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Control of viral haemorrhagic disease of rabbits in Poland

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Summary: *The authors present an epizootiological analysis of viral haemorrhagic disease of rabbits in Poland. The biological, physical and chemical properties of virus isolates used for the production and control of the vaccine 'Cunivac' (produced in Poland) are also presented. The safety and efficacy of this vaccine are demonstrated. Laboratory experiments and large-scale field observations yielded satisfactory results.*

KEYWORDS: Disease control – Epidemiology – Rabbits – Vaccination – Viral haemorrhagic disease.

INTRODUCTION

The first outbreaks in Poland of viral haemorrhagic disease (VHD), an acute and fatal infectious disease of rabbits, were recognised in the regions of Krakow and Silesia in the south of the country in spring 1988 (9). A similar disease affecting rabbits had been reported in 1987 in Slovakia and Ukraine (23, 24, 27). In 1988, the spread of the infection in Poland was very rapid, with high morbidity and mortality. The Ministry of Agriculture therefore included VHD on the national list of notifiable diseases. The first vaccination attempts were made in autumn 1988, and large-scale production of the commercial inactivated vaccine developed at the National Veterinary Research Institute (NVRI) began in 1989.

MATERIALS AND METHODS

Epizootiological analysis

The number of outbreaks, numbers of dead or slaughtered rabbits and the territorial range of VHD were based on reports of infectious diseases issued by the Veterinary Service of the Ministry of Agriculture. Data concerning vaccine production were obtained from the NVRI.

Virus

The following strains of VHD virus were isolated from internal organs of animals which died during outbreaks: SGM, KGM, PD and ZW (7, 9). Viruses were stored as 20% homogenates or freeze-dried at –18°C and 4°C.

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Estimation of virus titre

The haemagglutination (HA) titre of VHD virus strains was determined using human erythrocytes. The 50% lethal dose (LD_{50}) of this virus was also evaluated.

Serological tests

All serological data were obtained by the haemagglutination inhibition (HI) test (7, 20, 28).

Animals

Experiments were performed on New Zealand and California breeds, and cross-bred rabbits of various ages and weights, mostly 2.0-3.0 kg. Rabbits were infected with VHD virus by the subcutaneous, intramuscular, intraperitoneal, intracerebral, oral and intranasal routes. In some experiments, other animals (dogs, cats, guinea-pigs, mice, blue foxes and hares) were also used.

Post-mortem examination and sampling

All the animals, both dead animals and those which survived experimental infection, were subjected to necropsy. Internal organs were sampled for use in histopathological and electron microscopic examination, and in the HA test.

Electron microscopy

Ultra-thin sections were prepared and stained according to the method described by Reynolds (22). The sections were examined and photographed at a magnification ranging from 8,000× to 62,000×.

Chemical properties

Resistance of the virus to ether (1) and chloroform (2) was examined. The inactivating properties of formalin, biethylenoimine (BEI), ethyl alcohol, sodium hydroxide (NaOH), iodine in Pollena JK and potassium persulfate in Virkon-Naturan were tested at various temperatures, using different concentrations of chemicals and times of inactivation.

Vaccine tests

'Cunivac', a vaccine against VHD virus, was tested for sterility, safety, level of virus inactivation and efficacy (10).

Calculation of results

LD_{50} titres were calculated by the method described by Reed and Münch (21). HA and HI titres were expressed as a reciprocal of the highest dilution. Means were presented as $\bar{x}$ (arithmetic) and $\bar{x}_g$ (geometric).

RESULTS

Occurrence and spread of viral haemorrhagic disease in Poland

In contrast to myxomatosis (12), VHD is characterised by rapid territorial spread. The first two outbreaks of the disease in Poland were recognised in April 1988 and, by the end of the year, 865 outbreaks and a total of 27,752 dead rabbits in 18 regions had been reported (Figs 1 and 2). The mortality rate following natural infection was variable, ranging from a few cases to 99.5% losses. The highest mortality was observed

