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Natural Equine Viral Arteritis in Foals

Witold Golnik¹, Zofia Michalska and Tomas Michalak

Introduction

Equine arteritis (EA) was previously referred to as «Fièvre typhoïde», «Epizootic cellulitis» or «Rotlaufseuche» (*Brion et al.*, 1967). First publications dealing more in detail with the disease came from the United States. They are reports by *Doll et al.* who in 1957 described an epizootic of equine arteritis in Pennsylvania (*Doll et al.*, 1957, 1957a; *Jones et al.*, 1957). At a later date, the occurrence of the disease was reported from Switzerland, Austria and Poland (*Bürki and Gerber*, 1966; *Jaksch et al.*, 1973; *Golnik and Michalak*, 1979). Serologic studies revealed the presence of antibodies against EA virus in horse sera from many European and extra-European countries (*McCullum and Bryans*, 1973; *Hyllseth and Patterson*, 1970; *Moraillon and Moraillon*, 1978).

EA mostly occurs in English thoroughbred or standard horses. This was confirmed by recent reports of *McCullum and Swerczek* (1978), as well as by our own findings (*Golnik and Michalak*, 1979). Only exceptionally is the disease lethal. In spite of its mild course, fetal abortion is no rarity. The percentage of aborted or still-born foals is high, ranging from 40% to 80% (*Doll et al.*, 1957a; *Golnik and Michalak*, 1979).

Experimental infection with large doses of virus results in high mortality of adult horses and foals, and the disease eventually takes a stormy course with shock symptoms (*Cheville*, 1975). Our report deals with one of the first EA enzootics and the pertinent diagnostic investigations. This is the first description of fatal EA virus-infection in foals.

Own Studies

Earlier suggestions prompted by serologic results (*McCullum and Bryans*, 1973; *Gerber et al.*, 1978; *Moraillon*, 1979) and indicating that EA virus infection occurs in Poland, were confirmed by the demonstration of inflammatory changes in arterial walls of two dead foals from the stud S (*Madej*, 1976). This has initiated extensive investigations into the aetiology and pathomorphology of the disease, conducted in our Institute. Initial virologic studies concerned fetuses, aborted in 1976 by mares from the stud P. From 7 successively examined fetuses, 7 virus strains with similar

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characteristics were isolated, one of which, identified as EA virus, has been referred to as Wroclaw-2 strain (*Golnik and Michalak, 1979*).

In 1976–1979 periodical abortions in mares and morbidity of horses and foals were noted in the race horse studs P and S. Among adult animals, the disease was mild with slight fever, apathy, occasional indigestion, colics or respiratory symptoms (nasal discharge, cough). In 1976–1977, the percentage of abortions in the stud P amounted to 40%. More pronounced clinical manifestations were seen in foals from the stud S; in addition to general symptoms, there were signs of acute or complicated pneumonia and gastro-intestinal disorders.

Material

Two dead foals from the stud S (1,5 months old referred to as 4/78, and 6 months old referred to as 105/79) were used for diagnostic studies; they were English thoroughbred horses.

Clinical Symptoms

As indicated by anamnestic data, foal 4/78 had been ill for a few days before death with the symptoms of anorexia and weakness. The rectal temperature was not elevated; there was a seromucous discharge from the nostrils and cough, suggesting the presence of pneumonia. In foal 105/79, temperature rise was also absent, anorexia, apathy and colics being the main symptoms; diarrhea was observed one day before death.

Autopsy Findings

In both animals the main changes were oedema and congestion of all lymph nodes, especially of the mesenteric ones; parenchymal degeneration of the liver, kidneys and myocardium, and subacute stasis-oedema of the spleen. Moreover, foal 4/78 showed abundant sero-sanguineous exudates in all body cavities and uncomplicated interstitial pneumonia. In foal 105/79 there was a gelatinous oedema and congestion of the posterior mesenterial radix; the ileum and the entire colon showed a haemorrhagic inflammation of the mucosa. The changes were particularly pronounced in the mucosa of the caecum, in the form of a fibrinous and necrotic enteritis.

Pathomorphologic Studies

Specimens from liver, spleen, kidney, lungs, lymph nodes, mesenterium, myocardium, adrenals and brain were collected, embedded in paraffin and stained with HE.

