Haematological profiles of pigs of different age in relation to the presence or absence of porcine reproductive and respiratory virus, porcine circovirus type 2 and hepatitis E virus

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ABSTRACT
This study was aimed to assessing haematological parameters in pigs of different age categories from six farms differing in the presence or absence of selected pathogens. The following categories were included: 5 age groups of growers, fatteners and breeding sows. Individual blood samples for determining complete blood count and white blood cell differential count were taken. Group samples of oral fluid and faeces were collected from each farm and tested for detection of Porcine Reproductive and Respiratory Virus (PRRSV), Porcine Circovirus Type 2 (PCV2), and Hepatitis E Virus (HEV) using PCR, RT-PCR, and qRT-PCR protocols. On farms free of PRRSV, PCV2 and HEV, the age of pigs had significant effect on: white blood cell count (WBC), haemoglobin concentration (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration, platelet count (PLT), percentage of neutrophils, lymphocytes and eosinophils and absolute numbers of neutrophils, lymphocytes, monocytes, basophils and ‘large unstained cells’ (LUCs). On positive farms all observed blood parameters except the percentage of LUCs were significantly affected by the age. The percentages of lymphocytes, mean corpuscular volume (MCV) and haematocrit were significantly lowered by PRRSV, while WBC, PLT, percentage and absolute numbers of neutrophils, basophils and LUCs were increased. Nine-week-old pigs with PCV2 had significantly lower red blood cell count, PLT, percentage and absolute numbers of monocytes and LUCs, and significantly higher MCV, MCH, percentage of neutrophils and percentage and absolute number of eosinophils than 9-week-old pigs with PCV2 and HEV. Age-related changes in haematological parameters occurred in PCR negative and positive farms.

HIGHLIGHTS
• Age-related changes in haematological parameters were present in PCR negative farms and in PCR positive farms in relation to selected pathogens.
• Values for several haematological parameters in 9-week-old pigs with a monoinfection differed significantly from those in pigs of the same age with two viral infections.
• To interpret results of haematological analyses correctly, it is important to consider the haematological analyser used, the pig age and, health history, and clinical data.

Introduction
Measurement of haematological parameters in pigs is rarely performed. There are several reasons for this, such as the costs associated with labour and laboratory testing, especially due to the low economic value of an individual animal and the limited availability of reference intervals for different age categories in pigs required for correct interpretation of laboratory results (Friendship et al. 1984; Eze et al. 2010; Klem et al. 2010; Thorn 2010; Cooper et al. 2014; Perri et al. 2017; Ježek et al. 2018). Ranges for most haematological parameters are quite wide. They vary since they depend on many factors, including diet, age, gender, physiological appearance, different husbandry techniques, biosecurity, season, restraint, sample collection technique, transport time or sample preparation, and the type of the analyser used for haematological analyses (Friendship et al. 1984; Thorn 2010; Ježek...
et al. 2018). All these factors must be considered when interpreting the results of haematological analyses.

There are many important reasons for determining haematological parameters in pigs. First, assessment of these parameters can be used to establish a proper diagnosis and to assess not only the health status of a pig but also the health status of a herd (Perri et al. 2017; Ježek et al. 2018). In addition, assessment of haematological parameters can contribute to early identification of diseases or poor growth performance (Perri et al. 2017; Sanchez et al. 2019) and may be highly valuable in the treatment or prognosis of many diseases (Eze et al. 2010).

The values of haematological parameters can vary considerably in the presence of some pathogens manifesting as inflammation and infection, even if the disease is subclinical (McKenzie and Laudicina 1998; Buzzard et al. 2012; Stukelj et al. 2013; Norbury and Moyer 2015). Furthermore, several haematological parameters are strongly related to the chronic disease status. Haematological data provide information that is useful both in the detection and monitoring of animals diagnosed with bacterial, viral, fungal, and parasitic infections (McKenzie and Laudicina 1998). Pathogens often cause changes in white blood cell counts (WBC) and red blood cell counts (RBC), haematocrit (Hct), haemoglobin concentration (Hb) and white blood cell differential count (WCDC) (Nielsen and Bøtner 1997; Thorn 2010).

