# Non-specific immunity in rabbits experimentally infected with Czech strains of the rabbit haemorrhagic disease virus

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***Abstract***

*The purpose of this study was to evaluate some parameters of non-specific immunity [myeloperoxidase (MPO) activity in PMN cells and lysozyme (LZM) concentration and activity in serum] in rabbits experimentally infected with 4 Czech strains of the rabbit haemorrhagic disease (RHD) virus: CAMPV-351 (reference strain), CAMP-561, CAMPV-562, CAMPV-558. Furthermore, mortality was recorded among the animals infected with four Czech strains of the RHDV. Statistical analysis revealed that the most numerous changes were caused by reference strain CAMPV-351, followed by strains CAMPV-562 and CAMPV-558, and strain CAMPV-561. The highest mortality (100%) was observed in animals infected with this last strain.*

***Key words:*** *RHD virus, rabbit, MPO, LZM.*

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## Introduction

At present, the rabbit haemorrhagic disease (RHD) virus is present in all continents, and since its description in 1984 in China [1], over 400 strains are identified [2]. So far, in the aspect of specific and non-specific cellular and humoral immunity induced in rabbits, 22 strains of RHDV have been studied [3-22] including 3 French strains (Fr-1, Fr-2, 9905RHDVa), 10 Polish strains (K-1, Kr-1, KGM, SGM, MAŁ, BLA, PD, GSK, Ż, ŻD), 4 German strains (Hagenow, Frankfurt, Triptis, Hartmannsdorf), 3 Italian strains (BS89-reference strain, Vt97, PV97), 1 English strain (Rainham), 1 Spanish strain (Asturias) and 1 French strain (9905 RHDVa). The following immunological parameters were studied: various functions of granulocytes, concentration and activity of LZM, and lymphocyte membrane markers. Studies of lymphocyte markers were also performed in rabbits infected with 4 Czech strains of RHD virus, CAMPV-351 (reference strain), CAMP-561, CAMPV-562, CAMPV-558, and concerned examination of CD5, CD4, CD8, CD25 markers and the number of lymphocytes B with IgM receptors [23, 24]. The research on RHDV strains indicates the presence of immunotypes (immunogroups) within this virus. The first classification of RHDV strains into immunogroups was performed by Tokarz-Deptuła [6]. Considering the recorded changes to non-specific and specific cellular and humoral immunity in rabbits infected with 10 studied strains (SGM, MAŁ, KGM, ŻD, PD, GSK, KR-1, BLA, Fr-1, Fr-2) of the RHD virus, Tokarz-Deptuła [6] identified three immunogroups (immunotypes). The first immunogroup (immunotype I) was formed by the most immunogenic French strain – Fr-2, the third immunogroup (immunotype III) was formed by the least immunogenic Polish strain Kr-1; while the second immunogroup (immunotype II) was formed by medium-immunogenic strains – Fr-1, SGM, MAŁ, KGM, ŻD, PD, GSK, BLA. Furthermore, Hukowska-Szematowicz [3] and Niedźwiedzka-Rystwej [16] pointed to a different immunological response within 10 European strains (Bs89, Hagenow, Rainham, Framkfurt, Asturias, Vt97, Triptis, Hartmannsdorf, Pv97, 9905RHDVa) [16] and 4 Czech strains (CAMPV-351, CAMPV-561, CAMPV562, CAMPV-558) [3].

The purpose of this study was to evaluate some parameters of non-specific immunity (myeloperoxidase (MPO) activity in PMN cells and lysozyme (LZM) concentration

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and activity in serum) in rabbits experimentally infected with 4 Czech strains of the RHD virus: CAMPV-351 (reference strain), CAMP-561, CAMPV-562, CAMPV-558. Furthermore, mortality was recorded among the animals infected with four Czech strains of the RHDV.

