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Prevalence and eradication of equine infectious anemia (EIA) in Slovenia

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Summary

The paper reviews history of diagnostic methods and prevalence of equine infectious anemia (EIA) in Slovenia from 1973 to 1995.
During the initial period (1950–1972) clinical diagnostic methods, such as hemogram, erythrocyte sedimentation rate, detection of siderocytes in peripheral blood, liver biopsy and pathohistological examinations were used. Since 1973 the disease has been etiologically diagnosed with the Coggins test.
In the general epidemiological survey in 1981 and 1982, a total of 18 368 horses were examined and 620 (3,38 %) positive animals were reported. In accordance with planned serological examinations and eradication the percentage of positive animals in the last 10 years rapidly decreased to less than 1 %. Individual positive animals originate mainly from import.
Determination and eradication of EIA in Slovenia is based on the Act of veterinary practice, Regulations on eradication of EIA and on the annual state Decree on the execution of preventive measures.

Keywords: horse, equine infectious anemia, prevalence, diagnostics, Coggins test, eradication

Prävalenz und Ausrottung der Infektiösen Anämie der Einhufer (EIA) in Slovenien

Diese Abhandlung gibt einen Überblick über die Geschichte der diagnostischen Methoden und Prävalenz der Infektiösen Anämie der Einhufer (EIA) in Slovenien von 1973 bis 1995.
In den vorausgegangenen Jahren (1950–1972) hatte man klinische diagnostische Methoden wie Blutbild, Erythrozyten-Sedimentationsrate, Nachweis von Siderozyten im peripherem Blut, Leberbiopsie und pathohistologische Untersuchugen angewendet. Seit 1973 wurde die Erkrankung mittels des Coggins-Testes diagnostiziert.
In der allgemeinen epidemiologischen Studie von 1981 und 1982 wurden 18.368 Pferde untersucht, von diesen wiesen 620 Tiere (3,38 %) ein positives Ergebnis auf.
In Übereinstimmung mit geplanten serologischen Untersuchungen und Ausrottung konnte der Prozentsatz der positiven Tiere auf weniger als 1% gesenkt werden. Einzelne positive Tiere sind hauptsächlich durch den Import bedingt. Alle Pferde in größeren Gestüten und alle Zuchthengste werden jährlich getestet. Zusätzlich werden alle importierten Pferde sowie alle Pferde , die innerhalb des Landes ihren Standort wechseln, mittels des Coggins-Testes überprüft.
Feststellung und Ausrottung der EIA in Slovenien sind geregelt durch ein Gesetz für die tierärztliche Praxis, Anordnungen über die Ausrottung der EIA und jährliche staatliche Verordnungen über die Durchführung vorbeugender Maßnahmen.

Schlüsselwörter: Pferd, Infektiöse Anämie der Einhufer, Prävalenz, Diagnose, Coggins Test, Ausrottung

Introduction

Equine infectious anemia (EIA) is a contagious disease of horses caused by a virus and characterized by a chronic illness and intermittent acute attacks of increased body temperature. The virus is a non-oncogenic retrovirus inducing disease only in *Equidae.*
The virus can be localized in acute cases in all tissues and persists in blood leucocytes. The disease is detected sporadically, but it may spread also in epidemic form from groups of ill horses by bloodsucking flies or transmitted by the use of contaminated surgical instruments or needles.
In the year 1938, a disease very similar to equine infectious anemia was observed in Slovenia, especially in marshy areas around Ljubljana. Prevalence of the disease was considerable before and during World War II because of large movements of horses. After the war the disease was encountered in association with two diseases of horses similar to EIA which caused considerable differential diagnostic problems: avitaminosis B (horse-tail poisoning) and swamp jaundice or the so-called swamp madness (*Gregorovič,* 1953, 1959).
On November 23, 1946, the veterinary Administration of Slovenia convened a session to address the problem of equine infectious anemia. Discussed were the history of the disease and the whole complex of the so-called „swamp diseases" and their interrelationships.
Because the number of suspect horses increased also in other parts of Slovenia, the solution of establishing the diagnosis and differential diagnosis became the primary objective in this area of research.
Soon after World War II (1950), the Central Veterinary Hospital at the Veterinary Scientific Institute of Slovenia attempted to define specific diagnostic tools for „swamp diseases". The Clinical laboratory at the Central Veterinary Hospital began immediately after its foundation in 1950 to implement hematological (E and L cell

count, Hb concentration, erythrocyte sedimentation rate, detection of siderocytes) and other laboratory methods. Eradication program and diagnostics have been carried out by the Veterinary Faculty in Ljubljana since 1958.

The literature on EIA from the year 1935 to 1994 comprises 32 scientific papers by Slovene authors: *S. Malenπek*, 1935; S. *Žibert,* 1947; *J. Koren*, 1949; *V. Gregorović* and *Gregorović* et al., 1953, 1955, 1957, 1958, 1959, 1960; *O. Böhm,* 1955, 1956, 1958; *L. ˇenk*, 1955, 1958; *F. Skuπek*, 1956, 1957, 1963; *M. Dolenc*, 1963; *Z. Železnik,* 1972; *Jukić* et al., 1981; *M. Kopitar,* 1982; *I. Jazbec* et al., 1983, 1984 and *J.Jurkovič*, 1985 (*Jazbec* et al, 1983, 1984).