Porcine Reproductive and Respiratory Virus (PRRSV), Porcine Circovirus Type 2 (PCV2) and the Hepatitis E Virus (HEV) are pathogens quite common in the pig industry (Kurmann et al. 2011; Holtkamp et al. 2013; Salines et al. 2017), which are either economically important for the health of pigs or constitute a potential threat to food safety, such as HEV. Infections with PCV2 and HEV in pigs often occur subclinically (Steiner et al. 2009; Kurmann et al. 2011; Raspor Lainšček et al. 2017; Salines et al. 2017). Infections caused by PRRSV and PCV2 only affect pigs (Alonso et al. 2013; Holtkamp et al. 2013), but HEV is potentially fatal to humans in certain populations in terms of chronic hepatitis (Colson et al. 2010; Plut et al. 2020). In addition, contaminated pork and contaminated meat products are potential sources of human infection (Colson et al. 2010). There are few reports on haematological parameters in pigs in the presence of various viruses (PRRSV, PCV2, HEV) (Segalés et al. 2001; Halbur et al. 2002; Stukelj et al. 2013; Adekola et al. 2019).

The aim of the present study was to assess haematological parameters in pigs of various age categories (growers, fatteners and breeding sows) from six farms in relation to presence or absence of PRRSV, PCV2 and HEV. The group of growers was further subdivided according to age, as this is the period when maternal antibodies decrease, and pigs begin to be infected with various pathogens. We hypothesised that haematological parameters differ significantly between groups of pigs classified according to the age, to the presence of the pathogens considered, and to the age and presence of the pathogens. Furthermore, we hypothesised that haematological parameters differ significantly between groups with monoinfections and those with polyviral infections.

### Materials and methods

#### Animals and farms

All procedures in the research were performed according to the Directive 2010/63/EU of the European Parliament and the Council on the Protection of Animals used for Scientific Purposes and Slovenian Animal Protection Law (Official Gazette of the Republic of Slovenia no. 38/2013 and 21/2018) and accepted by the National Ethics Committee.

The study was carried out in six commercial farms (Table 1) in Slovenia: two large one-site farms, one with 1000 and the other with 1850 breeding sows, one two-site farm with 600 breeding sows and three small one-site farms with 100, 95 and 90 breeding sows. The sows from all farms were maternal hybrids (H12), where dam is Slovenian Landrace - line 11 and sire Slovenian Large White. Piglets were weaned on

| Farm | Sows | Growers 5 weeks old | Growers 7 weeks old | Growers 9 weeks old | Growers 11 weeks old | Growers 13 weeks old | Fatteners | Total (farm) |
|------|------|---------------------|---------------------|---------------------|---------------------|---------------------|-----------|-------------|
| A    | 10   | 9                   | 10                  | 10                  | 8                   | 9                   | 9         | 65          |
| B    | 10   | 10                  | 10                  | 10                  | 9                   | 10                  | 9         | 68          |
| C    | 10   | 9                   | 9                   | 9                   | 7                   | 9                   | 0         | 53          |
| D    | 10   | 10                  | 10                  | 10                  | 10                  | 10                  | 10        | 70          |
| E    | 10   | 10                  | 10                  | 9                   | 9                   | 9                   | 10        | 68          |
| F    | 10   | 7                   | 9                   | 18                  | 0                   | 9                   | 10        | 54          |
| Total/age group | 60   | 55                  | 49                  | 66                  | 43                  | 57                  | 48        | 378         |
day 28. All farms were farrow to finish and had an all-in-all-out system.