## Material and methods

The study was conducted on 120 mixed-breed rabbits designated as conventional animals, originating from a farm run under zoo-technical and veterinary supervision [25]. The weight of animals ranged from 2.5 kg to 3.0 kg. During the study, the rabbits remained at the vivarium of the Department of Microbiology and Immunology, Department of Natural Sciences, University of Szczecin, where zoohygienic parameters corresponded to standards applicable in Poland [26]. Experimental animals designated for infection were divided into 4 groups of 20 rabbits each. Animals in group 1 were administered intramuscularly (leg muscles) with lyophilisate of reference strain CAMPV-351 diluted in 1 ml sterile physiological saline, animals in group 2 were administered in the same manner the strain CAMPV-561, the ones in group 3 – with CAMPV-562, while in group 4 – with CAMPV-558. Each group of infected animals had a corresponding group of control animals comprising 10 animals each, whereas each animal was intramuscularly administered (leg muscles) 1 ml of sterile physiological saline.

Each of the virus strains: CAMPV-351 (obtained in 1987), CAMPV-561 (1996), CAMPV-562 (1992), CAMPV-558 (1988) originated from a naturally dead animal. With these strains, in the form of liver homogenate, rabbits were experimentally infected, from which (after their death) liver was sampled. Next, liver was prepared as 20% homogenate cleared by centrifugation at 3000 rpm, 10% chloroforming for 60 minutes and centrifugation again, and then lyophilisation in 24-hour procedure [27]. All the antigens prepared had the same number of viral particles specified by density, within the range from 1.310 to 1.340 g/cm$^3$ [16].

Blood from infected and control animals was drawn through a port from the marginal vein of the ear onto an anti-coagulant or without it, depending on the needs and requirements of the method. For all groups of the studied experimental animals, blood was drawn at hour "0", namely before the administration of the RHD virus in the group of infected rabbits and sterile physiological saline in the group of control animals, and then at hours 4, 8, 12, 24, 36, 48, 52, 56, 60, 72; except for CAMPV-561 strain, in which case after hour 48 all the infected rabbits died. In the case of strains CAMPV-351, CAMP-562, and CAMPV-558, the research was continued until the appearance of the first clinical symptoms characteristic of the RHD virus.

Myeloperoxidase activity (MPO) in PMN cells was assessed according to the Graham method described by Zawistowski [28]. The lysozyme concentration in serum was determined by the method of plate diffusion according to Hankiewicz [29], while the use of *Micrococcus lysodeikticus*, while LZM activity index was calculated according to Szmigielski [30]. All the results of the studies were subjected to statistical analysis with Student *t*-test at $p = 0.05$ in the Statistica software 6.0 (StatSoft, Poland), by comparing the results obtained in infected and control rabbits (Tables 1-4). In animals infected with the RHD virus, mortality was recorded at particular hours of the experiment, on the basis of which records the mortality index was calculated.

For all experiments, the animals were handled according to the Polish law on the protection of animals and NIH standards. Experiments were accepted by the local Ethical Committee.

## Results

Results of the study (Tables 1-4) indicated that changes in the form of increase and/or decrease occurred for all indices and with high frequency occurred at hour 24, 36, 48, 52, and slightly less frequently at the end of the experiment, namely at hour 56, 60 and 72.

Myeloperoxidase activity in rabbits infected with four Czech strains of the RHD virus was similar (Tables 1-4). In the case of CAMPV-351 strain, this was a single change in the form of increase (12 h), whereas in the case of CAMPV-558 strain, two changes were recorded in the form of increase (24 h) and decrease (52 h).

Results regarding LZM concentration (Tables 1-4) indicate that among four analysed Czech strains of the RHDV, three yielded small changes to this index. CAMPV-351 strain caused few changes in the form of increase (24 h, 36 h and 72 h), similarly as CAMPV-561 strain – decrease (12 h), and CAMPV-558 – decrease (52 h). CAMPV-562 strain did not cause changes of statistical significance. Lysozyme activity in serum (Tables 1-4) recorded significant changes, both in the form of increase and decrease. Reference strain CAMPV-351 caused most changes in the form of increase (36, 48, 56, 60, 72 h), followed by CAMPV-562 strain causing decrease (36, 48, 56, 60 h), and CAMPV-558 strain, which causes increase (36, 48, 52 h). The lowest number of changes in the form of decrease was observed for CAMPV-561 (8 h).

