Acta Veterinaria Hungarica 51 (2), pp. 153–163 (2003)

COMPARISON OF METHODS FOR THE DIAGNOSIS OF EQUINE HERPESVIRUS TYPE 1 INFECTION

L. SZEREDI*, V. PÁLFI and T. MOLNÁR

Central Veterinary Institute, H-1149 Budapest, Tábornok u. 2, Hungary

(Received July 24, 2002; accepted December 10, 2002)

The objective of the investigations was to study the occurrence of the equine herpesvirus type 1 (EHV-1) infection in aborted equine fetuses and in newborn foals and to compare the sensitivity of virus isolation, immunohistochemistry and histology in 101 cases and of fetal serology in 68 cases in the diagnosis of the infection. Out of the 93 aborted equine fetuses and 8 weak foals, 15 (14.9%) (14 fetuses and 1 foal) proved to be EHV-1 infected by immunohistochemical and 13 (12.9%) by virological investigation. Characteristic microscopic changes were seen in several organs in all cases, while intranuclear inclusion bodies could be found only in 25 (35.2%) of the 71 virus-positive tissue samples. Four (5.9%) cases proved to be positive by fetal serological investigation, but none of these cases showed any EHV-1 specific lesions and in none of these cases could the virus be detected by virus isolation or by immunohistochemistry. According to the results, fetal serology does not seem to be a useful test in virus-positive cases, while the immunohistochemical method seems to be a reliable and a slightly more sensitive method than virus isolation in the diagnosis of EHV-1 infection.

Key words: Equine herpesvirus type 1 infection, abortion, virus isolation, immunohistochemistry, fetal serology

Equine herpesvirus type 1 (EHV-1) is the most important infectious cause of abortion in horses; the infection is also associated with perinatal mortality in foals (Smith, 1997). Infected fetus, fetal membranes and lochia provide a high risk for in-contact mares to become infected (Crabb and Studdert, 1996). Because the virus can rapidly spread in a horse farm, the early, accurate diagnosis of EHV-1 infection in a herd is important so that control and preventive measures can be initiated (Schultheiss et al., 1993). Routine diagnostic methods to confirm EHV-1 infections are virus isolation (VI) or direct fluorescent antibody test (IF) to detect viral antigens in cryostat sections (O.I.E., 2000). Sometimes these techniques fail to confirm the presence of EHV-1, especially when the tissues are autolytic (Gimeno et al., 1987; Galosi et al., 2001). Polymerase chain reaction (PCR) was also developed for rapid detection of EHV-1 infection (Bal-

*Corresponding author; E-mail: szeredil@oai.hu; Fax: +36 (1) 222-6071

lagi-Pordány et al., 1990). This technique is reliable even in poorly preserved fetal tissues, where virus isolation was negative (Galosi et al., 2001). The technique seems to be as sensitive as the indirect fluorescent antibody test in fetal organs (Schröer et al., 2000). The immunohistochemical (IH) method is another useful tool for detecting the virus in tissue samples (Gimeno et al., 1987). This technique enables detection of the virus and the histological changes at the same time. Fetal serology is also suggested for the detection of EHV-1 infection (Crandell and Angulo, 1985; O.I.E., 2000); while others found this method inappropriate for this purpose (Whitwell et al., 1992).

The objective of this investigation was to study the occurrence of the EHV-1 infection in all aborted equine fetuses (n = 93) or weak foals (n = 8) submitted to the Central Veterinary Institute, Budapest in the three-year period between 1998 and 2000 and to compare the value of different laboratory methods (VI, IH, histology and fetal serology) in the diagnosis of this infection.

Materials and methods

The samples came from 57 different farms. In eight cases only the organs were submitted, while in the remaining cases the entire body was sent in for examination. In 76 cases fetal membranes were also sent to the laboratory, but they were not examined in this study. All aborted fetuses were over 8 months. The weak foals were between half an hour and 8 days old. Fourteen fetuses and one newborn foal (one hour old) proved to be infected by EHV-1. One aborted mare (Case 10) showed severe colic before abortion and the placenta was partly retained in the uterus; all other mares aborted without showing any previous clinical signs. Two mares (Cases 4 and 10) were kept alone on the farms while the other 13 lived in groups. No data were available on whether the mares had received preventive vaccination against EHV-1.