On all farms, breeding animals were fed twice a day, but growers and fatteners were fed ad libitum, all with commercially produced feed. All feed contained ground corn, wheat meal, barley meal, soybean meal and were supplemented with complementary feed and mineral-vitamin mixtures in different amounts but according to the recommendations of the National Research Council US, Committee on Nutrient Requirements of Swine (2012).

On all farms, randomly selected animals were divided into three main groups, including growers, fatteners, and breeding sows. Sows were in mid or late-gestation and multiparous. The group of growers was further subdivided according to age to: 5 weeks old (w/o), 7 w/o, 9 w/o, 11 w/o and 13 w/o growers. These groups of growers were housed in different rooms on the farms. Thus, we had 7 age groups of pigs, including 5 age groups of growers, 1 group of fatteners and 1 group of breeding pregnant sows.

All farms were certified as free from classical and African swine fever, Aujeszky’s disease, Clostridium perfringens C, Brachyspira hyodysenteriae, Salmonella sp. and in all farms vaccination against Mycoplasma hyopneumoniae was performed. Preventive vaccination against PCV2 and PRRSV was not implied. The clinical examination of the herd was carried out on farm site visit. The animals were clinically healthy.

However, we have not taken any on-site preventive measures against the pathogens observed in this study.

**Samples**

Individual blood samples (Table 1) for determining complete blood count (CBC) and WCDC were taken from the anterior vena cava into tubes containing the anticoagulant EDTA (Vacuette, Greiner Bio-One, Kremsmunster, Austria). The samples were transported in a refrigerated box at 4°C and the analyses were performed on the day of sampling.

The group’s oral fluid (OF) and faeces samples were collected to determine the health status of the herd and to confirm the presence of PRRSV, PCV2 and HEV. The samples were collected as described in the study by Plut et al. (2020). Animals were placed in groups of ten individual pigs in separate crates and divided into six age dependent categories at all farms: 5 w/o, 7 w/o, 9 w/o, 11 w/o weaners; fatteners; and breeding sows. Group samples of fresh faeces were collected from these pigs also, at random pen sites. A total of 36 OF samples and 36 faeces samples from the animals in the study were collected and examined from each of the animal categories on each of the six farms. Previously described PCR, RT-PCR and qRT-PCR protocols were used to detect PCV2, PRRSV and HEV (Plut et al. 2020).

**Haematological analyses**

The individual blood samples were analysed using an automated laser-based haematology analyser ADVIA 120 with a species-specific setting for pigs in the multi-species software developed by the manufacturer of the analyser (Siemens, Munich, Germany). Factory software settings were used without adjustments or modifications. The analyser utilises the principle of automated cytochemistry coupled with flow cytometry. The ADVIA 120 veterinary software does not distinguish between segmented and band neutrophils, and the neutrophil count reflects the total neutrophils. The ADVIA 120 haematology analyser quantitates the size and Hb content of individual RBCs, allowing quantification of the number and percentage of RBCs outside the normocytic-normochromic range. The following haematological parameters are reported in the Results section: WBC, RBC, Hb, Hct, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), and percentage and number of neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells (LUCs). The category LUC consists of a heterogeneous population of all large cells that lack peroxidase activity (atypical lymphocytes, immature granulocytes, and blasts).

**Statistical analyses**

Statistical analyses were performed using the software package R (R Core Team 2013). First, we analysed the haematological parameters using unbalanced one-way ANOVA with the null hypothesis stating that all groups of pigs classified by age (5 w/o, 7 w/o, 9 w/d, 11 w/o and 13 w/o growers as well as fatteners and breeding sows) have the same mean haematological parameter values, that is, one ANOVA for each haematological parameter as the response variable was performed. To verify the assumptions of normality, frequency histograms and Chi-squared goodness of fit test were used; to verify the assumption of equal group variances, the Bartlett’s test was used, and the assumption of independence was determined from the design of the study (Montgomery and Runger 2003). We reject the
null hypothesis of one-way ANOVA if $P$ value is less than a pre-specified threshold (significance level), which we set to the usual value of 0.05. To find pairs of groups with significantly different means we used Tukey’s honestly significant difference test (HSD). When the assumption of equal group variances is violated, the Welch’s ANOVA and the pairwise $t$-tests with no assumption of equal variances are used instead.