In the case of Czech strain CAMPV-351, the first symptoms of the disease were observed at 36 h of the experiment. The first deaths were recorded between 24/36 h of the study (6 rabbits). Mortality for this strain in the course of the entire experiment, namely up to 72 h amounted to 80%. In the case of the second of the analyzed strains, CAMPV-561, the first symptoms were recorded as early as at 24 h of the experiment, and they preceded very numerous deaths. Between 12/24 h, one death took place, whereas between 24 and 36 h of the experiment, as many as

**Table 1.** Parameters of non-specific immunity in rabbits infected with CAMPV-351 strain of the RHD virus

| | | Values of parameters in hours | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | | **0** | | **4** | | **8** | | **12** | | **24** | | **36** | | **48** | | **52** | | **56** | | **60** | | **72** | |
| | | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** |
| ***n*** | | **20** | **10** | **20** | **10** | **20** | **10** | **20** | **10** | **20** | **10** | **14** | **10** | **6** | **10** | **5** | **10** | **4** | **10** | **4** | **10** | **4** | **10** |
| Myeloperoxidase activity | $\bar{x}$ ±SD | 2.0 | 1.7 | 2.0 | 1.9 | 2.1 | 1.9 | 2.1* | 1.8 | 2.1 | 2.1 | 2.2 | 2.1 | 2.3 | 2.1 | 1.8 | 2.0 | 1.7 | 1.6 | 1.5 | 1.6 | 1.3 | 1.7 |
| (MPO) (l.b.) | | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | 0.6 | 0.3 | 0.4 | 0.4 | 0.4 | 0.3 | 0.4 | 0.4 | 0.2 | 0.7 | 0.2 | 0.7 | 0.2 | 0.4 | 0.2 | 0.1 | 0.3 |
| Lysosyme (LZM) | | | | | | | | | | | | | | | | | | | | | | | |
| Concentration (mg/l) | $\bar{x}$ ±SD | 2.4 | 2.3 | 2.5 | 2.4 | 2.7 | 2.0 | 2.8 | 2.2 | 3.4* | 2.2 | 3.1* | 1.9 | 3.4 | 2.3 | 2.2 | 1.8 | 2.0 | 1.9 | 2.0 | 1.7 | 1.7 | 3.5* |
| | | 0.9 | 0.7 | 1.0 | 0.9 | 0.9 | 0.8 | 0.2 | 0.6 | 0.3 | 0.8 | 0.9 | 0.5 | 0.3 | 0.4 | 0.3 | 0.7 | 0.5 | 0.5 | 0.5 | 0.5 | 0.6 | 0.6 |
| Activity index (l.b.) | $\bar{x}$ ±SD | 0.029 | 0.026 | 0.033 | 0.025 | 0.028 | 0.022 | 0.030 | 0.028 | 0.044 | 0.034 | 0.039* | 0.016 | 0.050* | 0.013 | 0.042 | 0.034 | 0.058* | 0.017 | 0.054* | 0.019 | 0.049* | 0.022 |
| | | 0.009 | 0.090 | 0.004 | 0.006 | 0.005 | 0.004 | 0.006 | 0.005 | 0.006 | 0.007 | 0.008 | 0.006 | 0.018 | 0.001 | 0.004 | 0.006 | 0.003 | 0.007 | 0.008 | 0.005 | 0.006 | 0.004 |

*Z – infected animals; K – control animals; n – number of animals; * – statistically significant change; $\bar{x}$ – mean value; ±SD – standard deviation*

**Table 2.** Parameters of non-specific immunity in rabbits infected with CAMPV-561 strain of the RHD virus

| | | Values of parameters in hours | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | | **0** | | **4** | | **8** | | **12** | | **24** | | **36** | |
| | | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** |
| ***n*** | | **20** | **10** | **20** | **10** | **20** | **10** | **20** | **10** | **19** | **10** | **5** | **10** |
| Myeloperoxidase activity (MPO) (l.b.) | $\bar{x}$ ±SD | 2.0 | 2.0 | 2.0 | 2.0 | 2.1 | 2.0 | 1.9 | 1.9 | 2.1 | 2.1 | 2.0 | 2.1 |
| | | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 |
| Lysosyme (LZM) | | | | | | | | | | | | | |
| Concentration (mg/l) | $\bar{x}$ ±SD | 4.2 | 4.9 | 4.8 | 4.4 | 4.1 | 4.8 | 3.3 | 4.2* | 4.3 | 4.5 | 5.1 | 4.3 |
| | | 1.5 | 1.4 | 1.6 | 1.9 | 1.4 | 1.5 | 0.7 | 1.0 | 1.3 | 1.5 | 0.9 | 0.7 |
| Activity index (l.b.) | $\bar{x}$ ±SD | 0.021 | 0.026 | 0.025 | 0.027 | 0.012 | 0.027* | 0.020 | 0.021 | 0.045 | 0.037 | 0.065 | 0.043 |
| | | 0.005 | 0.008 | 0.007 | 0.004 | 0.005 | 0.009 | 0.009 | 0.008 | 0.006 | 0.005 | 0.009 | 0.006 |