Macroscopic and microscopic examination

After gross inspection of the fetuses and foals or organs, tissue samples were taken from several organs (Table 1) and fixed in 10% formalin solution. The samples were embedded in paraffin, then 4 μm thick sections were cut and stained with haematoxylin and eosin.

Bacteriology

Samples were taken from the stomach content, liver, spleen, lungs and/or kidney in all cases. The samples were inoculated onto common agar and onto blood agar containing 10% sheep blood, and the cultures were evaluated after incubation under aerobic conditions at 37 °C for 48 h.

Table 1

Occurrence of EHV-1 antigen and intranuclear inclusion bodies in the organs of aborted fetuses and a newborn foal infected by EHV-1

Fetus no.	Lungs IH/i. b.	Liver IH/i. b.	Spleen IH/i. b.	Heart IH/i. b.	Kidney IH/i. b.	Thymus IH/i. b.	Lymph node IH/i. b.	Brain IH/i. b.	Adrenal gland IH/i. b.	Small intestine IH/i. b.
1*	+/±	±/–	–/–	–/–	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.
2	+/+	±/–	–/–	±/–	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.
3	+/–	+/–	±/–	–/–	–/–	±/–	+/±	–/–	n. t.	n. t.
4	+/±	+/–	±/–	–/–	–/–	+/–	n. t.	–/–	n. t.	n. t.
5	+/+	±/–	–/–	–/–	–/–	n. t.	n. t.	±/–	n. t.	n. t.
6	+/–	+/–	+/–	–/–	–/–	n. t.	n. t.	±/–	n. t.	n. t.
7	+/+	+/±	+/–	±/–	±/–	n. t.	n. t.	n. t.	n. t.	n. t.
8	+/+	+/±	+/–	±/–	±/–	n. t.	n. t.	±/–	n. t.	n. t.
9	+/±	+/+	±/–	±/–	±/–	+/+	n. t.	n. t.	+/±	n. t.
10*	+/±	+/+	+/+	+/–	±/–	n. t.	n. t.	n. t.	n. t.	n. t.
11	+/+	+/+	+/±	±/–	±/–	+/+	n. t.	±/–	n. t.	+/±
12	+/–	+/–	+/–	+/–	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.
13#	+/–	±/–	±/–	–/–	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.
14	±/–	+/±	+/–	±/–	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.
15	+/–	+/+	+/±	+/–	±/–	+/–	+/±	+/–	n. t.	n. t.
Positive cases (+, ±)	15/9	15/7	12/3	9/0	6/0	5/2	2/2	5/0	1/1	1/1
%	100/60	100/47	80/20	60/0	60/0	100/40	100/100	71/0	100/100	100/100

*EHV-1 could not be isolated; #newborn foal; IH = immunohistochemistry; i. b. = intranuclear inclusion body in sections stained with haematoxylin and eosin; + = high amount of antigen or intranuclear inclusion bodies; ± = low amount of antigen or intranuclear inclusion bodies; – = no antigen or intranuclear inclusion bodies; n. t. = not tested

Virology

Virus isolation was attempted from the spleen, lungs and liver in all cases. Homogenates (10 per cent) of each organ were prepared in minimum essential medium (MEM) supplemented with 100 U penicillin and 100 µg streptomycin/ml. The homogenates were prepared in individual sterile bags, containing minced organs and appropriate volumes of MEM, using a stomacher (Lab-Slender, Seward Laboratory) for 3 min at room temperature. The resulting suspension was then purified by centrifugation at 5000 rpm for 10 min at 4 °C and the purified supernatants were inoculated on rabbit kidney cell line RK 13. Cultures were incubated at 37 °C for up to 7 days or until the cytopathic effect (CPE) was observed. Cytopathogenic agents were characterised by direct immunofluorescence analysis using FITC-labelled polyclonal anti-EHV-1 antibodies (BIOVETA, Nitra, Slovakia).