Second, we analysed the haematological parameters using unbalanced one-way ANOVA with the null hypothesis stating that all groups of pigs classified by the presence of the considered pathogens (PCR-negative farms, PCR-positive farms with PRRSV, PCR-positive farms with PCV2 and PCR-positive farms with both HEV and PCV2) have the same mean haematological parameter values, that is, one ANOVA for each haematological parameter as the response variable was performed.

Third, we analysed the samples using unbalanced one-way factorial ANOVA with the null hypothesis stating that all groups of pigs classified by age and the presence of the considered pathogens at the same time (being the 2 factor levels) have the same mean haematological parameter values, that is, one unbalanced one-way factorial ANOVA for each haematological parameter as the response variable was performed, where the observed groups of pigs were obtained according to all possible combinations of age and the presence of pathogens (e.g. 5 w/o growers on PCR-negative farms). In addition, the unpaired Welch Two Sample $t$-test was used to test the hypothesis that two populations (with or without the presence of the observed pathogens) have equal mean parameter values. In particular, the unpaired Welch Two Sample $t$-test was used to detect possible influence of a second pathogen on the mean parameter values, but our sample contained only one type of dual infection (i.e. HEV and PCV2).

### Results

There were two farms (Farm A and Farm C) where the pigs (growers, fatteners, and breeding sows) were PCR-negative for all three pathogens, PRRSV, PCV2 and HEV. One farm (Farm F) was PCR-positive for HEV and PCV2, two farms were PCR positive for PCV2 (Farm B and Farm D), and one farm was PCR positive for PRRSV (Farm E) (Table 2).

The haematological parameters WBC, Hb, MCH, MCHC, PLT, the percentage of neutrophils, lymphocytes, and eosinophils, and the absolute numbers of neutrophils, lymphocytes, monocytes, basophils and LUCs differed significantly between PCR-negative farms and farms where at least one of the pathogens was detected (Tables 3–5).

Every age category of growers was compared to fatteners and sows. Cells corresponding to statistically different pairs of age groups are shown in Tables S1–S3.

On PCR-negative farms, significantly higher WBCs were observed in the growers than in sows (Table 3). In addition, significantly lower Hb and MCHC levels and significantly higher PLT and lymphocyte counts were observed in growers than in sows and fatteners (Tables 3 and 5). Significantly lower percentages of neutrophils and eosinophils and significantly higher percentage of lymphocytes and absolute numbers of neutrophils, monocytes, basophils, and LUCs were found in growers than in sows on the same farms (Tables 4 and 5).

In contrast, on the farms that were PCR-positive for PRRSV, PCV2 and/or HEV, age (i.e. breeding sows; 5 w/o, 7 w/o, 9 w/o, 11 w/o and 13 w/o growers; fatteners) significantly affected the values of all observed blood parameters, except for the percentage of LUCs (Tables 3–5). In sows, significantly lower WBC, RBC, absolute numbers of lymphocytes and basophils, and significantly higher MCH were observed than in growers and fatteners (Tables 3 and 5). Significantly lower Hb, MCHC, percentages of neutrophils and eosinophils and significantly higher PLT, percentages of lymphocytes and neutrophils were observed in growers than in sows and fatteners (Tables 3 and 4). A significantly higher Hct was observed in fatteners than in growers and sows, and a significantly lower percentage of basophils were observed in sows than in fatteners (Tables 3 and 4).