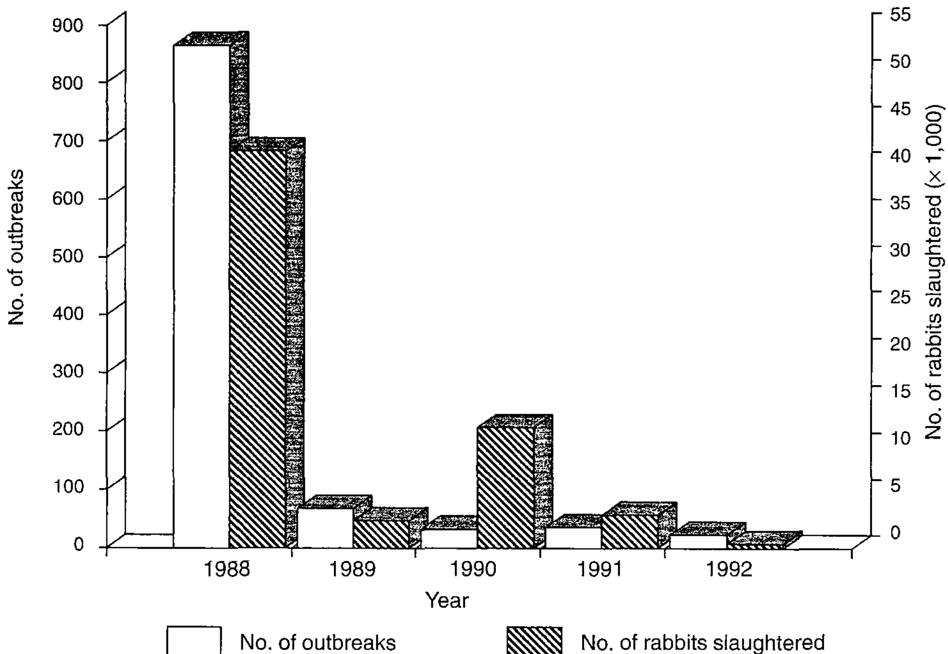

FIG. 1

Number of outbreaks of viral haemorrhagic disease and number of rabbits slaughtered as a control measure between 1988 and 1992

in Angora rabbits and cross-breeds. At first, only adult rabbits were affected, while young animals became ill one to three weeks later. On one farm with 200 rabbits, 199 died within 15 days, showing typical signs (9, 15, 17). The highest mortality rate was noted between 7 and 12 days after the appearance of the first clinical symptoms. An acute form of VHD was observed after parenteral inoculation. Of 481 rabbits, 3 died before 18 h post-inoculation (p.i.), 31 between 18 and 24 h p.i., 273 between 38 and 56 h, 35 after approximately 72 h, and 9 animals after 96 h p.i. There were no differences in the timing of the occurrence of the first clinical signs in rabbits infected by various routes.

Epizootiological analysis revealed that rabbits are not the only source of infection. The transport of infected animals, meat or objects may also have spread the infection.

Seasonal prevalence of the disease is presented in Figure 3. The greatest number of outbreaks occurs during the summer. This was observed both in 1988 and in the following years. The decline in the number of outbreaks is shown in Figures 1 and 2.

CHARACTERISTICS OF THE POLISH VACCINE AGAINST VIRAL HAEMORRHAGIC DISEASE

The positive results achieved by other investigators (13, 29) encouraged the preparation of a Polish vaccine. Replication of the native isolates in various cell cultures proved impossible, and internal organs of experimentally-infected rabbits were