Immunohistochemical examination

A total of 212 organ samples were examined by this technique. At least lungs and/or liver were investigated in all cases; other organs were also examined in some cases. In the EHV-1 positive cases several organs were tested by IH (Table 1). After dewaxing of sections, antigen retrieval was performed in citrate buffer (pH 6.0), by heating in a microwave oven (750 W) for 20 min. The samples were incubated in 3% H_2O_2 solution for 10 min and then blocking was performed with a 2% solution of skimmed milk powder for 20 min. The sections were incubated overnight with a 1 in 12,000 dilution of EHV-1-specific goat serum at room temperature (VMRD, Pullman, WA, USA). As control, another series of sections was incubated in a similar manner with an irrelevant goat serum. Antigen-antibody coupling was detected by the peroxidase-antiperoxidase method (Dako Co., Glostrup, Denmark). The sections were treated at room temperature for 10 min with 3-amino-9-ethylcarbazole solution (Sigma Aldrich Co.) containing also 0.01% H_2O_2. Counterstaining was done with Mayer's haematoxylin, and the preparations were covered with glycerol-gelatin and examined at ×100 to ×400 magnification.

Fetal serology

In a total of 68 cases heart blood samples were tested for the presence of EHV-1 antibodies by microneutralisation test (O.I.E., 2000). Seven out of the 15 EHV-1 infected cases were investigated by this method.

Results

Macroscopic examination

Macroscopic examination of the EHV-1 virus positive cases revealed petechial haemorrhages on the serous membranes, icterus, enlargement of the liver and spleen, pronounced pulmonary oedema, presence of small necrotic foci in the liver, prominent lymphoid follicles in the spleen, and the accumulation of straw-coloured serum in the thoracic and abdominal cavities. Of the changes listed above, the fetuses and the foal exhibited one or more at the same time.

Bacteriology

Abortifacient bacteria could not be cultured from any of the 15 EHV-1 virus positive cases.

Virology

Cytopathogenic agents were isolated in thirteen (12.9%) cases. The isolates grew well on RK 13 cells showing cytopathic effect of rounded cells and syncytia. Identification of EHV-1 virus in the infected cells was confirmed by direct IF. The presence of viral antigen was detected as a specific staining both in the nucleus and in the cytoplasm of the infected cells.

Histological examination

In the EHV-1 virus positive cases the following changes could be seen. In the lungs, intra-alveolar and interlobular oedema was found in all cases, while diffuse necrotic inflammation was found in five cases (1, 5, 7, 8 and 11) and intra-alveolar, interstitial inflammation accompanied by some necrotic foci in the alveolar and bronchial epithelium occurred in the remaining ten cases. In the liver, slight to severe lympho-histiocytic inflammation in the area of the portal triad was seen in all cases; in addition, in one fetus (15) diffuse vacuolar degeneration of the hepatocytes and in eight fetuses (4, 7, 8, 10, 11, 13, 14 and 15) fresh necrotic foci in the parenchyma were also found (Fig. 1). Necrosis of varying severity and lymphoid hyperplasia occurred in the follicles of the spleen in all fetuses and in the foal, while in seven cases (3, 6, 10, 11, 12, 14 and 15) focal necrosis was seen also in the red pulp. Fetuses 6 and 8 exhibited multiple focal lympho-histiocytic myocarditis, while in fetus 6 a circumscribed, fresh necrosis surrounded by glial cell proliferation and in fetus 11 multiplex glial cell proliferation was observed in the brain. In fetus 4 mild while in fetuses 9 and 11 severe necrosis was seen in the thymus. Necrosis occurred also in the mesenteric lymph nodes as well as in the capsule, cortex and medulla of the adrenal gland. Mild lympho-histiocytic inflammation and necrosis of many enterocytes was seen in the small intestine. No lesions could bee seen in the kidneys. The occurrence of intranuclear inclusion bodies in the different organs is summarised in Table 1. In the four EHV-1 antibody positive fetuses no histological changes specific for EHV-1 infection could be observed.

Immunohistochemistry

The EHV-1 antigen could be detected in 15 (14.9%) cases. The results of these positive cases are presented in Table 1. The virus was detected both in the cytoplasm and the nucleus of cells. Intranuclear inclusion bodies showed particularly strong staining (Fig. 2). The viral antigen was seen in the macrophages and in the alveolar and bronchial epithelial cells of the lungs and in the hepatocytes and in macrophages located in the area of the portal triad of the liver. The virus was detected in the interstitial macrophages of the heart and kidney and in the macrophages as well as in the reticular cells of the thymus, spleen and mesenteric lymph nodes. Virus dispersed in the microglia cells was found in the