*Z – infected animals; K – control animals; n – number of animals; * – statistically significant change; $\bar{x}$ – mean value; ±SD – standard deviation*

**Table 3.** Parameters of non-specific immunity in rabbits infected with CAMPV-562 strain of the RHD virus

| | | Values of parameters in hours | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | | **0** | | **4** | | **8** | | **12** | | **24** | | **36** | | **48** | | **52** | | **56** | | **60** | | **72** | |
| | | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** |
| ***n*** | | **20** | **10** | **20** | **10** | **20** | **10** | **20** | **10** | **19** | **10** | **7** | **10** | **4** | **10** | **3** | **10** | **3** | **10** | **3** | **10** | **3** | **10** |
| Myeloperoxidase activity | $\bar{x}$ ±SD | 2.1 | 2.1 | 2.1 | 2.0 | 2.3 | 1.9 | 2.0 | 2.1 | 2.0 | 2.1 | 2.1 | 2.0 | 2.1 | 2.0 | 2.2 | 2.1 | 2.0 | 2.1 | 2.0 | 2.3 | 2.1 | 2.2 |
| (MPO) (l.b.) | | 0.2 | 0.1 | 0,2 | 0.2 | 1.1 | 0.2 | 0.4 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.3 | 0.1 | 0.2 |
| Lysosyme (LZM) | | | | | | | | | | | | | | | | | | | | | | | |
| Concentration (mg/l) | $\bar{x}$ ±SD | 3.0 | 3.1 | 3.2 | 2.9 | 2.4 | 2.6 | 2.3 | 3.3 | 1.9 | 2.3 | 3.6 | 2.9 | 4.0 | 1.6 | 6.1 | 3.3 | 3.1 | 2.9 | 2.5 | 3.3 | 3.1 | 3.3 |
| | | 1.2 | 1.1 | 1.1 | 1.0 | 1.0 | 0.3 | 1.1 | 1.0 | 1.6 | 1.1 | 1.6 | 0.6 | 1.0 | 0.1 | 1.7 | 0.1 | 2.3 | 0.1 | 0.6 | 0.6 | 1.4 | 0.6 |
| Activity index (l.b.) | $\bar{x}$ ±SD | 0.014 | 0.017 | 0.013 | 0.021 | 0.010 | 0.018 | 0.016 | 0.013 | 0.013 | 0.018 | 0.027* | 0.019 | 0.024* | 0.017 | 0.020 | 0.015 | 0.038* | 0.019 | 0.025* | 0.018 | 0.018 | 0.019 |
| | | 0.005 | 0.006 | 0.004 | 0.005 | 0.004 | 0.006 | 0.007 | 0.005 | 0.003 | 0.004 | 0.010 | 0.006 | 0.008 | 0.006 | 0.010 | 0.006 | 0.009 | 0.005 | 0.008 | 0.006 | 0.007 | 0.004 |

*Z – infected animals; K – control animals; n – number of animals; * – statistically significant change; $\bar{x}$ – mean value; ±SD – standard deviation*

