioroni, 1983; Pennycott, 1994 and El-Sisi et al., 1995). The more serious problem is the possibility of viral transmission to other domestic poultry through infected or diseased pigeons. This is in keeping with the findings of Alexander et al. (1985) who reported ND outbreaks in fowls in Great Britain caused by pigeon PMV-1 viruses.

Differentiation of avian paramyxovirus isolates may be made by HI and a comparison of other properties including structural polypeptide profile (Alexander and Collins, 1981).

In this respect the isolated viruses with HA and HI activity expressed different protein and glycoprotein antigens. LaSota strain was used for control and comparison. SDS-PAGE polypeptide profiles of the isolated viruses and LaSota virus revealed separation of nine proteins or glycoproteins. The isolated viruses were closly similar to each others regarding their polypeptide profile. Furthermore, these viruses shared seven bands of mol. wts. 180, 135, 110, 75, 60, 55 and 40 KDa, indicating their similarities. There were additional bands expressed by isolated viruses at mol wts. of 47 and 42 kDa. Many authors described the structural polypeptides recorded in present investigation specially those of mol. wts. 180, 110, 75, 60, 55, 47, 42 and 40 kDa (Mount Castle et al., 1971; Moore & Burke, 1974; Hightower et al., 1975; Shortridge et

al., 1980 and Alexander & collins, 1981). They also found these similarities among groups of PMV-1 and NDV. The high percentage of similarity between isolated viruses and LaSota virus in HA, HI patterns, TEM of the virus and SDS-PAGE profile gave great support to the nature of the isolated viruses as PMV-1 similar to NDV variants infecting pigeons. This openion may be explained by Russell and Alexander (1983) who clarified the difference between pigeon PMV-1 (PPMV-1) and NDV in possession of 7 specific antigen components by NDV while PPMV-1 has only 3. Furthermore, only 2 of the components of PPMV-1 were shared with those of LaSota. Also, Alexander & Collins (1981) reported that NDV have two envelop glycoproteins and 7 polypeptides. For more explanation, the relationships among the polypeptides of NDV were studied using both kinetic and tryptic peptide analysis, there were at least six unique viral polypeptides named L, HN, F (60 kDa), NP (56 kDa), 47 kDa and N (41 kDa) proteins (Hightower et al., 1975). In this work, similar molecular weights were demonstrated.

In brief, polypeptide analysis of avian PMVs cannot be considered as a method suitable for routine typing but may will be usefull for confirming the uniqueness of any future avian PMV isolates that appears to represent a new serotype.

The observed morphopathological changes in the inoculated embryos conform with those reported on paramyxovirus infections (Beard & Easterday, 1967; Wilczynski et al., 1977 and Beard & Hanson, 1984). The histological changes involved degenerative neuronal changes, demyelination, microglial reaction and perivascular lympoid cell cuffing. In other words, the histological picture was that of non-purulent encephalitis.

Electron microscopy of the virus-infected neuronal cells indicated that the replication of the studied virus had taken place exclusively in cytoplasm. No evidence of intranuclear viral replication was detected. Cytoplasmic assembly confirms that the investigated virus belongs to RNA-group of viruses. Some RNA viruses can bud from intracytoplasmic membranes such as cytoplasmic vacuoles derived from endoplasmic reticulum or Golgi complex (Murphy et al., 1970). The currently examined virus was obviously seen budding from plasma membrane of the infected neuronal cells. Also, the virus was noticed free in cytoplasm and also in extracellular locations. As in case of other RNA viruses, the viral core (nucleocapsid, viral protein) seemed to be assembled in cytoplasm and thereafter acquire its membranous coat by

budding through plasma membrane of the infected cells. This concept is in line with the reported replication strategy of paramyxoviruses (Holmes et al., 1969; Grimley & Friedman, 1970; Zlotnik & Harris, 1970; Boulton & Webb, 1971 and Whitefield et al., 1971). The viral envelope proteins are incorporated into discrete regions of cell membrane and then viral nucleocapsid aligns beneath these area. Spikes (surface projections) appear at the same time on the outer surface and finally the mature viral particles can bud. The similarity in structure between the host cell membrance and external viral membrane substantiates this replication strategy. The configuration and assembly mode of the presently demonstrated virus is consistent with that of the members of Family Paramyxoviviridae, Genus Paramyxovirus (Finch & Gibbs, 1970; Donelly & Yunis, 1971; Mclean & Doane, 1971; Cheville & Beard, 1972; Seto et al., 1980; Castleman, 1984 and Hanson et al., 1989).