Both animals showed marked venous stasis, vacuolar degeneration of hepatocytes, degeneration of the renal tubules, adrenals and myocardium. Venous stasis with numerous haemosiderin granules and atrophy of the white pulp were seen in the spleen. The lymph nodes were oedematous, congested, with reduced number of lymphocytes in their proliferation centres. There was also congestion of the adrenals and cerebral meninges. In foal 4/78, the microscopical changes in the lungs consisted of diffuse congestion of the lobules and serous-haemorrhagic and cellular infiltration of

the interstitial tissues. A serous oedema was also visible in the alveolar walls. Additional findings were proliferation and desquamation of the respiratory epithelium. Infiltration by lymphocytes and histiocytes was mainly found in the peribronchial tissue. In foal 105/79, the lungs were only passively congested. The pulmonary tissue of both foals showed neither inclusion bodies of Dimock nor antigens of rhino-pneumonitis equorum virus in the direct immunofluorescence test. Marked inflammatory changes were found in the alimentary tract of foal 105/79. The walls of the ileum and colon were oedematous, congested and contained infiltrates of lympho- and histiocytes. The Peyer's plaques exhibited a reduced number of lymphocytes, whereas in the caecum, a superficial necrosis with karyorhexis was an additional finding.

In all examined organs, as well as in the intestinal mesenterium, particular attention was paid to arterioles of muscular type. Most arterioles, especially in the mesenterium, lungs, spleen and lymph nodes, showed serous oedema and loosening of the tunica adventitia. Poor stainability and hyalinization were observed in smooth muscle cells of the tunica media. In the lungs of foal 4/78, the adventitia of some arterioles contained abundant infiltrates of lymphocytes. Neither necrotic nor inflammatory changes were seen in the media of the vessels; there was no damage of the endothelium.

Virologic Studies

Attempts at Virus Isolation

The material to be examined was lung, liver and spleen samples from both foals referred to as 4/78 and 105/79. The tissues were homogenized and 10% suspensions in phosphate buffered saline (PBS) were prepared according to weight/volume formula. The suspensions were centrifuged for 10 minutes at 2000 r.p.m. and 200 I.U./ml of penicillin and 200 microgram/ml of streptomycin were added. The supernatant was used to infect monolayer cultures of rabbit kidney (RK-13)² grown in a medium of the following composition: Eagle's solution MEM 95%, bovine fetal serum 5% and cell cultures of monkey kidney (Vero)³ in Parker's solution 95%, inactivated calf serum 5%. To both media we added penicillin and streptomycin in the above doses. The infected cell cultures in rubber-stoppered tubes were incubated for 60 minutes at 37°C and then supplemented with Eagle's or Parker's solution containing 1% of appropriate serum and antibiotics. The cultures were kept for 4-7 days at 37°C and then frozen (at -70°C) and thawed 3 times. Thus, processed material was passaged 8 times in cell cultures.

The results are shown in table 1. The virological examination of tissues from foal 4/78 yielded the isolation (from RK-13 cells) of two isolates: one from the lungs, designated 4/78a and the other from spleen, referred to as 4/78c. In foal 105/79, the Vero cell culture yielded one strain from the lungs referred to as 105/79a; it was adapted to the RK-13 cells and used for further studies.

Identification of Strains 4/78c and 105/79a by the Neutralization Test

The test was carried out by the method described elsewhere (Hyllseth and Patterson, 1970) using slight modifications of our own. Serial 10-fold increasing virus dilutions in Eagle's MEM were

² RK-13 cell cultures were obtained from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wroclaw