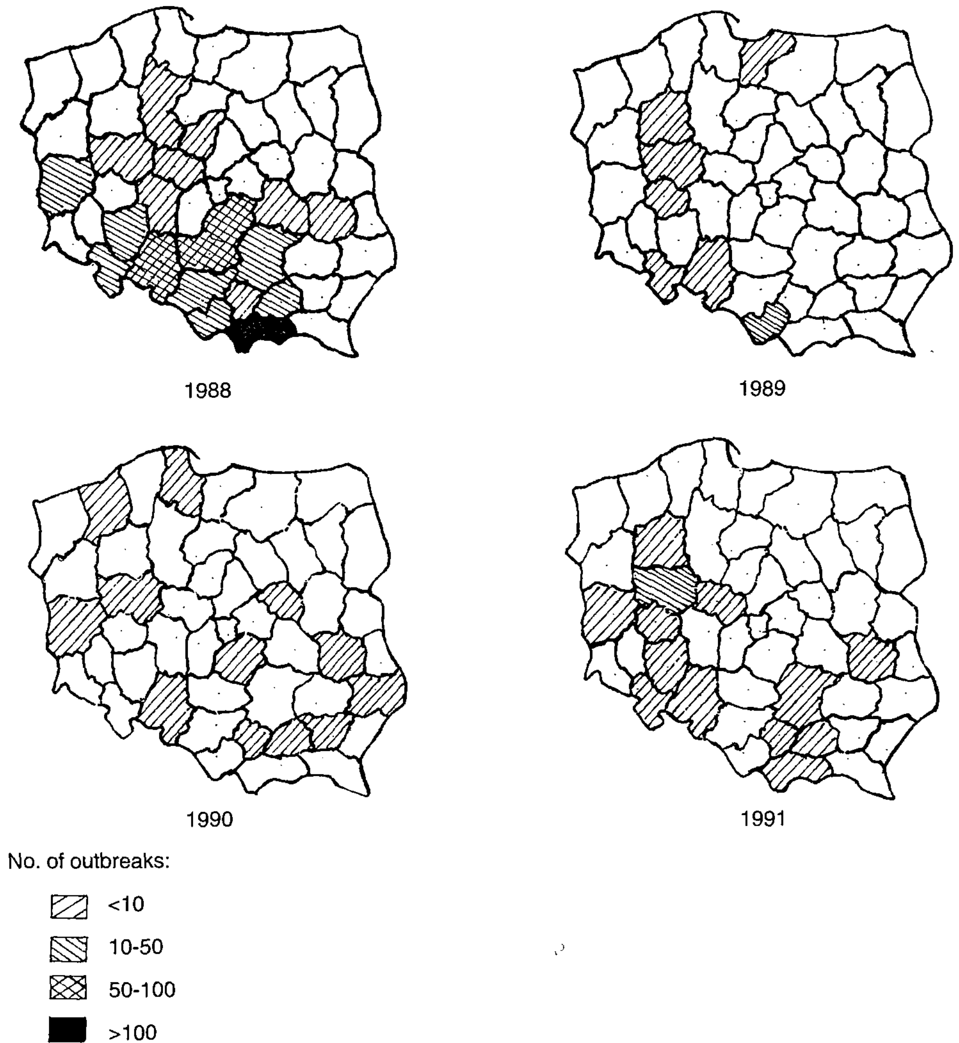

FIG. 2

Analysis of the spread of viral haemorrhagic disease in Poland between 1988 and 1991

therefore used as a source of antigen. Both transmissible clinical symptoms and morphological changes in tissues of rabbits were noted after experimental infection. These were similar to those described by other authors (15, 24, 27, 31). The titre of the virus in liver homogenates was equal to $10^{3.0}$-$10^{6.0}$ ID_{50}, and HA titres ranged from 1/160 to 1/10,240. Results were repeatable.

As shown in Table I, four different isolates agglutinated human erythrocytes not only of type O, but also of types A, B and AB (both Rh+ and Rh–). The estimated HA titre of each isolate at 4°C and at room temperature was the same. The test performed at 37°C did not give regular or repeatable results (7). HA titres examined at various