Conclusively, the relationship in HA and HI activities, pathological features, viral morphology and polypeptide profile suggest that the presently isolated viruses belong to PMV-1 or their NDV variant.

REFERENCES

Ahmed, A.A.S. and Reda, I.M. (1967): response of pigeons to eight strains of Newcastle disease virus. Avian Dis., 11: 734-740.

Alexander D. J. and M.S. Collins (1981): The structural polypeptides of Avian Paramyxovirus. Arch. Virol, 67 : 309 - 323.

Alexander D.J. (1985): The classification, host range and distribution of avian paramyxoviruses Proc. of the Symb. on Acute Virus Diseases of Poultry, Brussel, 52-66.

Alexander, D. J.; Russell, P.H.; Parsons, G.; Abu-Elzain, E.M.E.; Fleury, H.J.A.; *Guittet, M.; Kaleta, E.F.; kihm, u.; Kosters, J.; Lomniczi, B.; Maister, J.; Meullemans, G.; Nerome, K.; Petek, M.; Pokomunski, S.; Polten, E.; Prip, M.; Richter, R.; Saghy, E.; Samberg, Y.; Spanoghe, L. and Tumova, B. (1985):* Antigenic and biological characterization of avian paramyxoviurs type 1 isolates from pigeons (An international collaborative study). Avian Pathol., 14 : 365-376.

Avian Study Group (1984): Paramyxovirus infection of pigeon. Vet. Rec., 114 : 232.

Bancroft, D. and Stevens, A. (1982): Theory and Practice of Histological Techniques. 2 nd Edn. Churchill Livingstone (Edinburgh, London, Melbourne).

Beard, C.W. and Easterday, B.C. (1967): The influence of route of administration Newcastle disease virus on host response. J. Infect. Dis., 117: 55-70.

Beard, C.W. and Hanson, R.P. (1984): Newcastle disease. In: Diseases of poultry. M.S. Hofstad, H.J Barnes, B.W. Calnek, W.M. Reid & H.W. Yoder (Eds.) 8th Edn. Pp. 425-470. Iowa State Univ. Press, Ames..

Beard, E.W. and Wilkes, W.J. (1973): A simple and rapid microtest procedure for determining Newcastle haemagglutination-inhibition (HI) antibody titers. Proc. 77 th Ann. Meet. U.S.An. Hlth. Assoc., Pp. 596- 600.

Biancifiori, F. and Fioroni, A. (1983): An occurance of Newcastle disease in

pigeons : virological and serological studies on the isolates. Comp. Immun. Microbiol. Infec. Dis., 6: 3: 247-252.

Boulton, P.S. and Webb, H.E. (1971): An electron microscopic study of langat virus encephalitis in mice. Brain, 94: 411.

Castleman, W.L. (1984): Alterations in pulmonary ultrastructure and morphoinetric parameters induced by parainfluenza (Sendai) virus in rats during postnatal growth. Am. J. Path., 114: 322.

Cheville, N.F. and Beard, C.W. (1972): Cytopathology of Newcastle disease. Lab. Inset., 27: 129.

Donelly, W.H. and Yunis, E.J. (1971): The morphogenesis of virulent Newcastle disease virus in the chick embryo. An ultrastructural study. Am. J. Path., 62, 87.

El-Sisi, M. A.; Shakal, M., Aly, A and Abou Hashem, N. (1995): Pathogenicity of recent isolates of pigeon paramyxovirus in pigeon in Egypt. Vet. Med. J., Giza, 43: 2, 207-212.

El-Zanaty, T.Y.; Åbd-El-Motelib; B. Salem; M. Shahata and M. Seif-El-Din (1988): A natural outbreak of Newcastle disease in pigeon in upper Egypt. Proceding 3rd. Sci. Cong. Fac. Vet. Med., Assiut University.

Finch, J. T. & Gibbs, A.J. (1970): Observations on the structure of the nucleocapsids of some paramyxoviruses. J. Gen. Virol., 6, 141-150.

Grimley, P.M. and Friedman, R.M (1970): Developments of Semliki Forest virus in mouse brain: an electron microscopic study. Expl. Molec. Path., 12, 1.