Experimental infection was accomplished the same year by *Böhm* and *Kovač* (1958) via transmission of the disease from an infected animal to negative foals for confirmation that the disease was infectious.

In 1956, *Englert, Gregorović* and *ˇenk* (1957) introduced liver biopsy, and, in 1959, *Gregorović* (1959) developed the method of detection of siderocytes in the peripheral bloodstream which was the most advanced diagnosis method of EIA in vivo in that time.

Until serological methods were applied (1960–1970), the only confirmatory objective diagnostic test was the bio-assay on homologous animals. But because it is very expensive and time-consuming, it never received wide usage. Isolation of the virus which was identified 24 years ago is, however, possible on cell cultures, yet proved unsatisfactory for routine diagnostic purposes.

A decisive progress in the diagnosis of EIA was made since the year 1970 with the use of agar gel immunodiffusion test - the AGID test (*Coggins* 1970, *Issel* 1993).

First such experiment was carried out by *Saxer* in 1960, while the method was perfected by *Coggins* (1970,1975) and recognized as the Coggins test. It has proved to be very accurate and enables detection of antibodies to EIA virus. The use of the Coggins test was obligatory in the United States already in 1971 and was that year introduced in the laboratory for virology at the Veterinary Faculty in Zagreb (*Petrović* et al, 1972, 1973).

The Veterinary Faculty in Zagreb (*Jukić*, 1982; *Jurkovič,* 1985) carried out serological tests of EIA for the needs of the veterinary service in Slovenia until 1981.

The Clinic for Ruminants at the Faculty in Ljubljana began to introduce the technique of the Coggins test in 1980, and as of January 1, 1982 it undertook the routine diagnostics of EIA for Slovenia.

In 1981, the Republican Veterinary Administration (*Kopitar,* 1982) imposed by decree serological testing of all horses in $^1/_5$ of all Slovene communes which was carried out at the Veterinary Faculty in Zagreb. The primary objective was to determine prevalence of the disease in Slovenia. In compliance with the Decree from 1982, serological testing of all horses in the remaining $^4/_5$ of Slovene become obligatory. These examinations were already carried out in the laboratory of the Clinic for Ruminants (*Jazbec* et al, 1983, 1984).

The purpose of this paper was to present the history of the disease diagnostics with special emphasis on determination of prevalence and the effectiveness of the eradication program from the year 1950 to 1995.

Materials and methods

Veterinarians collected blood samples for serological testing according to the one-year program of the Veterinary Administration of Slovenia which enabled an even distribution of work load in the lab. Of 60 Slovene communes, 12 collected blood samples in 1981 and forwarded them for testing to the Veterinary Faculty in Zagreb (*Jukić* et al, 1982). During 1982, the horses from the remaining 48 communes were examined; serological testing included also a greater number of animals which were not tested in 1981. Blood samples were sent to the diagnostic laboratory of the Clinic for Ruminants in Ljubljana. Most of the samples were brought, only a smaller number was sent by mail. For serological testing the Coggins-Norcross (AGID) test with antigens from producers IFFA Merieux, Pitman-Moore and Veterinary Faculty of Zagreb was used.

The reliability of the diagnostic procedure was indirectly supervised by the international center of EIA in Alfort, and directly by the reference laboratory at the Veterinary Faculty in Zagreb under guidance of Prof. *Jukić*.

In addition to obligatory serological testing of horses in Slovenia, all imported animals and all horses migrating within state are submitted for the AGID test.

After general obligatory serological testing of horse population during 1981–1982, all horses in greater stud farms and all stud horses are regularly tested on an annual basis. These examinations are prescribed by special annual state Decree.

In compliance with special Regulations prescribing the diagnosis and eradication of EIA, all horses are serologically tested at the expense of the owners who buy them (obligatory 30 days quarantine) as well as those that migrate occasionally (sporting events, shows, sales). For every intra/inter state movement the animals are required to have a certificate not older than 3 months.

Results and discussion

In 1982, a total of 13.418 horses were serologically examined using the Coggins test. Privately owned were 12.248 animals (91,28 %) and 1170 (8,72 %) were socially owned. During 1982, 11.802 examinations were conducted in 45 communes including 1616 animals from 12 communes which were not tested in 1981 as programmed.

Serological examinations of horses from 12 communes carried out in Zagreb in 1981 and the examinations from 1982 have shown that horses were infected in 45 communes (90 %). All samples (n=137+128+2+97=364) from 4 communes were negative. No samples were tested from 2 communes. Six hundred twenty of 18.368 samples (3,83 %) tested positive. The commune with the lowest percentage had 1,08 % and with the highest 11,43 % positive specimens.