**Table 4.** Parameters of non-specific immunity in rabbits infected with CAMPV-558 strain of the RHD virus

| | | Values of parameters in hours | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | | **0** | | **4** | | **8** | | **12** | | **24** | | **36** | | **48** | | **52** | | **56** | | **60** | | **72** | |
| | | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** | **Z** | **K** |
| ***n*** | | **20** | **10** | **20** | **10** | **20** | **10** | **20** | **10** | **20** | **10** | **19** | **10** | **14** | **10** | **13** | **10** | **13** | **10** | **13** | **10** | **11** | **10** |
| Myeloperoxidase activity | $\bar{x}$ ±SD | 2.1 | 2.0 | 2.1 | 2.1 | 2.2 | 2.1 | 2.1 | 2.2 | 2.1* | 2.0 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.2* | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.3 |
| (MPO) (l.b.) | | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 |
| Lysosyme (LZM) | | | | | | | | | | | | | | | | | | | | | | | |
| Concentration (mg/l) | $\bar{x}$ ±SD | 2.1 | 3.7 | 2.2 | 3.1 | 2.9 | 3.7 | 2.7 | 4.0 | 3.0 | 3.3 | 3.2 | 4.3 | 3.0 | 3.4 | 2.7 | 5.3* | 2.4 | 2.9 | 3.1 | 4.5 | 2.8 | 3.9 |
| | | 0.9 | 0.6 | 0.9 | 1.0 | 0.8 | 1.1 | 0.8 | 1.6 | 0.3 | 1.0 | 0.8 | 1.5 | 0.8 | 0.7 | 1.3 | 1.5 | 0.8 | 0.8 | 1.0 | 1.2 | 0.9 | 1.0 |
| Activity index (l.b.) | $\bar{x}$ ±SD | 0.019 | 0.017 | 0.020 | 0.021 | 0.013 | 0.017 | 0.021 | 0.015 | 0.022 | 0.018 | 0.028* | 0.015 | 0.032* | 0.014 | 0.038* | 0.019 | 0.018 | 0.019 | 0.018 | 0.017 | 0.017 | 0.016 |
| | | 0.007 | 0.005 | 0.008 | 0.007 | 0.003 | 0.008 | 0.007 | 0.008 | 0.004 | 0.007 | 0.005 | 0.004 | 0.009 | 0.004 | 0.008 | 0.007 | 0.006 | 0.008 | 0.007 | 0.004 | 0.006 | 0.004 |

*Z – infected animals; K – control animals; n – number of animals; * – statistically significant change; $\bar{x}$ – mean value; ±SD – standard deviation*

14 animals died, while between 36/48 h – 5 animals. The mortality ratio amounted to 100%. Experimental infections of rabbits with CAMPV-562 strain caused the occurrence of symptoms in animals starting from 36 h of the experiment, whereas a single death without symptoms was recorded between 12/24 h. Between 24/36 h, 12 deaths were observed, while in other hours of the experiment, single deaths were recorded. Mortality rate in the case of this strain amounted to 85%. The Czech strain CAMPV-558 caused excitability of the infected animals, and increased blood coagulability already starting from 24 h of the experiment. The first deaths were recorded between 24/36 h of the experiment (1 rabbit), and then between 36/48 h (5 rabbits), between 48/52 h (1 rabbit), and between 60/72 h (2 rabbits). At 72 h of the experiment, mortality rate in the case of this strain amounted to 45%.

## Discussion

The increase in MPO activity (12 h) obtained in own study for reference strain CAMPV-351 is completely different from the one obtained for reference Italian strain BS89 (decrease 8, 12, 24 h) [16]. Contrary to the Italian strain, changes recorded for strains Fr-1 (increase at 4-56 h), Fr-2 (4-8, 24-48 h), Kr-1 (increase at 24-36, 52 h), BLA (increase at 8-60 h), ŻD (increase at 24, 36, 52 h) [6, 15] are similar to changes in the case of CAMPV-351. In turn, the results of the studies obtained for CAMPV-558 are partly similar to the ones obtained for SGM strain (decrease at 52 h), KGM (increase at 24, 52 h, decrease at 60 h) and Triptis (decrease at 8, 12, 24 h) [6, 16, 21]. The recorded lack of changes to the MPO activity after infecting rabbits with CAMPV-561 and CAMPV-562 strains is similar to results obtained for strains MAŁ, PD, GSK, Vt97, Hartmannsdorf and HA-Hagenow, Rainham, Frankfurt, Asturias [6, 16, 21].