brain of five fetuses. In case 6, virus-infected microglia cells occurred in particularly high numbers in areas close to a recent necrosis. In the adrenal gland, the virus was detected in macrophages dispersed in the capsule, cortex and medulla, as well as in epithelial cells of the cortex. The antigen was visible also in the endothelium of blood vessels of the brain, lungs, liver, kidney, heart and adrenal glands (Fig. 3). Virus-infected peripheral blood monocytes were seen in blood vessels of the brain, lungs, liver, heart, liver, spleen and mesenteric lymph node, as well as in the sinusoids of liver and lymph node. The antigen was especially abundant in and around the necroses. At the same time, by this method virus was only occasionally, or not at all, found in the areas of extensive cell necrosis seen in the follicles of the spleen and mesenteric lymph node.

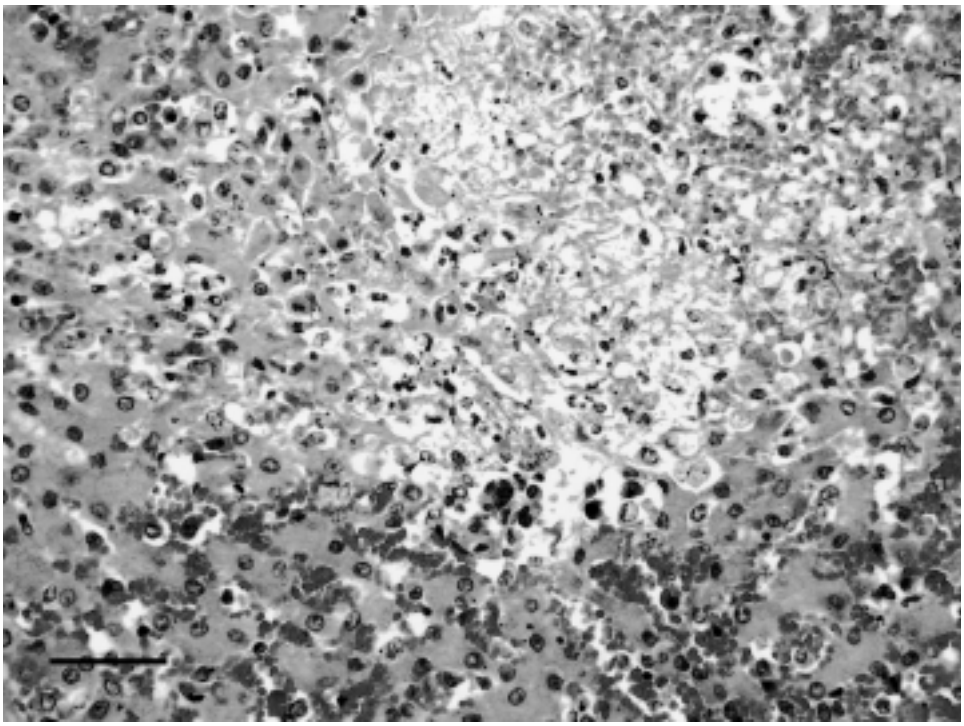

Fig. 1. Fetal liver. Fresh necrosis in the parenchyma. Haematoxylin-eosin. Bar: 40 μm

Fetal serology

Four (5.9%) samples contained specific antibodies to EHV-1; the titre range was 1:3–1:23. None of these were positive in any of the virus detection tests.