³ Vero cell cultures came from the State Hygiene Department in Warsaw

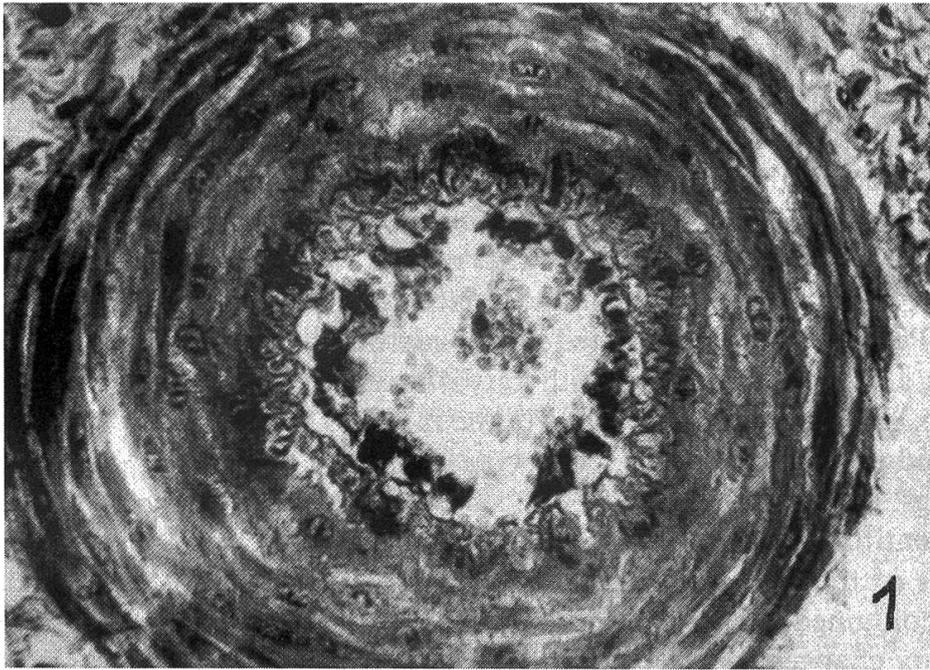


Fig. 1 Early hyaline changes in muscle cells of media in an artery of the lungs. HE, approx. $\times 250$.

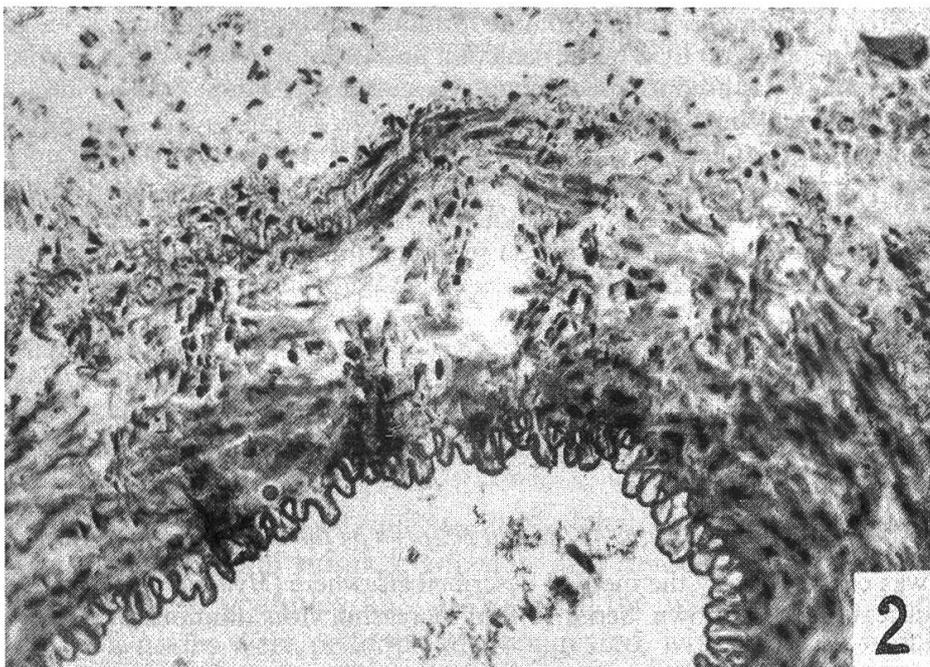


Fig. 2 Lymphocytic infiltration and oedema in adventitia of a lung artery. Few lymphocytes penetrating in the media. HE, approx. $\times 250$.