Sows on PCR-positive farms with PRRSV, PCV2 and/or HEV had significantly higher WBC, absolute numbers of neutrophils, monocytes, eosinophils, and a significantly lower percentage of lymphocytes than sows on PCR-negative farms (Tables 3–5). Growers in PCR-positive farms with PRRSV, PCV2 and/or HEV had significantly higher MCHC, PLT, percentage and absolute

### Table 2. Detection of pathogens (PRRSV, PCV2, HEV) in different age groups in OF and faeces.

| Farm | 5 weeks old | 7 weeks old | 9 weeks old | 11 weeks old | 13 weeks old |
|------|-------------|-------------|-------------|--------------|-------------|
| PRRSV | E | neg | pos | pos | pos | pos | pos | neg |
| PCV2  | B | neg | neg | pos | neg | neg | neg | neg |
|       | D | neg | neg | neg | pos | neg | neg | neg |
| HEV   | F | pos | pos | pos | neg | neg | neg | neg |

HEV: Hepatitis E virus; PCV2: Porcine circovirus Type 2; pos: Positive; PRRSV: Porcine reproductive and respiratory virus; neg: negative.
In the presence of the pathogens considered, several differences between age groups emerged and were shown to be significant (Tables 3–5). For instance, in the absence of all the pathogens considered, the differences between RBC and MCH values of different age groups (i.e., sows, growers and fatteners) were not significant, but they became significant for all age-group pairs (i.e., sows-growers, sows-fatteners, growers-fatteners) in the presence of the pathogens considered.

numbers of neutrophils and basophils but lower percentage and absolute number of lymphocytes than growers on PCR-negative farms (Tables 3–5). Fatteners on PCR-positive farms with PRRSV, PCV2 and/or HEV had significantly higher WBC, PLT, percentage of eosinophils, absolute numbers of monocytes, eosinophils and basophils and lower MCV than fatteners on farms without any pathogens (Tables 3–5).
and growers-fatteners) if any of the pathogens was present on the farm. In the absence of all the pathogens considered, the differences between Hct, MCV, percentage of basophils and absolute number of eosinophils of different age groups (i.e. sows, growers and fatteners) were not significant, but they became significant for at least one age-group pair (i.e. sows-growers, sows-fatteners or growers-fatteners) if any of the pathogens was present on the farm. In the absence of all the pathogens considered, there were significant differences between the percentages of lymphocytes and eosinophils and the absolute numbers of neutrophils and basophils of only sows and growers, but in the presence of the pathogens, at least one new age-group pair had significantly different parameter values. Parameter values for WBC, Hb, PLT, percentage and absolute numbers of monocytes, percentage, and absolute numbers of LUCs were not significantly different for any of the age-group pairs where the pathogens was present on the farm. The presence of the pathogens led to eliminations of the differences between different age groups in two haematological parameters: MCHC and the absolute number of lymphocytes. On PCR-positive farm infected with PRRSV (Farm E) we found a significantly lower percentage of lymphocytes, MCV and Hct, and increased WBC, PLT, percentage and absolute numbers of neutrophils, basophils and LUCs than on PCR-negative farms. On PCR positive-farms with PCV2 (Farms B, D, F) a significantly lower percentage of lymphocytes and increased percentage and absolute numbers of neutrophils, eosinophils and basophils were detected. On PCR-positive farm with HEV (Farm F) significantly lower MCV and percentage of lymphocytes and increased RBC, Hb, percentage and absolute number of basophils and percentage of neutrophils were observed.

The results of haematological parameters measured in a group of animals with a PCV2 monoinfection and a group of animals with two viral infections (PCV2 and HEV) in 9 w/o growers (Table 2) are presented in Table 6. Nine-week-old pigs infected with PCV2 had significantly lower RBC, PLT, percentage and absolute number of monocytes and LUCs, and significantly higher MCV, MCH, percentage of neutrophils, percentage and absolute number of eosinophils than 9 w/o pigs with PCV2 and HEV.