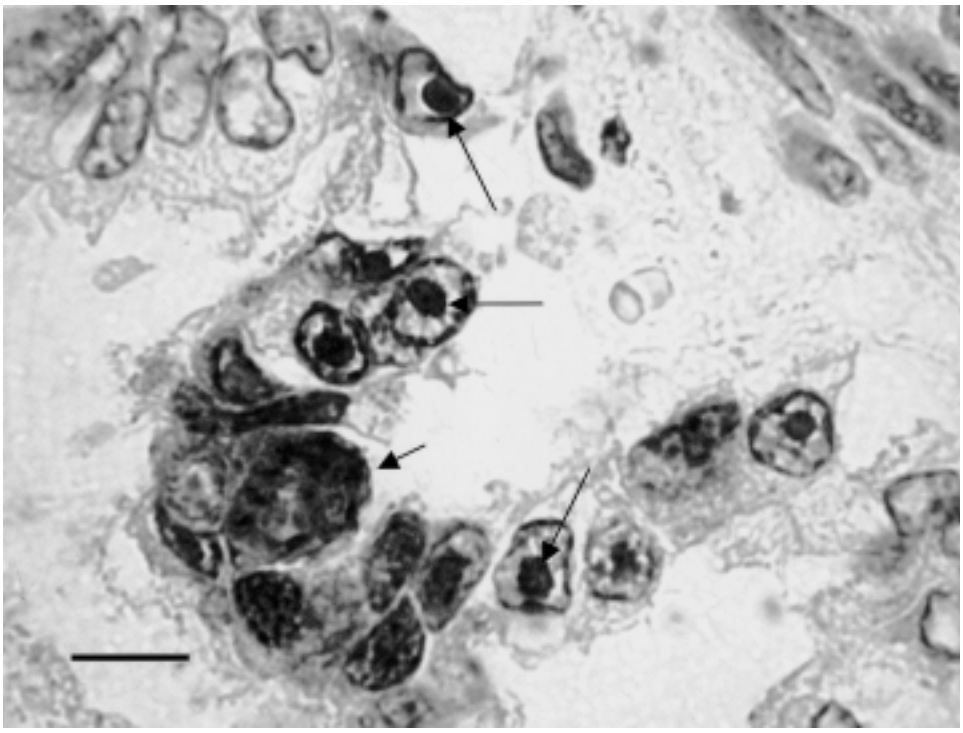

Fig. 2. Fetal lung. Positive signal in the cytoplasm and nucleus of the bronchial epithelial cell (small arrow). Strong signal can be seen in the intranuclear inclusion bodies (long arrows). Immunohistochemistry. Bar: 13 µm

Discussion

The observed macroscopic and microscopic processes were similar to the changes already described in EHV-1 induced abortion and in newborn foals (Kennedy and Miller, 1993; Smith, 1997). EHV-1 induced abortion and death of newborn foals have been shown to occur in Europe with a frequency of 3–25% (Petzoldt et al., 1968; Luttmann et al., 1971; Platt, 1973; Benten et al., 1977; Petzoldt et al., 1987; Pospischil et al., 1992; Pálfi and Christensen, 1995) and in the USA with a frequency of 3–9% (Giles et al., 1993; Hong et al., 1993; Tengelsen et al., 1997), while in Hungary the abortion rate was found to be 20% (Glávits et al., 1984; Rusvai et al., 1996). We found this infection in 14.9% of the cases. Although vaccines against EHV-1/4 are regularly used, our results show that EHV-1-induced abortion is still a very important cause of fetal loss in equine herds in Hungary.