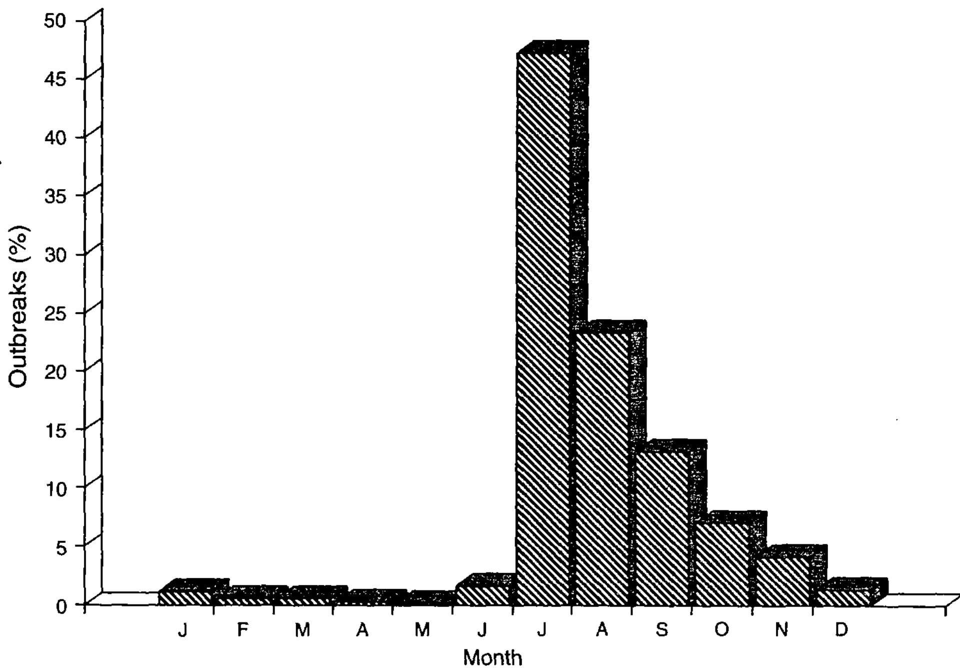

FIG. 3

Seasonal prevalence of viral haemorrhagic disease in Poland between 1988 and 1992

intervals following infection are shown in Table II. All internal organs can serve as potential sources of the antigen. Sampling should be performed just prior to death of the animal. Electron microscopic examinations demonstrated viral particles of 28-30 nm replicating in the cytoplasm of infected cells in all internal organs (11, 17, 24).

TABLE I

Mean haemagglutination titre of various strains of viral haemorrhagic disease virus with human erythrocytes of types O, A, B and AB

Type of HRBC	Strain: SGM No. of examinations	SGM $\bar{X}$ titre	KGM No. of examinations	KGM $\bar{X}$ titre	PD No. of examinations	PD $\bar{X}$ titre	ZD No. of examinations	ZD $\bar{X}$ titre
O	14	3,072	3	3,328	15	5,120	3	4,352
A	8	3,072	1	2,560	9	1,920	2	3,840
B	8	3,584	1	2,560	3	6,656	1	10,240
AB	8	2,560	1	2,560	7	5,120	–	–

$\bar{X}$: arithmetic mean

HRBC: human red blood cells

TABLE II

Dynamics of haemagglutination (HA) titre in rabbits infected with viral haemorrhagic disease virus

Origin of tissue homogenate	HA titre *						
	6 h	**12 h**	**18 h**	**24 h**	**30 h**	**36 h**	**42 h**
Lung	<10	20	320	640	640	1280	2560
Heart	<10	20	320	320	320	ND	320
Liver	10	20	320	640	640	1280	5120
Spleen	20	20	40	40	640	640	640
Kidney	<10	10	320	320	640	640	1280

ND: no data
* figures at the head of each column indicate number of hours post-inoculation

Long-term stability is typical of VHD virus. The virus may survive for 51 months at –18°C, over 50 months at 4°C and 10-19 months when kept at room temperature. The KGM and SGM strains were resistant to ether, chloroform and pH 3. The virus was not inactivated after exposure for 2 h to 2% Virkon, 3% Pollena JK and 70% ethanol (8). The following were infallible inactivators of the virus: 0.2% formaline (24 h at 37°C), 0.4% BEI, 1% NaOH and 4% Virkon. The agglutination properties of the virus were not affected by the above disinfectants. The KGM, SGM, PD and ZW strains were apathogenic for suckling and adult mice, and guinea-pigs. KGM and SGM were also apathogenic for hares, dogs, cats and blue foxes (17).

Determination of the conditions for viral replication and inactivation, the physico-chemical and biological properties of the virus, and methods of virus purification (11) enabled preparation of the vaccine. In 1988, five experimental batches of the inactivated, gel-adjuvanted vaccine were prepared. The tests for sterility, safety and efficacy were satisfactory and the vaccine was comparable to that produced in Czechoslovakia (23, 26). Experimental and reference vaccines were used for 66 rabbits. All the animals remained healthy and survived challenge. Field observations were performed on 604 rabbits which remained healthy for over six months (10). In 1989, large-scale production of Cunivac began at the NVRI, and 765,200 doses (13 batches) had been produced by 1993. All 128 vaccinated rabbits remained resistant to challenge, while 87 of 108 controls died. Further investigations indicated that immunity was developed as early as four days after vaccination. Rabbits were also resistant to challenge when vaccinated with 1 ml of the vaccine as well as with 0.5 ml and 0.1 ml.

The immunogenic properties of the vaccine were shown after 27 months of storage at 4°C, and 50% of rabbits were immune when vaccinated with Cunivac stored at 4°C for 31 months (A. Fitzner, unpublished findings). The expiry date of the vaccine is 18 months from the date of production.

The efficacy of prophylactic vaccination was confirmed by veterinary practitioners. The use of the vaccine in a contaminated area did not reduce mortality in rabbits. However, the mortality rate decreased to 31% on one infected farm where rabbits were kept in separate buildings.