### Discussion

To the authors’ knowledge, there are not many studies in which haematological parameters in pigs of different age groups are evaluated. Haematological reference values for different age groups of pigs have already been reported (Friendship et al. 1984; Klem et al. 2010; Cooper et al. 2014; Perri et al. 2017; Ježek et al. 2018); however, there are few studies where the authors performed the determination of the pathogens (Nielsen and Bøtner 1997; Stukelj et al. 2013; Adekola et al. 2019), usually in a small number of animals. Here, the pigs were tested by PCR for PRRSV, PCV2 and HEV. Some of the age groups of pigs were
free of all three pathogens, some of the groups had PCV2 or PRRSV monoinfection and one age group of pigs had two viral infections (PCV and HEV). At the level of the farms, two were PCR negative for all pathogens tested, four of them were PCR positive for PRRSV or PCV2 or/and HEV but animals were clinically healthy.

The results of our study show that the age of pigs on PCR-negative farms (i.e. breeding sows; 5 w/o, 7 w/o, 9 w/o, 11 w/o and 13 w/o growers; fatteners) significantly affects the values of haematological parameters. This is consistent with other studies (Thorn 2010; Ježek et al. 2018). Comparison of the values of haematological parameters of sows from PCR-negative farms with the reference values from the literature (Jazbec 1990; Thorn 2010) shows that most of them were in accordance with reported reference ranges. However, the PLT was below the lower limit of the reference range reported in the literature (Jazbec 1990; Thorn 2010), but in accordance with the reference range established by the producer of the analyser (138.2 – 467.8 x 10^9/L; Advia 120, Siemens, Germany). This discrepancy can be explained by the difference between types of haematological analysers used in our and previously published studies. Furthermore, the intensification of pig production and the rapid weight gain have a major impact on the physiological functions in pigs and may lead to disturbances in the mechanisms of haemostasis and consequently PLT (Pliszczak-Król et al. 2016). Haemostatic processes play an important role in many physiological and pathological phenomena, including healing of damaged tissue, inflammatory reactions, and antimicrobial responses. A slightly lower mean WBC in sows from PCR-negative farms than those published in a study carried out on five pig farms in Slovenia (Ježek et al. 2018) is probably due to the use of different haematological analysers, as well as to differences in the sows included in these two studies (gestation period, age, different farms). White blood cell decreases during gestation and with age—it is higher in younger animals (Thorn 2010).

The values of haematological parameters for PCR-negative growers and fatteners obtained in our study are consistent with those reported by Ježek et al. (2018) for the same types of pigs. Only higher MCV values were found compared with the reported reference values (Thorn 2010; Ježek et al. 2018), which may be explained by preanalytical issues and differences in the haematological analysers used in different studies. In addition, some previously published reference values (Jazbec 1990) are not specified in terms of the age of pigs. Our resulting mean values for haematological parameters obtained for growers and fatteners from PCR-negative farms differ from those determined in sows, which is in accordance with the data from the literature (Thorn 2010). The group of growers (5 w/o) had the lowest absolute number of eosinophils. The highest WBC, absolute number of lymphocytes, basophils, monocytes, and neutrophils had 9 w/o growers, and the highest PLT 7 w/o growers. Age significantly influenced most haematological parameters, which is consistent with the results of other studies (Stukelj et al. 2010; Yeom et al. 2012; Ježek et al. 2018) and is related to physiological changes during maturation processes (Evans 2006; Thorn 2010; Ježek et al. 2018).

The haematological parameters of sows, 5 w/o, 7 w/o, 9 w/o, 11 w/o and 13 w/o growers, and fatteners without clinical signs from PCR-positive farms correspond to the reported reference ranges (Jazbec 1990; Thorn 2010; Ježek et al. 2018). On PCR-positive farms we also found that the age of pigs (i.e. breeding sows; 5 w/o, 7 w/o, 9 w/o, 11 w/o and 13 w/o growers; fatteners) significantly influenced the values of all observed blood parameters except the percentage of LUCs.