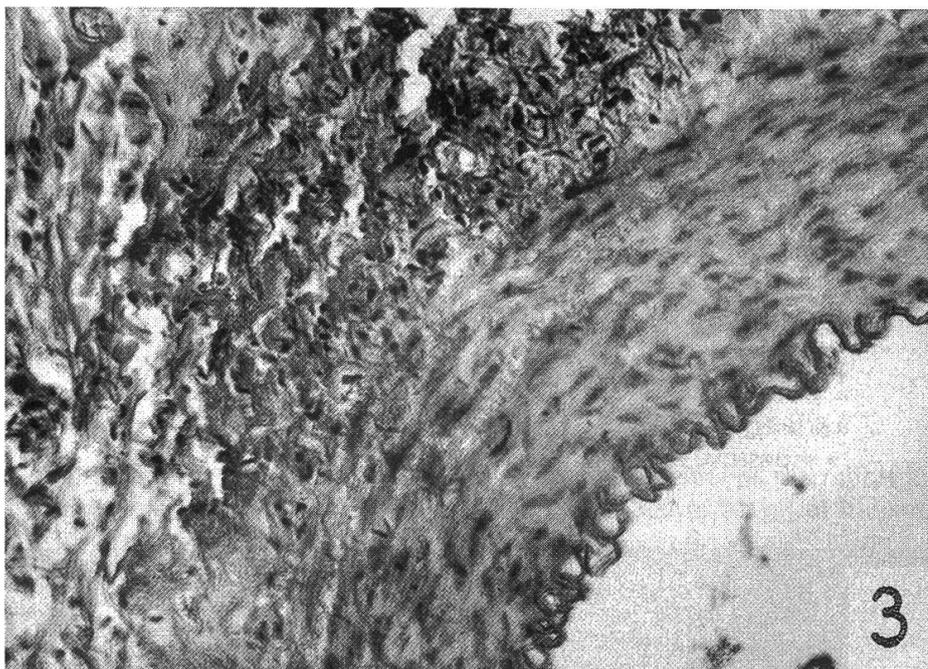


Fig. 3 Slight infiltration with lymphocytes in adventitia of a mesenteric artery. HE, approx. $\times 250$.

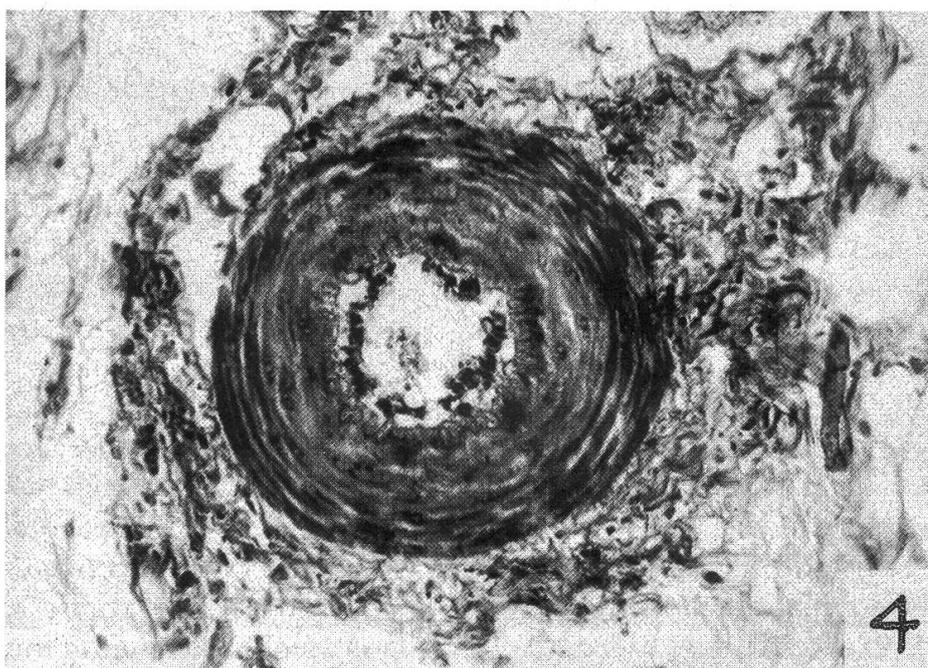


Fig. 4 Oedema of adventitia and early hyaline changes in media of an artery in the lungs. HE, approx. $\times 160$.

Table 1: Virus isolation trials

| Material | Cell culture | |
|----------|--------------|------|
| | RK-13 | Vero |
| 4/78a | + | - |
| 4/78b | - | - |
| 4/78c | + | - |
| 105/79a | - | + |
| 105/79b | - | - |
| 105/79c | - | - |