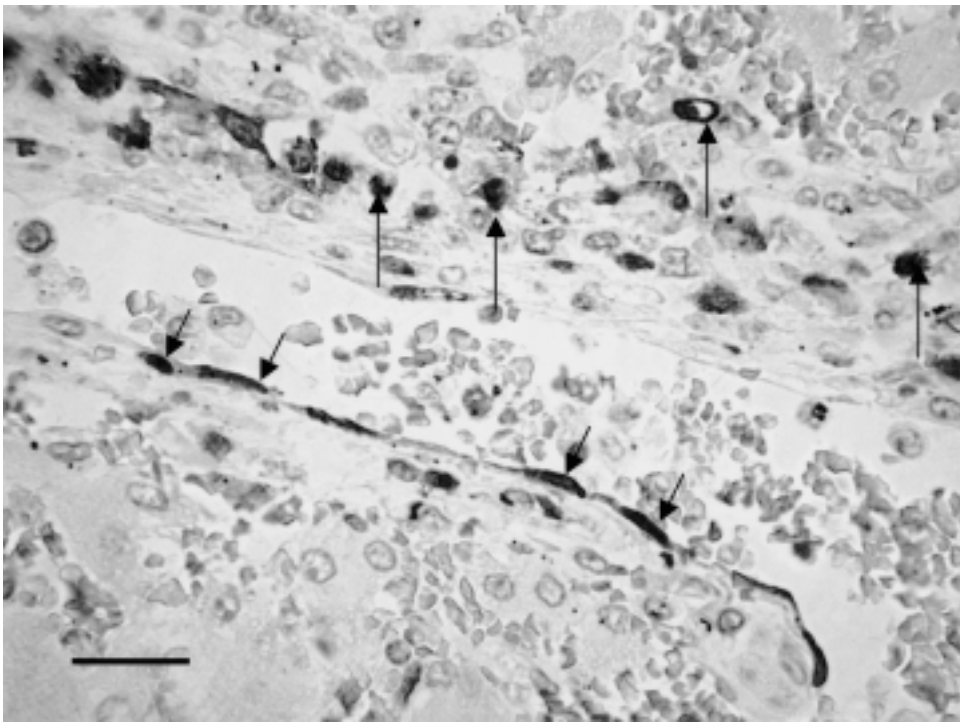

Fig. 3. Fetal liver. Positive signal in the endothelial cells in a blood vessel (small arrows) and in the macrophages (long arrows). Immunohistochemistry. Bar: 30 μm

Comparing the different diagnostic methods Rimstad and Evensen (1993) found that antigen detection and VI had similar sensitivity in the diagnosis of EHV-1-induced abortion, although in some rare cases they could detect the virus either by one or by the other method. In accordance with the findings of other authors (Whitwell, 1982; Gimeno et al., 1987; Edington et al., 1991), antigen detection was found to be slightly more sensitive than VI in this study, as in two cases virus could be detected only by IH. The primary site of virus replication is the fetal lung (Westerfield and Dimock, 1946). This is supported also by the findings of the present study, namely that virus was consistently detected in large amounts in the lungs. The next best choices for antigen detection are the liver and the lymphoid organs. For antigen retrieval microwave heating of the de-waxed sections in citrate buffer solution was used. With the help of this method we could detect much more positive cells than by the digestion of sections with 0.01% protease solution at 37 °C which was used in most of the earlier studies. Additionally, out of the 71 EHV-1 positive organ samples from the 15 EHV-1 infected cases 16 (22.5%) samples were positive (low amount of viral antigen) only by the microwave heating method (unpublished).

The specific microscopic changes were always present in all EHV-1 positive cases. The most consistent lesions were found in the lungs and liver. In contrast, the acidophilic intranuclear inclusion bodies occurred only in 35.2% (25 organ samples) of the virus-infected organs. Necrosis of varying severity was seen in the follicles of the spleen and mesenteric lymph node in almost all cases, without the simultaneous detection of large amounts of virus antigen in this area. This supports the earlier statement according to which these changes come from fetal hypoxia preceding abortion, rather than from the direct effect of the virus (Whitwell, 1982; Smith et al., 1992).

EHV-1 may be present in numerous organs of aborted fetuses, including the brain and the heart (Schmidt et al., 1994; Machida et al., 1997; Del Piero and Dubovi, 1998). Glial cell proliferation and/or necrotic encephalitis were observed in two fetuses, accompanied by the presence of virus antigen in the brain. In three other cases no histological changes could be seen in the EHV-1 infected brain. Schmidt et al. (1994) detected the virus in the endothelium and in epithelial cells of the choroid plexus; in the present study virus was found in the endothelium as well as in the microglia cells and in peripheral blood monocytes within the blood vessels. In two cases lympho-histiocytic inflammation accompanied by viral antigen was seen in the heart while in the other seven cases only the virus could be detected without any histological changes in this organ.

Crandell and Angulo (1985) regard the serological examination of fetuses as a very useful method for diagnosing abortion induced by EHV-1. Our results were at variance with this, but consistent with the observations of Whitwell et al. (1992). EHV-1 specific antibodies could be detected in equine fetuses without the presence of the virus or the virus-specific changes. Whitwell et al. (1992) considered the EHV-1 specific antibodies as a response to non-infectious virus antigens derived from the mare. This can be a non-pathogenic or a vaccine virus strain. The latter case can happen when a non-immune mare is vaccinated with a live vaccine strain.

The macroscopic and microscopic changes were highly suggestive of EHV-1 infection in the aborted fetuses and the newborn foal. Because they cannot be considered diagnostic for the virus infection, diagnosis should be supported by a specific laboratory test (Schultheiss et al., 1993). The VI technique is time consuming and expensive; additionally, false-negative results may be caused by post-mortem changes (Gimeno et al., 1987; Galosi et al., 2001). IH is relative cheap, fast and seems to be more sensitive than the VI method. This test enables the retrospective study of formalin-fixed paraffin-embedded material and also the precise identification of the localisation of the virus in EHV-1 infected animals.