When comparing haematological parameters of pigs of different age categories between PCR-positive and PCR-negative farms, significant differences were found in several of them. Interestingly, significantly higher percentage of WBC and absolute numbers of neutrophils, monocytes and eosinophils, and a significantly lower percentage of lymphocytes in sows on PCR-positive farms were measured compared to sows on PCR-negative farms. This was observed although sows on PCR-positive farms were not PCR-positive for any of the pathogens tested on any of the farms included in our study. These results may be explained by differences in husbandry techniques, handling, physiological status, biosecurity and general health of the herd. White blood cells play a primary role in the body’s defence mechanisms. Apart from pathological conditions, an increase in WBC can also be observed in animals after strenuous exercise or feeding. It also occurs in sows in the final stage of gestation and immediately after farrowing, as well as in suckling piglets (Czech et al. 2017).

When comparing the haematological parameters of growers on PCR-positive farms with the parameters of growers on PCR-negative farms, we found significantly higher MCHC, PLT, percentages and absolute numbers of neutrophils and basophils and significantly lower percentage and absolute number of lymphocytes on
PCR-positive farms. This is possibly due to infection by PRRSV, PCV2 and HEV as well as infection by some other pathogens that were not determined in our study. Differences in husbandry techniques should also be taken into account. All three pathogens were present in growers. Nielsen and Bøtner (1997) described transient lymphopenia in four and a half months old pigs experimentally inoculated with PRRSV, and Segalès et al. (2000) reported a lower percentage of lymphocytes in naturally infected animals with PCV2 compared to healthy pigs. Halbur et al. (2002) also observed a slight increase in neutrophil, in PRRSV-infected pigs. In our study, we found a significantly reduced percentage of lymphocytes in growers on PCR-positive farms compared to growers on PCR-negative farms; PRRSV was found in 7 to 13 w/o pigs and PCV2 in 5 to 11 w/o pigs.

When comparing the haematological parameters of fatteners on PCR-positive farms with the haematological parameters of fatteners on PCR-negative farms, we found that WBC, PLT, percentage and absolute number of eosinophils, monocytes, and basophils on PCR-positive farms, were significantly higher. This was possibly due to infection by PRRSV. This pathogen was detected only in fatteners. It was reported that experimental inoculation of pigs with different PRRSV isolates resulted in decreased values of Hb, Hct and RBC (Halbur 2002), but this was not observed in our study. The increase in WBC observed in our study and in the infected pigs from the study of Halbur et al. (2002) was most likely due to the increased demand and their subsequent production by bone marrow. The increased WBC on PRRSV-positive farm could be explained by a secondary infection. PRRSV is able to modulate or alter the immune system’s ability to control other pathogens (Chase and Lunney 2012). The increase in eosinophils in infected pigs is similar to the observation of Halbur and others (2002) and may be due to an increase in myeloid activity. Eosinophilia is also frequently observed in helminth infections, which are not unusual in pigs (Kalai et al. 2012; Masure et al. 2013).

Pigs (9 w/o) from different farms that were PCR positive (PRRSV or, PCV2 or HEV) had an increased percentage of neutrophils and basophils and a decreased percentage of lymphocytes, which may be related to the viral infection (Jazbec 1990; Segalès et al. 2000). The time between weaning and growing is particularly susceptible to infection, as maternal antibodies are withdrawn (Done et al. 2012).