SIMULTANEOUS VACCINATION AGAINST MYXOMATOSIS AND VIRAL HAEMORRHAGIC DISEASE

Ten rabbits were vaccinated simultaneously against myxomatosis and VHD, using 'Myxovac M' and Cunivac. All vaccinated rabbits remained healthy and gained weight throughout the 21-day observation period, together with twelve rabbits which were vaccinated only with Myxovac M or Cunivac. Challenge with virulent KGM and ZA strains (VHD and myxomatosis virus, respectively) was performed in two separate rooms. Results are presented in Table III.

TABLE III

Prevalence of immunity against challenge with viral haemorrhagic disease (VHD) virus and/or myxoma virus, 20-21 days after vaccination with Myxovac M and Cunivac

No. of rabbits	Vaccine		Result of challenge *	
	Cunivac	Myxovac M	VHD virus	Myxoma virus
6	+	+	6/6	6/6
2	+	+	2/2	NC
2	+	+	NC	2/2
3	+	–	3/3	NC
3	–	+	NC	3/3
4	–	+	0/4	NC
2	+	–	NC	0/2
2 (controls)	–	–	2/2**	2/2**

* number of healthy rabbits/number of rabbits challenged
** control rabbits not challenged
NC: not challenged

The level of immunity following simultaneous vaccination was the same as following the use of monovalent vaccines (16). Of 450 vaccinated rabbits, 150 were immunised with both Myxovac M and Cunivac. In field experiments, approximately 5,000 rabbits were vaccinated in the same way, and no post-vaccinal complications were reported by veterinary practitioners.

DIAGNOSIS AND CONTROL OF VHD

Diagnosis of VHD is based on clinical and epidemiological observations, as well as pathological examinations. Other tests – HA test using human erythrocytes, immunofluorescence, immunoprecipitation and inoculation of rabbits – are also used in laboratory diagnosis to confirm infection with VHD virus.

Legal regulations for VHD are the same as those which apply for myxomatosis (12).

DISCUSSION

The rapid spread and clinical course of VHD in Poland were similar to those described in other countries (4, 14, 18, 23). Laboratories using rabbits for diagnosis, and for the production and control of biological preparations and drugs, suffered heavy losses due to VHD. The largest number of outbreaks was reported in 1988, followed by significant regression. Between 1988 and 1990, the number of outbreaks in Germany increased (14). Experimental inoculation of different species revealed that only rabbits were susceptible to this virus infection. None of 21 infected hares showed clinical symptoms of VHD, and no virus was detected in the internal organs of these animals for 21 days after challenge. VHD and European brown hare syndrome are caused by antigenically similar viruses, but cross-infection between rabbits and hares does not occur (3, 4, 5, 6, 19, 26). The high resistance of VHD virus to physico-chemical factors was confirmed by other research workers (26, 30, 31). The present study revealed long viability of the virus and low efficacy of some of the disinfectants used. The use of human erythrocytes of all types – not only O (20) – for the diagnosis of VHD was also demonstrated (7).

The use of efficacious Polish vaccine against VHD reduced the economic losses in the rabbit population. Rabbits developed immunity as soon as four days after vaccination with Cunivac, confirming the reports of Šmíd *et al.* (26) and Huang (13). Preliminary observations indicated that simultaneous vaccination with Myxovac M and Cunivac, together with elimination of biological vectors, is an effective method for the control of both myxomatosis and VHD in Poland.

CONCLUSION

A number of factors – the experimental work which has been performed, the efforts of the Veterinary Services, increased familiarity with the problem among breeders (through publications), the improvement of diagnostic methods, and the use of safe and efficacious vaccines – have all contributed to a reduction of the economic losses caused by myxomatosis and VHD in Poland.

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LUTTE CONTRE LA MALADIE HÉMORRAGIQUE VIRALE DU LAPIN EN POLOGNE. – J. Górski, B. Mizak et M. Chrobocińska.

***Résumé** : Les auteurs présentent une étude épizootiologique de la maladie hémorragique virale du lapin en Pologne, et analysent les propriétés biologiques, physiques et chimiques des isolats utilisés pour la fabrication et le contrôle du vaccin « Cunivac » produit dans ce pays. Ils démontrent également l'innocuité et l'efficacité du vaccin. Les expériences en laboratoire et les observations de terrain, effectuées à grande échelle, ont donné des résultats satisfaisants.*

MOTS-CLÉS : Epidémiologie – Lapins – Maladie hémorragique virale du lapin – Prophylaxie – Vaccination.