Acknowledgements

We gratefully acknowledge the help of veterinarians submitting the samples and the excellent technical assistance of Mrs Ágnes Mészáros.

References

Ballagi-Pordány, A., Klingeborn, B., Flensburg, J. and Belák, S. (1990): Equine herpesvirus type 1: detection of viral DNA sequences in aborted fetuses with the polymerase chain reaction. Vet. Microbiol. **22,** 373–381.

Benten, C., Petzoldt, K., Sonnenschein, B. and Benten, K. (1977): Investigations on causes of abortions in German warm-blooded horses for several years (in German). Deutsche Tierärztl. Wschr. **84,** 453–492.

Crabb, B. S. and Studdert, M. J. (1996): Equine rhinopneumonitis (equine herpesvirus 4) and equine abortion (equine herpesvirus 1). In: Studdert, M. J. (ed.) Virus Infections of Equines. Elsevier Science B.V., Amsterdam, pp. 11–37.

Crandell, R. A. and Angulo, A. B. (1985): Equine herpesvirus-1 antibodies in stillborn foals and weak neonates. Vet. Med. **80,** 73–75.

Del Piero, F. and Dubovi, E. J. (1998): The diagnosis of equine herpesvirus-1 (EHV-1) abortion and neonatal infection with emphasis on immunoperoxidase histochemical findings. Vet. Path. **35,** 444.

Edington, N., Smyth, B. and Griffiths, L. (1991): The role of endothelial cell infection in the endometrium, placenta and foetus of equid herpesvirus 1 (EHV-1) abortions. J. Comp. Path. **104,** 379–387.

Galosi, C. M., Vila Rosa, M. V., Oliva, G. A., Pecoraro, M. R., Echeverría, M. G., Corva, S. and Etcheverrigaray, M. E. (2001): A polymerase chain reaction for detection of equine herpesvirus-1 in routine diagnostic submissions of tissues from aborted foetuses. J. Vet. Med. B **48,** 341–346.

Giles, R. C., Donahue, J. M., Hong, C. B., Tuttle, P. A., Petrites-Murphy, M. B., Poonacha, K. B., Roberts, A. W., Tramontin, R. R., Smith, B. and Swerzek, T. W. (1993): Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986–1991). J. Am. Vet. Med. Assoc. **203,** 1170–1175.

Gimeno, E. J., Nosetto, E. O., Martin, A. A., Galosi, C. M., Ando, Y. and Etcheverrigaray, M. E. (1987): Demonstration of equine herpes virus 1 (EHV-1) in histological sections and tissue cultures by the peroxidase-antiperoxidase (PAP) technique. J. Vet. Med. B **34,** 740–742.

Glávits, R., Molnár, T. and Pálfi, V. (1984): Observations in the abortion of mares caused by equine rhinopneumonitis virus 1. Data to the pathology and diagnosis of the disease (in Hungarian, with English abstract). Magyar Állatorvosok Lapja **39,** 361–365.

Hong, C. B., Donehue, J. M., Giles, R. C., Petrites-Murphy, Jr., M. B., Poonacha, K. B., Roberts, A. W., Smith, B. J., Tramontin, R. R., Tuttle, P. A. and Swerczek, T. W. (1993): Equine abortion and stillbirth in central Kentucky during 1988 and 1989 foaling seasons. J. Vet. Diagn. Invest. **5,** 560–566.

Kennedy, P. C. and Miller, R. B. (1993): Equine herpesvirus-1. In: Jubb, K. V. F., Kennedy, P. C. and Palmer, N. (eds) Pathology of Domestic Animals, 4th edition, 1993. Academic Press, San Diego, pp. 436–439.

Luttmann, U., Weiland, E., Dimitriadis, I. and Petzoldt, K. (1971): The fluorescent antibody technique for the diagnosis of equine herpesvirus abortion in comparison to conventional diagnostic methods (in German). Deutsche Tierärztl. Wschr. **78,** 623–627.

Machida, N., Taniguchi, T., Nakamura, T. and Kiryu, K. (1997): Cardio-histopathological observations on aborted equine fetuses infected with equid herpesvirus 1 (EHV-1). J. Comp. Path. **116,** 379–385.