When discussing the results in the area of LZM concentration obtained for reference strain CAMPV-351 (increase at 24, 36, 72 h), one may determine that they differ from the results obtained for reference strain BS89 (decrease at 8, 12, 24 h) [16]. Just as in the case of infection with CAMPV-351, increase in LZM concentration was observed for other strains – Fr-2 (increase at 4-56 h), MAŁ (increase at 8, 24-56 h), PD (increase at 24, 48, 52, 60, 72 h), Ż (increase at 52, 56, 60 h), ŻD (increase at 8, 48-56 h), Vt97 (increase at 4, 12, 24, 36 h), Hatrmannsdorf (4, 8 h) and Rainham (increase at 4, 24 h), Hagenow (increase at 4, 8, 12 h), Frankfurt (4, 8, 12, 24 h), Asturias (increase at 8, 12, 24 h) [4, 6, 16, 21]. CAMPV-562 strain did not cause changes to LZM concentration, which conforms to the observations by other authors for strains Fr-1 [6], Triptis [6, 16], 9905RHDVa [6, 16, 21]. In turn, the decrease to this parameter recorded for strains CAMPV-561 and CAMPV-558 conforms to changes observed for strains SGM (8-60 h), KGM (8-60 h), BS89 and BLA (12, 48, 52 h), Pv97 (4-36 h) [6, 16]. Increase in LZM activity was recorded both for reference Czech strain CAMPV-351, and for Italian BS89 strain, whereas in the case of CAMPV-351 changes commenced later and lasted longer (36-72 h), while BS89 strain caused changes earlier (4 h) [16]. Increase in LZM activity was also observed for strains CAMPV-562 and CAMPV-558, and the result was very similar to strains BS89 (4 h), Ż (56 h) and HA-Rainham (4-36 h), BLA (56, 60 h) and Hagenow (12 h) [4, 6, 16, 21]. In turn, the decrease in LZM activity (8 h) recorded for CAMPV-561 strain conforms to the result observed for strains Fr-1 (decrease at 12, 48, 52 h), Fr-2 (decrease at 4-56 h), SGM (decrease at 24, 36 h), MAŁ (decrease at 8, 24, 56 h), PD (decrease at 4, 8, 24, 36, 52, 56, 72 h), GSK (decrease at 48, 56, 60 h), Vt97 (decrease at 8, 36 h), Hartmannsdorf (24 h) and Asturias (decrease at 8, 24 h), 9905RHDVa (36 h) [4, 6, 16]. However, the changes recorded for all four Czech strains analysed do not confirm the results obtained for strains Kr-1 (increase at 4, 12, 24; decrease at 48 h), KGM (increase at 12, 36, 48 h; decrease at 8, 24 h), Frankfurt (increase at 4, 12 h; decrease at 36 h), Triptis (no changes) and K-1 (no changes) [6, 16, 21].

The time and number of deaths of rabbits infected with four Czech strains of the RHDV points to differences in their pathogenicity. The highest 100% mortality was recorded in the group of animals infected with CAMPV-561, while the lowest (45%) – in the group of animals infected with CAMPV-558 strain. The recorded mortality was similar to the one recorded by other authors [6, 16, 31] for Polish and foreign strains. Mortality of 100% (CAMPV-561) was also observed for strains ŻD, BS89, Pv97, Frankfurt, Triptis, Hartsmanndorf, Rainham, Asturias [16] and Fr-2 [6]. In turn, it was slightly lower for Kr-1 (90%), SGM (95%), GSK (95%), Hagenow (90%), 9905RHDVa (90%), Fr-1 (90%). However, mortality for reference strain CAMPV-351 (80%) and CAMPV-562 (85%) was the same as in the case of MAŁ (80%) and American strains (70-95%) [31]. Mortality recorded for CAMPV-558 (45%) was so far the lowest recorded mortality among Czech strains of the RHDV. Lower mortality was only recorded in the case of infection with Italian strain Vt97 (30%) [16]. In the case of French strains – Fr-1, Fr-2 – 90% mortality was recorded for Polish strains: MAŁ, SGM, K-1 – 80-95%, PD – 25%, BLA – 32%, GSK – 93%, Ż – 87%, ŻD – 100% and 90-100% mortality for foreign strains. The time of occurrence of clinical symptoms and their type did not differ from clinical symptoms recorded by other authors [4-7, 10, 13].