With PCR we detected two pathogens at the same time in only one farm, Farm F, and only one pathogen in three other farms. The two pathogens detected simultaneously were PCV2 and HEV. The degree of infection by HEV in Farm F was high, which could be due to PCV2 coinfection that led to modification of the immune system caused by the immunosuppressive effect of PCV2 (Yang et al. 2015). In the study by Moldal et al. (2009) pigs with PCV2-associated disease (PCVAD) had significantly lower RBC, number of eosinophils, lymphocytes, monocytes and lower Hct and, a significantly higher number of neutrophils than healthy pigs. When we compared haematological parameters between a group of 9 w/o pigs with a mono-infection (PCV2) in Farm B and a group of pigs of the same age with two viral infections (PCV2 and HEV) in Farm F, we noted that a group with PCV2 mono-infection had significantly lower RBC, percentage and absolute number of monocytes and a significantly higher percentage of neutrophils than the group with PCV2 and HEV. In our study 9 w/o growers with PCV2 mono-infection had significantly lower percentage and absolute number of eosinophils. The eosinophil results contrast with the study by Moldal et al. (2009) and may be due to helminth infections, which are not uncommon in pigs (Kalai et al. 2012; Masure et al. 2013). In the PCV2-positive group, PLT was also significantly lower, which could be due to infection or mycotoxicosis (Jazbec 1990). Furthermore, in the group with PCV2 and HEV, MCV and MCH were significantly lower compared to the group with PCV2 mono-infection. The differences could be due to the presence of two pathogens instead of one, as well as to the different farm management. In the study by Adekola et al. (2019), HEV seropositivity in pigs was associated with a significant decrease in erythrocyte parameters (packed cell volume, Hb, RBC, MCV and MCH). The somehow surprising finding in our study was that RBCs were significantly lower in the group of 9 w/o pigs with PCV2 mono-infection than in the group of pigs of the same age infected with PCV2 and HEV. Both Farm B and Farm F are smaller farms with about 100 breeding sows. Biosecurity measures on Farm B are poorer, which may have led to additional subclinical infections that were not tested in our study. All haematological parameters differing significantly between the group of pigs with PCV2 mono-infection and the group with PCV2 and HEV were within the reported reference ranges (Jazbec 1990; Thorn 2010; Ježek et al. 2018). Although we detected PCV2 and HEV by PCR in Farm F, the animals were clinically healthy, meaning that the diseases were subclinical. This could be the reason for the haematological values being within the reference ranges.
Conclusions

Our study showed that age-related changes in haematological parameters occurred in pigs in which the pathogens were present or absent according to PCR being used for detection of the nucleic acids of selected pathogens (PRRSV, PCV2 and HEV). The values of several haematological parameters differed significantly between PCR-negative and PCR-positive animals and between the 9 w/o animals with PCV2 monoinfection and the 9 w/o animals with two viral infections (PCV2 and HEV). There is wide variation in reported haematological reference values for pigs due to selection of individual, instrumentaion and preanalytical factors. Therefore, it is important to establish laboratory’s own reference values for haematological parameters in pigs of different ages and sex using the available haematological analyser and according to the stated preanalytical procedure.

Nevertheless, the anamnestic and clinical data of the herd should be considered when interpreting the results of haematological analyses.

Etichal approval

Blood samples, OF and faeces samples were taken as part of regular diagnostics on six farms participating in the Slovenian Target Research Program CRP V4-1604 (Animal Welfare including the health of poultry and pigs in conventional and alternative housing systems). The Ethics Committee, which approves and supervises the animal experiments, is part of the Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection under the Ministry of Agriculture, Forestry and Food. The content of the abovementioned research project was supervised by that administrative authority, and all participants, procedures and objectives of the program were constantly monitored through periodic reports. In accordance with Directive 2010/63/EU of the European Parliament and the Council on the Protection of Animals used for Scientific Purposes and Slovenian Animal Protection Law (Official Gazette of the Republic of Slovenia no. 38/2013 and 21/2018), non-experimental clinical veterinary practices and practices that are unlikely to cause pain, suffering, distress or lasting harm equivalent to or greater than that caused by the introduction of a needle are not considered as an experiment on animals and approval by the National Ethics Committee is considered unnecessary. This is stated in the document Resolution: 5-5-2020/3 issued by Committee for Animal Welfare of Veterinary Faculty, which also includes the discussion about the verbal and written consent to the participation of animal owners. Written consent from the farm owners was obtained and is attached to the Resolution.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

All data are fully available without restriction.

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