O.I.E. (2000): Equine rhinopneumonitis. In: Manual of Standards for Diagnostic Tests and Vaccines. 4th edition. Office International des Epizooties, Paris, pp. 565–575.

Pálfi, V. and Christensen, L. S. (1995): Analyses of restriction fragment patterns (RFPs) and pathogenicity in baby mice of equine herpesvirus 1 and 4 (EHV-1 and EHV-4) strains circulating in Danish horses. Vet. Microbiol. **47,** 199–204.

Petzoldt, K., Dieckmann, W. and Lindemann, L. (1968): Comparative diagnostic investigations in fetuses and foals with rhinopneumonitis virus infection (equine virus abortion) (in German). Deutsche Tierärztl. Wschr. **74,** 545–549.

Petzoldt, K., Merkt, H., Müller, E. and Kirpal, G. (1987): New diagnostic observations in equine herpesvirus abortions (in German). Tierärztl. Prax. **15,** 393–397.

Platt, H. (1973): Aetiological aspects of abortion in the thoroughbred mare. J. Comp. Path. **83,** 199–205.

Pospischil, A., Lieb, A. and Corboz, L. (1992): Causes of foal abortion in Switzerland. Schweiz. Arch. Tierheilk. **134,** 401–409.

Rimstad, E. and Evensen, O. (1993): The identification of equid herpesvirus 1 in paraffin-embedded tissues from aborted fetuses by polymerase chain reaction and immunohistochemistry. J. Vet. Diagn. Invest. **5,** 174–183.

Rusvai, M., Kucsera, L. and Pálfi, V. (1996): Prevalence of equine viral diseases in Hungary and the main aspects of their control (in Hungarian, with English abstract). Magyar Állatorvosok Lapja **51,** 499–503.

Schmidt, P., Meyer, H., Hübert, P., Hafner, A., Andiel, E., Grabner, A. and Dahme, E. (1994): *In-situ* hybridization for demonstration of equine herpesvirus type 1 DNA in paraffin wax-embedded tissues and its use in horses with disseminated necrotizing myeloencephalitis. J. Comp. Path. **110,** 215–225.

Schröer, U., Lange, A., Glatzel, P., Ludwig, H. and Borchers, K. (2000): The relevance of equine herpesvirus 1 (EHV-1) infection in a German thoroughbred stud: vaccination, abortion and diagnostics (in German). Berl. Münch. Tierärztl. Wschr. **113,** 53–59.

Schultheiss, P. C., Collins, J. K. and Carnman, J. (1993): Use of an immunoperoxidase technique to detect equine herpesvirus-1 in formalin-fixed paraffin-embedded equine fetal tissues. J. Vet. Diagn. Invest. **5,** 12–15.

Smith, K. C., Whitwell, K. E., Binns, M. M., Dolby, C. A., Hannat, D. and Mumford, J. A. (1992): Abortion of virologically negative foetuses following experimental challenge of pregnant pony mares with equid herpesvirus 1. Equine Vet. J. **24,** 256–259.

Smith, K. C. (1997): Herpesviral abortion in domestic animals, review. The Vet. J. **153,** 253–268.

Tengelsen, L. A., Yamini, B., Mullaney, T. P., Bell, T. G., Render, J. A., Patterson, J. S., Steficek, B. A., Fitzgerald, S. D., Kennedy, F. A., Slanker, M. R. and Ramos-Vara, J. A. (1997): A 12-year retrospective study of equine abortion in Michigan. J. Vet. Diagn. Invest. **9,** 303–306.

Westerfield, C. and Dimock, W. W. (1946): The pathology of equine virus abortion. J. Am. Vet. Med. Assoc. **109,** 101–111.

Whitwell, K. E. (1982): An assessment of the criteria used to diagnose virus abortion: a review of 100 cases. J. Reprod. Fert. Suppl. **32,** 632–633.

Whitwell, K. E., Bowen, M. and Hannant, D. (1992): A search for antibody to equine herpesvirus 1 in equine foetuses. In: Plowright, W., Rossdale, P. D. and Wade, J. F. (eds) Proc. 7th Int. Conf. Equine Inf. Dis. R&W Publications, Newmarket, pp. 339–340.