## Conclusions

The analysis of changes in the MPO activity in PMN cells, and LZM concentration and activity, indicated that the most numerous changes to such indices were caused by reference strain CAMPV-351, followed by strains CAMPV-562 and CAMPV-558, and strain CAMPV-561. In turn,

when assessing the intensity of changes to such indices, one may state that it was the greatest in the case of LZM activity in blood serum, lower to the concentration of lysozyme, while it was the lowest in the MPO activity in PMN cells. Such four Czech strains of the RHDV also caused a different mortality, which proves that they include very pathogenic strains (100% mortality up to 48 h of the experiment – CAMPV-561 strain), less pathogenic strains (80-85% mortality up to 72 h – CAMP-351 and CAMPV-562), and the least pathogenic strains (45% mortality up to 72 h of the experiment – CAMPV-558). Immunological difference of the analysed strains as compared to the division into very pathogenic, less pathogenic and the least pathogenic strains, indicates that the strains causing the most numerous changes (CAMPV-351) and many changes to immunity indices (CAMPV-562, CAMPV-558) were in the group of less pathogenic strains (CAMPV-351 and CAMPV-562) and the least pathogenic strains (CAMPV-558). In turn, CAMPV-561 strain, which caused few changes to the immunological factors analysed, was classified into the group of very pathogenic strains (100% mortality).

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### References

1. Liu SJ, Xue HP, Pu BQ, et al. (1984): A new viral disease in rabbits. Anim Husb Vet Med 16: 253-255.
2. Annon (2011): Gen Bank, National Center of Biotechnology Information, Pub Med. http://www.ncb.nlm.gov/pubmed/ (date of last check 11.05.2011).
3. Hukowska-Szematowicz B (2006): Immunological and genetical characterization of selected strains of RHD (rabbit haemorrhagic disease) virus. Doctoral thesis. University of Szczecin, Poland.
4. Tokarz-Deptuła B, Niedźwiedzka P, Deptuła W (2005): Serum lysosyme (LZM) in rabbits experimentally infected four Polish strains RHDV. Conference Material VIII "Molecular Biology in diagnostics infectious disease and biotechnology". Warsaw 2005; 132-136.
5. Piekarski J (1994): The immunological and haematological picture and viral pathomorphogenesis and clinic investigations in rabbits experimentally infected with RHD (rabbit haemorrhagic disease) virus. Doctoral thesis. University of Warmia and Mazury, Olsztyn, Poland.
6. Tokarz-Deptuła B (2009): Immunitety phenomena in rabbits infected with the RHD (rabbit haemorrhagic disease) virus. Pol J Environ Stud 7: 1-81.
7. Deptuła W, Kęsy A, Tokarz-Deptuła B, et al (1999): Dynamics of selected parameters in rabbits infected with rabbit haemorrhagic disease virus. Folia Veterinaria 43: 186-190.
8. Hukowska-Szematowicz B, Tokarz-Deptuła B, Deptuła W (2005): Lymphocytes T and their subpopulations in peripheral blood in rabbits experimentally infected with two strains of VHD virus (viral haemorrgaic disease). Pol J Env Stud 14: 550-555.
9. Tokarz-Deptuła B, Deptuła W (2003): Dynamic alterations in peripheral blood lymphocytes in rabbits experimentally infected with VHD (viral haemorrhagic disease) virus – Polish strain Kr-1. Pol J Vet Sci 6: 67-69.
10. Tokarz-Deptuła B, Deptuła W (2003): Non-specific cell-mediated immunity in rabbits experimentally infected with four various doses of VHD (viral haemorrhagic disease) virus, French strain Fr-2. Pol J Vet Sci 6: 64-66.
11. Tokarz-Deptuła B, Deptuła W (2004): The immunity during immunization with the viral haemorrhagic disease (VHD) in rabbits. Centr Eur J Immunol 29: 58-62.
12. Tokarz-Deptuła B, Deptuła W (2004): T and B lymphocytes and their subpopulations in peripheral blood in rabbits experimentally infected with Fr-2 strain of viral haemorrhagic disease (VHD) virus. Bull Vet Inst Puławy 48: 367-370.
13. Tokarz-Deptuła B, Deptuła W, Kęsy A (2002): Rabbit plaque with particular attention given to immune phenomena. Medycyna Wet 58: 497-500.
14. Tokarz-Deptuła B, Hukowska B, Deptuła W (2003): Dynamic alterations in selected indices of non specific immunity in rabbits experimentally infected with VHD (viral haemorrhagic disease) virus. Pol J Vet Sci 6: 70-73.
15. Nahurska A, Tokarz-Deptuła B, Hukowska B, Deptuła W (2003): Selected indices of non-specific humoral immunity in rabbits experimentally infected with non-haemaglutinogenic strain of VHD (viral haemorrhagic disease) virus. Pol J Vet Sci 6: 25-27.
16. Niedźwiedzka-Rystwej P, Deptuła W (2010): Non-specific immunity in rabbits infected with 10 strain of the rabbit haemorrhagic disease virus with different biological properties. Centr Europ J Biol 5: 613-632.
17. Niedźwiedzka P, Tokarz-Deptuła B, Deptuła W (2008): Ingestion capacity and haematological parameters in rabbits experimentally infected with strains of RHD (rabbit Haemorrhagic disease) virus differing in biological features. Adv Agricult Sci 12: 99-106.
18. Tokarz-Deptuła B, Niedźwiedzka P, Hukowska-Szematowicz B, Deptuła W (2006): Indications of immunity in rabbit experimentally infected with RHDV strains with different haemagglutination properties. In: Man and the natural environment of Western Pomerania, I. Biotic Environment-environmental biology, experimental and applied. Editor Tarasiuk J, Kępczyński J. Publisher Print Group, Szczecin 2006; 598-601.
19. Tokarz-Deptuła B, Niedźwiedzka P, Hukowska-Szematowicz B, Deptuła W (2007): Indices of immunity in rabbits experimentally infected with strains of RHD virus differing in haemagglutination properties. Medycyna Wet 63: 1251-1254.
20. Niedźwiedzka-Rystwej P, Tokarz-Deptuła B, Deptuła W (2009): Specific immunity in rabbits infected with RHD (rabbit haemorrhagic disease) virus strains with various capacity of erytrocytes haemagglutination. Centr Eur J Immunol 34: 14-17.
21. Niedźwiedzka-Rystwej P, Deptuła W (2009): Non-specific humoral immunity in rabbits infected with the selected German strains of the RHD (rabbit haemorrhagic disease) virus. Centr Eur J Immunol 34: 218-221.
22. Niedźwiedzka-Rystwej P, Deptuła W (2010): Cytometric analysis of lymphocytes T and B in rabbits infected with non-haemagglutinogenic strains of RHD virus (rabbit haemorrhagic disease) – Rainham, Frankfurt and Asturias. Pol J Vet Sci 13: 157-162.
23. Hukowska-Szematowicz B, Deptuła W (2008): Peripheral blood lymfocytes in rabbits infected with Czech strains,