a = lung, b = liver, c = spleen,

+ = positive for CPE, - = negative for CPE

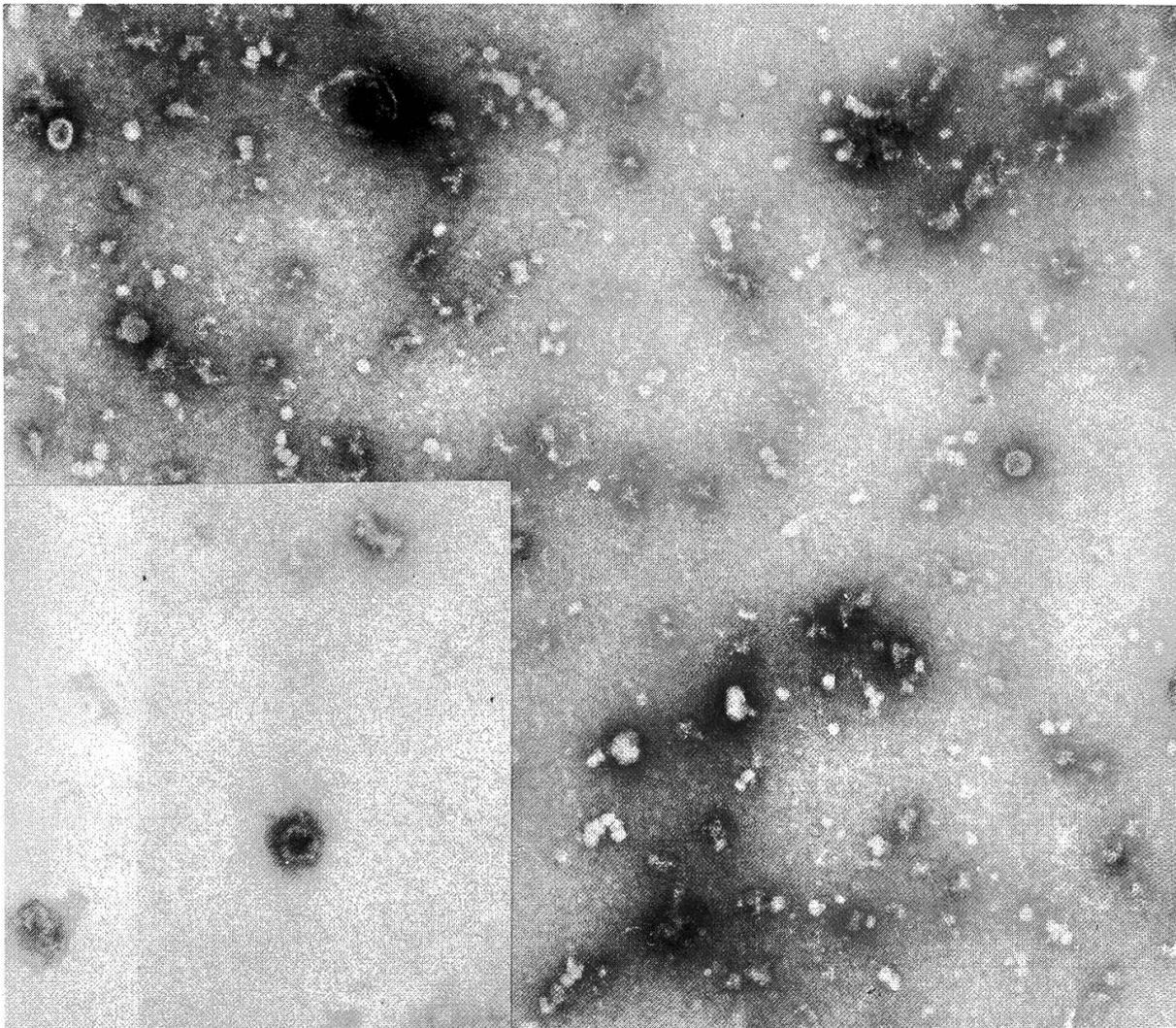


Fig. 5 Electron microscopic picture of particles of EA 105/79a virus strain. Magnification $\times 27000$.

Inset: A particle of virus strain EA 4/78c at a magnification $\times 40000$.

prepared and combined in equal volumes (0,5 ml each) with immune anti-arteritis equorum serum⁴ and normal serum. Both sera were heat inactivated and used in 1:10 dilution in Eagle's MEM fluid. The virus-serum mixture was incubated for 60 minutes in a water bath at 37 °C and then to each aliquot was added 10% of restituted, lyophilized guinea pig serum complement (Sera and Vaccine Manufacture, Cracow, batch 60175). The virus-serum-complement mixture was incubated for 60 minutes in a thermostate at 37 °C and then used to infect monolayer cultures of rabbit kidney cells (RK-13) prepared in tubes. The results were read 3,5 and 7 days after inoculation. From the obtained data, the neutralization index was calculated (NI); its values were NI-2,25 and NI-3,25 for 4/78c and 105/79a strains, respectively. This procedure was repeated routinely without using the guinea pig serum complement. In this case, a neutralizing effect of immune and normal sera could be seen neither against 4/78c nor 105/79a strains.

Electronmicroscopic Studies of Strains 4/78c and 105/79a

After centrifugation at 35000 × g for 3 hours, the supernatants of the rabbit kidney cell cultures (RK-13) inoculated with the tested strains were suspended in 0,2 ml of bidistilled water and precipitated overnight at 4 °C. A volume of 0,025 ml of the obtained suspension was transferred onto 100 mesh nets covered with carbonpowdered collodion film. After 1 minute incubation the nets were rinsed 3 times in distilled water, dried on filter paper and negative stained with 1% aqueous PTA solution for 20 minutes in wet chamber, pH 7.0. After withdrawal of excess fluid with filter paper, the nets were dried for 1 minute at room temperature and examined in an electron microscope Jem 100 C. Photographs were taken at magnifications of 27000 × and 40000 ×.