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CONTROL DE LA ENFERMEDAD HEMORRÁGICA VIRAL DEL CONEJO EN POLONIA. – J. Górski, B. Mizak y M. Chrobocińska.

Resumen: *Los autores presentan aquí un estudio epizootiológico de la enfermedad hemorrágica viral del conejo en Polonia. Analizan las propiedades biológicas, físicas y químicas de los virus aislados para la fabricación y el control de la vacuna «Cunivac», producida en el país, y demuestran su inocuidad y eficacia. Tanto las experiencias realizadas en laboratorio como las observaciones de terreno a gran escala dieron resultados satisfactorios.*

PALABRAS CLAVE: Conejos – Control de enfermedades – Enfermedad hemorrágica viral del conejo – Epidemiología – Vacunación.

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REFERENCES

1. ANDREWES CH. & HORSTMANN D.H. (1949). – The susceptibility of viruses to ethyl ether. *J. gen. Microbiol.*, **3**, 290.

2. BÖGEL K. & MAYR A. (1962). – Ein Besistenstest mit Detergetion zur Charakterisierung von Viren. *Zentbl. Bakt. Orig.*, **186**, 134.

3. BUCZEK J., DEPTUŁA W. & PIEKARSKI J. (1991). – Wirusowa enzootyczna bronchopneumonia królików. *Medycyna Wet.*, **47** (10), 447.

4. CANCELLOTTI F.M. & RENZI M. (1991). – Epidemiology and current situation of viral haemorrhagic disease of rabbits and the European brown hare syndrome in Italy. *In* Viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev. sci. tech. Off. int. Epiz.*, **10** (2), 409-422.

5. CHASEY D., LUCAS M., WESTCOTT D. & WILLIAMS M. (1992). – European brown hare syndrome in the UK; a calicivirus related to but distinct from that of viral haemorrhagic disease in rabbits. *Arch. Virol.*, **124**, 363-370.

6. DEPTUŁA W. & BUCZEK J. (1993). – Syndrom brązowych zajęcy europejskich – nowa jednostka chorobowa. *Medycyna Wet.*, **49** (5), 208-210.

7. FITZNER A., KĘSY A. & CHROBOCIŃSKA M. (1992). – Porównanie zdolności aglutynowania erytrocytów człowieka grupy O, A, B i AB przez wirus krwiotocznej bronchopneumonii królików (RHDV). *Medycyna Wet.*, **48** (2), 89-90.

8. FITZNER A., MIZAK B., KĘSY A., NIEDBALSKI W., PAPROCKA G. & GÓRSKI J. (1993). – Określenie wpływu wybranych środków odkażających na przeżywalność wirusa krwiotocznej bronchopneumonii królików (VHD). *Życie wet.*, **68** (12), 298-300.

9. GÓRSKI J., MIZAK B., MIZAK Z. & KOMOROWSKI A. (1988). – Obraz kliniczny oraz zmiany anatomo-patologiczne w przebiegu pomoru królików (wirusowej krwiotocznej bronchopneumonii krolików). *Życie wet.*, **63** (9), 266-269.

10. GÓRSKI J., MIZAK B., KĘSY A., FITZNER A. & ŁÓJ H. (1989). – Cunipest – krajowa szczepionka przeciwko pomorowi królików. *Medycyna Wet.*, **45** (9-10), 519-521.

11. GÓRSKI J., MIZAK B., CZUBA W., KĘSY A., FITZNER A. & ŁÓJ H. (1989). – Sposób wytwarzania szczepionki przeciwko pomorowi królików. Polish Republic Patent No. 281282, 1 September.

12. GÓRSKI J., MIZAK B. & CHROBOCIŃSKA M. (1994). – Control of rabbit myxomatosis in Poland. *Rev. sci. tech. Off. int. Epiz.*, **13** (3), 869-879.

13. HUANG HAI-BO (1991). – Vaccination against and immune response to viral haemorrhagic disease of rabbits: a review of research in the People's Republic of China. *In* Viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev. sci. tech. Off. int. Epiz.*, **10** (2), 481-498.