Gurkirpal - Singh (1993): Biological properties of avian paramyxovirus type 1 isolated from pigeon. Indian J. of Comparative Microbiology, immunology and Infections Diseases 14 : 34-35.

Hanson, J, Lowes, N., Chalmers, G. and Mclntosh, D. (1989): Paramyxovirus infection in pigeons. Canadian Vet. J., 30:12 , 970.

Hightower, L.E., Morrison, T.G. and Bratt, M.A. (1975): Relationships among the polypeptides of Newcastle disease virus. J. Virol. 16: 1599-1607.

Holmes, I.H., Work, M.C. and Warburton, M.F. (1969): Is rubella an arbovirus? II. Ultrastructural morphology and development. Virology, 37, 15.

Kaleta, E. F. and Baldauf, C. (1988); Newcastle disease in free-living and pet birds. In: Newcastle Diseases. D.J. Alexander (Ed.). Pp. 197-246. Kluwer Academic Publishers (Boston).

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227 : 680-685.

Lancaster, J.E. and Alexander, D.J. (1975): Newcastle disease : virus and spread. Monogr. No. 11, P. 26. Can. Dept. Agric., Ottawa.

Mclean, A.M. and Doane, F.W. (1971): The morphogenesis and cytopathology of bovine parainfluenza type 3 virus. J. Gen Virol., 12: 271-279.

Moore, N.F. and Burke, D.C. (1974): Charaeterization of the structural proteins of different strains of Newcastle disease virus. J. gen. Virol. 25: 275-289.

Mountcastle, W.E., Compans, R.W. and Ghoppin, P.W. (1971): Proteins and glycoproteins of paramyxoviruses : a comparison of Simian virus 5, Newcastle disease virus and Sendai virus. J. Virol. 7, 47-52.

Murphy, F.A., Harrison, A.K. and Collin, W.K. (1970): The role of extraneural arbovirus infection in the pathogenesis of encephalitis. Lab. Invest., 22, 318.

Pennycott, T.W. (1994): Avian Paramyxovirus type 1 in feral pigeons. Vet. Rec., 134 : 21, 260.

Richter, R. (1983): Paramyxovirus infectionen bei Rasse Tauben. Prakt. Tierarzt., 64 : 915.

Roy, P. and Venugopalan, A.T. (1998): Virulence of Newcastle disease vaccine virus (es) in the field. (Bibliogrophic citation) : Tropical Animal Health and Production. 30 : 41-44.

Russell, P. H. and Alexander, D. J. (1983): Antigenic variation of Newcastle disease virus strain detected by monoclonal antibodies. Arch. Virol., 75 : 243-245.

Seto, J. T., Wahn, K. and Becht, H. (1980): Electron microscope study of cultured cells of the CAM infected with representative paramyxoviruses. Arch. Virol., 65: 247-255.

Shakal, M.A. (1989): Zur Isolierung and Charakterisierung von aviaeren paramyxovirus stammen aud degypishenund deutschen Haustauben (Columba Livia Gmel. 1789 Var. Domestica) unter verwendung von permanentzellinen. Ph. D. Thesis, Tieraerztl. Fakultaet, Ludwig - Maximilians Universtaet, Muenchen, Germany.

Shortridge, K.F., Alexander, D.J. and Collins, M.S. (1980): Isolation and properties of viruses from poultry in Hong Kong which represent a new (sixth) distinct group of avian paramyxoviruses. J. gen. Virol., 49: 255-262.

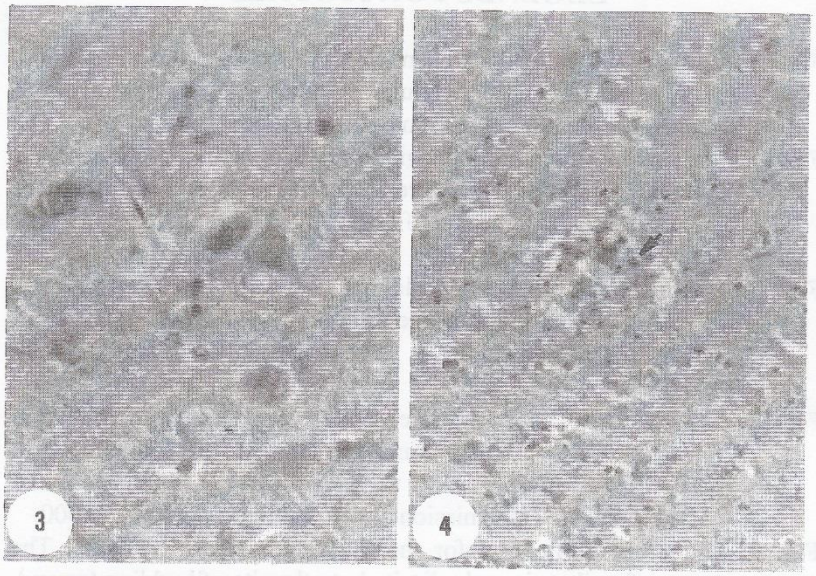
Vindevogel, H., Pastoret, P.P., Thiry, E. and Peeters, N. (1982): Reapparition de formes graves de la maladie de Newcastle chez le piogeon. Annales de Medecine veterinaire, 126: 5-7.