The strains 4/78c and 105/79a exhibited single, spherical virus particles; they were characterized by the presence of an electrondense, homogeneous core, about 34 nm in diameter and of a less electron-dense capsule of 15 to 34 nm thickness; the diameter of the entire particles ranged from 45 to 70 nm.

Discussion

First cases of EA in Poland were found in 1976 (*Madey, 1976*). In the following years, isolated cases of a disease suggesting the presence of EA were observed in studs of English thoroughbred or standard horses. In adult animals the course of the disease was mild, in accordance with some reports in the pertinent literature (*McCollum and Swerczek, 1978*). Pregnant mares were found to abort sporadically, but in one outbreak abortions occurred in about 40% of mares (*Golnik and Michalak, 1979*). Less typical was the course of the disease in foals; they exhibited clinical symptoms of the alimentary or respiratory tract, often without rise of the body temperature. Similarly, the autopsy findings in two foals, in which the presence of EA was confirmed by virologic studies, were not typical. In foal 4/78 there was an interstitial pneumonia and in the other case, referred to as 105/79, the main pathologic changes consisted of fibrinous and necrotising inflammation of the intestine, especially the caecum. No oedema, extravasation and infarction of intestinal vessels were noted, in contradistinction to the reports of *Jones et al. (1957)*. However, it must be mentioned that the observations of the above authors were made in foals inoculated with high virus doses. In experimental infections of foals and adult horses, morphologic changes are more pronounced (*Jones et al., 1957; Crawford and Henson, 1973; Prickett et*

⁴ Immune anti EA serum was obtained through the courtesy of Dr. A. Morailon, Ecole Nationale Vétérinaire d'Alfort, France.

al., 1973). Our histopathologic examinations demonstrated only slight changes in the arterioles of muscular type in the lungs, mesenterium and lymphatic organs. As compared with those described by *Prickett et al.* (1973), they seem to be in their early phase of development. The tunica media showed only degeneration of the muscular cells, with oedema and a few inflammatory infiltrates in the adventitia. This insignificance of the vascular damage may account for the absence of extensive circulatory disturbances in both foals.

Our material does not permit to establish the full morphologic picture in foals that succumbed to natural EA virus infection; it is, however, much less pronounced than that of the experimentally produced disease.

Attempts at isolation of EA virus from the tissues of both foals gave a positive result; cultures of rabbit kidney cells (RK-13) and of monkey kidney cells (Vero) were used. Both cultures showed to be adequate for primary isolation of EA strains; but the strains obtained differed with respect to their cytopathogenic effect. Strain 4/78c was found to cause CPE in RK-13 cells after 4 passages, whereas strain 105/79a failed to exert a cytopathogenic activity, in spite of 8-fold passages. On the other hand, it caused changes in Vero cell cultures, bringing about lysis of the cytoplasm and disintegration of cell nuclei in the 4th passage.

Our observations indicate that some EA virus strains, including strain 105/79a, lead to latent infection in the RK-13 cell cultures (*Golnik and Golnik, 1979*). The hitherto obtained EA virus strains were mostly isolated from cultures of homologous cells (*Bürki and Gerber, 1965; Jaksch et al., 1973*). The strains Wroclaw-2 (*Golnik and Michalak, 1979*), 4/78c and 105/79a were primarily isolated from RK-13 or Vero cell cultures. However, heterologous cell cultures are mainly used for studies on known viral strains (*Wilson et al., 1962; Doll et al., 1968; Maess et al., 1970; McCollum et al., 1971; Konishi et al., 1975; Moraillon and Moraillon, 1978*). Once its cytopathogenic effect had been established, the strain 4/78c could be readily adapted to Vero cells and the strain 105/79a to RK-13 cells. The HE stained preparations from RK-13 and Vero cell cultures infected with 4/78c and 105/79a strains showed similar changes; 48 hours after inoculation, vacuolization and lysis of the cytoplasm, karyorhexis and karyolysis were noted.