CAMPV-562 and CAMPV-558 of RHD virus. Centr Eur J Immunol 33: 8-13.
24. Hukowska-Szematowicz B, Deptuła W (2008): Dynamics of peripheral blood lymphocytes in rabbits experimentally infected with two Czech strains of RHD virus. Bull Vet Inst Pulawy 52: 23-29.
25. Annon (1987): Information and training materials of the Laboratory Animals Section, General Assembly of the Association of Agriculture Engineers and Technicians. In: Materiały informacyjno-szkoleniowe Sekcji ds. Zwierząt laboratoryjnych. ZG Stowarzyszenia Inżynierów i Techników Rolnictwa, Warszawa1987; 26-77.
26. Regulation of the Minister of Agriculture and Rural Development of 10 March 2006 on detailed conditioms for maintenance of laboratory Animals In experimental units, breeding units and suppliers (Polish Journal of Lows of 2006, No. 50, item 368).
27. Fitzner A, Kęsy A, Niedbalski W, et al. (1996): Identification of the dominating VP60 polypeptide in domestic isolates of the RHD virus. Medycyna Wet 52: 303-305.
28. Zawistowski S (1976): Histology-techniques and basics. PZWL, Warszawa 1976.
29. Hankiewicz J, Świerczek E (1975): Comparision analysis of lysozyme evaluation with agar diffusion and nefelometric metod. Przegl Lek 32: 376-378.
30. Szmigielski S (1972): The analysis of up and down regulated granulocythes. Postdoctoral thesis. Wojskowy Instytut Medycyny Lotniczej, Warszawa 1972.
31. McIntosh MT, Behan SC, Mohamed FM, et al. (2007): A pandemic strain of calcivirus threatens rabbit industries in the Americas. Virol J 4: 96-109.