14. LÖLIGER H.-CH. & ESKENS U. (1991). – Incidence, epizootiology and control of viral haemorrhagic disease of rabbits and the European brown hare syndrome in Germany. *In* Viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev. sci. tech. Off. int. Epiz.*, **10** (2), 423-434.

15. MARCATO P.S., BENAZZI C., VECCHI G., DELLA SALDA L., SIMONI P., AIELLO P. & TUMINO G. (1988). – L'epatite necrotica infectiva del coniglio. Profilo patogenitico di una nuova malattia emorragica. *Riv. Conigliocoltura*, **25** (9), 59-64.

16. MIZAK B., CHROBOCIŃSKA M. & GÓRSKI J. (1991). – Nieszkodliwość i skuteczność jednoczesnego szczepienia królików przeciwko pomorowi i myksomatozie. *Medycyna Wet.*, **47** (9), 395-397.

17. MIZAK B., GÓRSKI J. & KOZACZYŃSKI W. (1991). – Pathogenesis of viral haemorrhagic disease in rabbits and biological properties of the virus. *Bull. vet. Inst. Pulawy*, **34**, 37-44.

18. MORISSE J.-P., LE GALL G. & BOILLETOT E. (1991). – Hepatitis of viral origin in Leporidae: introduction and aetiological hypotheses. *In* Viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev. sci. tech. Off. int. Epiz.*, **10** (2), 283-295.

19. OHLINGER V.F. & THIEL H.J. (1991). – Identification of the viral haemorrhagic disease virus of rabbits as a calicivirus. *In* Viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev. sci. tech. Off. int. Epiz.*, **10** (2), 311-323.

20. PU B.Q., QUIAN N.H. & CUI S.J. (1985). – Micro HA and HI tests for the detection of antibody titres to so-called 'haemorrhagic pneumonia' in rabbits [in Chinese]. *Chin. J. vet. Med.*, **11** (10), 16-17.

21. REED L.J. & MÜNCH H. (1938). – A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.*, **27**, 493.

22. REYNOLDS E.S. (1963). – The use of lead citrate at high pH as an electronopaque stain in electron microscopy. *J. Cell Biol.*, **17**, 208.

23. RODÁK L., ŠMÍD B. & VALÍČEK L. (1991). – Application of control measures against viral haemorrhagic disease of rabbits in the Czech and Slovak Federal Republic. *In* Viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev. sci. tech. Off. int. Epiz.*, **10** (2), 513-524.

24. ŠEŠEVIČKOVÁ A., PECHAC O., MIROSSAY E., VOLOSIN M. & KORMAN J. (1988). – K problematike hynutia domacich kralikov na vychodnom Slovensku. *Veterinařství*, **38** (6), 250-251.

25. ŠMÍD B., VALÍČEK L., ŠTĚPÁNEK J., JURÁK E. & RODÁK L. (1989). – Experimental transmission and electron microscopic demonstration of the virus of haemorrhagic disease of rabbits in Czechoslovakia. *J. vet. Med., B*, **36**, 237-240.

26. ŠMÍD B., VALÍČEK L., RODÁK L., ŠTĚPÁNEK J. & JURÁK E. (1991). – Rabbit haemorrhagic disease: an investigation of some properties of the virus and evaluation of an inactivated vaccine. *Vet. Microbiol.*, **26**, 77-85.

27. SOŁTYSIAK Z. & MICHALSKA Z. (1989). – Obraz sekcyjny i histopatologiczny przy pomorze królików (pestis cuniculi) w toku epizootii na terenie Dolnego Śląska. *Medycyna Wet.*, **45** (9-10), 521-524.

28. WAWRZKIEWICZ J. & MAJER-DZIEDZIC B. (1992). – Swoiste przeciwciała w surowicy królików przeciwko krwotocznej chorobie logomorfów. *Medycyna Wet.*, **48** (6), 268-269.

29. WU G.D. (1986). – RHD inactivated tissue vaccine and heterogeneous antiserum: application in the field [in Chinese]. *Anim. Hus. vet. Med.*, **18** (3), 109-110.

30. XU WEI-YAN (1991). – Viral haemorrhagic disease of rabbits in the People's Republic of China: epidemiology and virus characterisation. *In* Viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev. sci. tech. Off. int. Epiz.*, **10** (2), 393-408.

31. XU Z.J. & CHEN W.X. (1989). – Viral haemorrhagic disease in rabbits: a review. *Vet. Res. Comm.*, **13** (3), 205-212.