The newly isolated strains were identified by the neutralization test; immune serum from EAV-infected horse and normal horse serum were used. The routine test gave a negative result. Neutralization of the newly isolated strains by antibody-containing immune serum was achieved only in the presence of guinea pig serum complement, as in the case of in vitro neutralization of the hitherto known EA virus strains (*Bürki, 1965; Hyllseth and Patterson, 1970; Maes, 1971; Radwan and Burger, 1973; Konishi et al., 1975; McCollum, 1978; Golnik and Michalak, 1979*). High neutralization indices were obtained for 4/78c (NI: 2.25) and 105/79a (NI: 3.25) strains. In spite of several repetitions of the test with anti-Bucyrus immune sera from various sources and with sera from horses with natural viral arteritis, the neutralization index of 105/79a strain was invariably higher than that of 4/78c strain, which may be due to slight antigenic differences between them. The hitherto known EA strains, when studied in the modified neutralization test, were found to be antigenically identical

with the standard Bucyrus strain (Bürki, 1965; Jaksch et al., 1973; Konishi et al., 1975; McCollum and Swerczek, 1978; Golnik and Michalak, 1979; Moraillon, 1979).

The electron microscopic studies of the 4/78c and 105/79a strains revealed the presence of identical spherical virus particles, 45–70 nm in diameter; they consisted of a core measuring 34 nm in diameter and a capsule. The morphology and dimensions of the particles of the strains suggest their identity with Bucyrus and Wroclaw-2 strains of EA virus examined by the same technique (Magnusson et al., 1970; Maess et al., 1970; Golnik and Michalak, 1979).

The morphologic and virologic findings justify the conclusion that a natural EA virus infection was responsible for the death of both foals.

Summary

A fatal natural EA virus infection in thoroughbred English foals is described. Clinically and at autopsy, changes in the respiratory and alimentary tracts were predominant in two animals. They consisted of interstitial pneumonia and necrotic inflammation of the large intestine. Other findings were degeneration of parenchymal organs and atrophy of the lymphatic centres of lymphnodes and spleen. In the walls of arterioles of muscular type, hyalinization of the tunica media and inflammatory infiltrates in the adventitia were the only changes.

From the tissue of both foals under study, two virus strains were isolated in RK-13 and Vero cell cultures. In a modified neutralization test and by electron microscopy, the virus was demonstrated to be identical with that of equine arteritis.

Zusammenfassung

Eine tödlich verlaufene, natürliche Infektion mit EA-Virus bei zwei englischen Vollblutfohlen in Polen wird beschrieben. Klinisch und autopsisch dominierte bei beiden Tieren die Erkrankung von Respirations- und Verdauungsapparat. Es bestand eine interstitielle Pneumonie und eine nekrotisierende Enteritis im Bereich des Dickdarms. Weiter wurden degenerierte Veränderungen in den parenchymatösen Organen und Atrophie der Lymphzentren in Lymphknoten und Milz festgestellt. Die Wände muskulärer Arteriolen zeigten als einzige Veränderungen Hyalinisation der Media und entzündliche Infiltration der Adventitia.

Aus den Geweben beider untersuchten Fohlen konnten zwei Virusstämme auf RK-13 und Vero-Zellkulturen isoliert werden. Durch einen modifizierten Neutralisationstest und mittels Elektronenmikroskopie wurde die Identität des Virus beider Stämme mit jenem der equinen Arteritis demonstriert.

Résumé

On décrit une infection naturelle à issue létale due au virus EA chez deux poulains de pur-sang anglais en Pologne. Du point de vue clinique et à l'autopsie, ce sont les lésions des appareils respiratoire et digestif qui prédominent chez ces deux animaux. On constatait la présence d'une pneumonie interstitielle et d'une entérite nécrotique au niveau du gros intestin. En outre, on trouvait des dégénérescences dans les organes parenchymateux et une atrophie des centres lymphoïdes des ganglions et de la rate. Les parois des artérioles du type musculaire présentaient comme seule modification une hyalinose de la couche moyenne et des infiltrations inflammatoires de l'adventice.

A partir des tissus des poulains examinés, on a pu isoler deux souches de virus sur des cultures de cellules RK-13 et Vero. Le virus des deux souches est identique à celui de l'artérite équine, ce qui a été démontré par le test modifié de la neutralisation et au moyen du microscope électronique.

Riassunto

Si descrive una infezione naturale, con decorso mortale, causata da virus EA in due puledri purosangue inglesi in Polonia. Da un punto di vista clinico e autoptico la malattia è risultata essere caratterizzata da lesioni degli apparati respiratorio e digestivo. Sono state riscontrate una polmonite interstiziale e una enterite necrotizzante a carico dell'intestino crasso. Inoltre sono stati osservati fenomeni degenerativi a carico degli organi parenchimatosi e atrofia dei follicoli linfatici nei linfonodi e nella milza. Le pareti delle arteriole di tipo muscolare hanno mostrato, come uniche lesioni, ialinizzazione della media e infiltrazione infiammatoria dell'avventizia.