Whitefield, S.G., Murphy, F.A. and Sudia, W.D. (1971): Eastern equine encephalomyelitis virus : an electron microsepic study of Aedes triseriatus (Say) salivary gland infection. Virology, 43, 110.

Wilczynski, S.P., Cook, M.L. & Stevens, J.G. (1977): Newcastle disease as a model for paramyxovirus induced neurologic syndrames. Am. J. Path., 89: 649-666.

Yang-ChengYao, Chang-PoaChun, Hwang-JyhMih, Chieh-HK, Yang-CY, Chang-PC and Hwang-JM (1997): Nucleotide sequence and phylogenetic analysis of Newcastle disease virus isolates from recent outbreaks in Taiwan. Avian Diseases, 41 : 365-373.

Zlotnik, I. and Harris, W.J. (1970): The changes in cell organelles of neurons in the brain of adult mice and hamesters during Semliki forest virus and louping ill encephalitis. Br. J. Exp. Path., 51: 37.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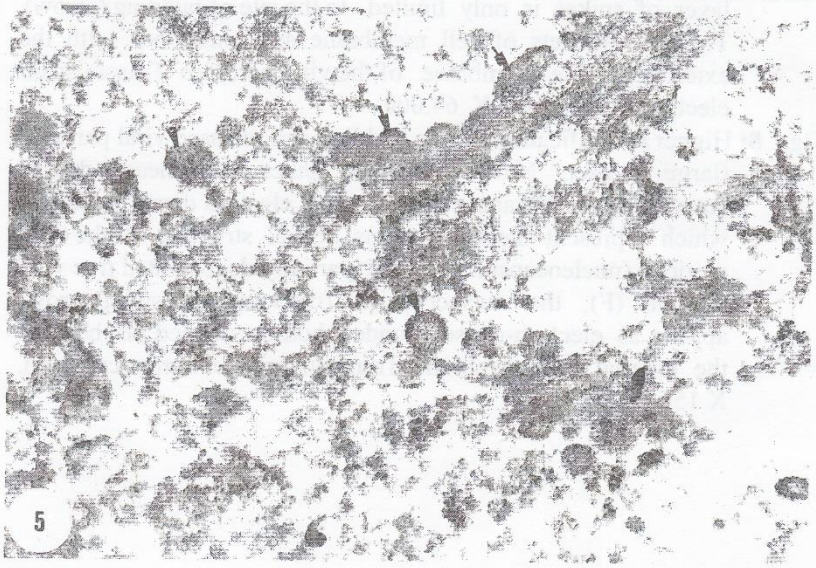
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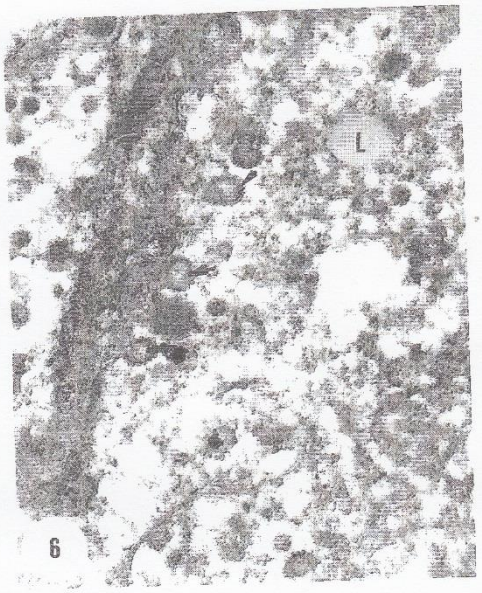

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