Among younger horses, up to 5 years of age 12,17 % were positive, from 6. to 10. year 27,72 %, from 11. to 15. year 34,51 %,

Table 1: Frequency of positive reactors regarding the years of age

Age in years	Positive animals	
	n	%
1– 5	8	2,17
6–10	102	27,72
11–15	127	34,52
16–20	89	24,18
21–25	36	9,78
26–30	6	1,63
Total	368	100,00

Tab. 2: Serologic examinations of EIA in Slovenia from 1973 to the end of 1980

Year	Property				Total	
	Socially-owned		Privately-owned			
	n	pos/%	n	pos/%	n	pos/%
1973	164	0	11	0	175	1/0,75
1974	20	0	44	0	64	17/26,60
1975	0	0	63	3/4,80	63	3/4,80
1976	36	4/11,1	53	5/9,40	89	9/10,10
1977	38	0	17	1/5,90	55	1/1,80
1978	48	0	55	10/18,20	103	10/9,70
1979	52	2/3,80	59	5/8,50	111	7/6,30
1980	326	0	1804	59/ 3,30	2130	59/2,80
Total	684	6/0,88	2106	83/3,94	2790	107/3,84

from 16. to 20. year 24,18 %, and from 21. to 30. year 11,41 % were reported positive. We assume that with several animals disease signs were clinically unapparent and so they reached old age. These clinically normal carriers could be the source for disease transmission to younger and susceptible animals (Table 1).

Among positive animals there were 63,84 % mares, 33,67 % geldings, 2,24 % stallions and 0,25 % donkeys.

Table 2 shows the development of serologic diagnosis of EIA in Slovenia including the very first examinations in 1973–1975 (*Jukić,*1982; *Jurkovič,* 1985) until the end of 1980 (*Jazbec* et. al. 1983) when a total of 2790 animals were serologically tested. During the period from 1973 to the end of 1980, 107 (3,84 %) positive animals were reported.

The eradication policy was based mainly on the fact that the disease is incurable and that positive animals are a constant source of infection.

We have never detected during that period or later any positive individuals in the famous stud farm Lipica nor in any greater stud herds. Positive animals were mainly privately owned; producers kept only a few animals, new ones were often imported.

The basic principle of the eradication program was to eliminate horses having positive reactions. Horse owners had to compensate for their loss themselves which caused, however, minor problems.

EIA being on the Office international des epizooties (OIE) list B 38, is also under the Act of veterinary practice in Slovenia and in accordance with the regulations all professional services and producers are responsible for eradication and systematic surveillance of the occurrence and spread of the disease. On the basis of the Act, the Regulations with specified provisions on EIA eradication were elaborated and have been in force since 1988.

The animals are infected with EIA when the clinical diagnosis is confirmed by the Coggins test. All positive animals should be immediately branded with the EIA mark (8 cm). Suspect animals are those that were in the past three months in contact with positive individuals. Suspect animals are free of EIA when four successive Coggins test results are negative. Horse populations are submitted to testing at least once in 5 years. In addition, an obligatory serological testing is carried out in all stud and sporting farms and all herds with more than 10 animals as well as twice annually in all institutes for biological preparations. The Coggins test is used to certify horses free of EIA 90 days prior to be exported or moved interstate, be sold or accepted at shows and tracks.

In accordance with the Regulations, all serologically positive and clinically abnormal animals should be eliminated, while those with subclinical signs (only serologically positive) are selected for slaughter. In stables where positive animals were detected great care must be taken that disinfection is satisfactory. The disease is considered suppressed when four months had elapsed since the last positive horse was eliminated and all remaining animals were serologically negative during that period.

In the most recent Slovene Act on veterinary practice from 1994, EIA is on the list of infectious diseases under no. 42 which should be eradicated in accordance with the general and specified regulations.

Every year the Veterinary Administration of Slovenia prescribes by the annual Decree the execution of preventive measures for detection and suppression of animal contagious diseases providing an article on specified obligations for EIA detection as foreseen by the Act and the Regulations.

Table 3 shows the period from 1981 to 1995 when independent etiologic diagnosis of EIA with the Coggins test was begun in Slovenia.

A close survey of EIA occurrence in Slovenia displays that eradication was executed to the extent that the majority of stud farms

Tab. 3: Epidemiological data on prevalence of EIA from 1981 to the end of 1995

Year	No. of tested animals	No. of positive reactors	
		n	%
1981	4.950	178	3,60
1982	13.418	442	3,29
1983	2.159	47	2,20
1984	1.424	15	1,10
1985	1.154	10	0,90
1986	1.149	12	1,04
1987	1.472	9	0,60
1988	1.373	8	0,60
1989	1.118	5	0,50
1990	1.427	3	0,20
1991	1.436	1	0,07
1992	1.684	3	0,20
1993	2.125	0	0,00
1994	2.251	10	0,44
1995	2.617	7	0,27

are free of the disease. Individual cases are reported only upon introduction of animals from neighboring countries.
Last but not least, permanent concern of veterinary service to determine the disease and the introduction of current etiological diagnosis proved very effective. This is confirmed by the fact, that we have managed to prevent the disease to be introduced into big and famous (Lipica) stud farms. All privately owned herds are also as a rule negative (Table 2 and 3). A negligible percentage of positive animals originates from purchases and import.

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