Dai tessuti dei due puledri si sono potuti isolare due ceppi virali su RK-13 e su culture di cellule Vero. Per mezzo di un test di neutralizzazione modificato e dell'indagine ultrastrutturale è stata dimostrata l'identità del virus di entrambi i ceppi con quello della arterite equina.

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Registration of the manuscript: June 29th 1981

PRESSEINFORMATION

Feierliche Rektoratsübergabe am 6. 11. 1981, Tierärztliche Hochschule Hannover.

Am 1. Oktober 1981 übernahm Prof. Dr. med. vet. *Horst Frerking* als Nachfolger von Prof. Dr. med. vet. Dr. med. vet. h. c. *Wilhelm Schulze* das Amt des Rektors der Tierärztlichen Hochschule Hannover.

Die feierliche Übergabe findet im Rahmen einer Akademischen Feier am 6. November 1981, 10.15 Uhr, in der Aula der Tierärztlichen Hochschule Hannover statt. Der scheidende Rektor wird während dieser Veranstaltung einen Bericht über seine Amtszeit geben. Der neue Rektor hält den Festvortrag: «Rind und Mensch».

Prof. *Frerking* ist an der Klinik für Rinderkrankheiten der Tierärztlichen Hochschule Hannover (Direktor: Prof. Dr. med. vet. *Matthaeus Stöber*) tätig. Er wurde 1980 vom Konzil der Tierärztlichen Hochschule Hannover für die Amtsperiode 1981–1983 zum Rektor gewählt. Seit 1. Oktober 1980 war er Prorektor der TiHo Hannover.

Prof. *Schulze*, Direktor der Klinik für kleine Klauentiere, Forensische Medizin und Ambulatorische Klinik, war 1977 zum Rektor gewählt worden. Vom 1. April 1977 bis 31. März 1978 war er Prorektor der Hochschule und hat zum 1. April 1978 sein Amt als Rektor angetreten. Bis zum 30. September 1982 wird Prof. *Schulze* nunmehr nochmals als Prorektor der TiHo Hannover tätig sein.

REFERAT

Halbzeit auf dem Weg zu einem neuen Antitumormittel?

Zürich (IC). – Mit der erfolgreichen Synthese von Quassin ist Professor Grieco und seinen Mitarbeitern von der Indiana University, Bloomington, der erste wichtige Schritt in der Herstellung neuer möglicher Antitumor-Mittel gelungen. Quassin ist eine farblose, kristalline Verbindung, die durch aufwendige Extraktions- und Reinigungsverfahren aus dem Holz des im tropischen Amerika und in Westindien vorkommenden Quassia-Baumes gewonnen wird. Die Droge wurde 1835 entdeckt, 1937 erstmals rein isoliert, 1960 in der chemischen Struktur aufgeklärt und wie bereits gesagt, vor kurzem erstmals synthetisiert.

Quassin weist einen bitteren Geschmack auf und zeigt ausserdem insektentötende Wirkung. Chemisch gesehen ist die Verbindung aus einem Kohlenstoffgerüst mit vier Ringen aufgebaut. Während Quassin selber keine Antitumor-Wirkung besitzt, konnte eine solche bei den um einen Ring erweiterten sogenannten Quassinoiden festgestellt werden. So wird zurzeit Bruceantin, ein Vertreter dieser Stoffgruppe, der aus dem Holz eines in Aethiopien heimischen Baumes isoliert wird, durch das nationale amerikanische Gesundheitsamt (NIH) an Leukämiepatienten getestet.

Dass die chemische Herstellung von Bruceantin und verwandter Quassinoide gegenwärtig über ein Dutzend Chemikerteams in aller Welt beschäftigt, kann deshalb kaum verwundern. Grosse Probleme bei der Synthese solcher Naturstoffe bietet erfahrungsgemäss die Stereochemie, das heisst der exakte, räumliche Bau der Moleküle. Gerade hier glaubt Professor Grieco, durch die erfolgreiche Synthese von Quassin eine ganze Reihe von Erfahrungen gemacht zu haben, die ihm bei der nun in Angriff zu nehmenden Synthese der komplizierter aufgebauten Quassinoide zugute kommen wird.

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