Haematological Profiles of Pigs on Different Farms Reflect Their Health Status

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Abstract

Background

Haematological examination is an important diagnostic tool in the assessment of pig health status. The present study aimed to assess haematological parameters in pigs of different age categories from six farrow-to-finish farms differing in herd health status. The following pig categories were included: 5 age groups of growers (5, 7, 9–10, 11 and 12–13 weeks-old), fatteners and breeding pregnant sows. Individual blood samples for determining complete blood count and white blood cell differential count were taken and group samples of oral fluid and faeces were collected from each animal category in each of the six farms and tested for the detection of Porcine Circovirus Type 2 (PCV2), Porcine Reproductive and Respiratory Virus (PRRSV), and Hepatitis E Virus (HEV) using PCR, RT-PCR, and qRT-PCR protocols. Individual blood samples were analysed using an automated laser-based haematology analyser. The following haematological parameters were reported: white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (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.

Results

On farms free of PRRSV, PCV2 and HEV, age had significant effect on the following parameters: WBC, Hb, MCH, MCHC, PLT, percentage of neutrophils, lymphocytes and eosinophils and absolute numbers of neutrophils, lymphocytes, monocytes, basophils and LUCs. On farms with PRRS, PCV2 and/or HEV, age significantly affected all observed blood parameters except the percentage of LUCs. The percentages of lymphocytes, MCV and Hct were significantly lower by PRRSV while WBC, PLT, percentage and absolute number of neutrophils, basophils and LUCs increased. Significantly lower percentages of lymphocytes and increased percentages and absolute numbers of neutrophils, eosinophils and basophils were caused by PCV2 presence. Significantly lower percentage of lymphocytes and MCV and increased RBC, Hb, percentage and number of basophils and percentage of neutrophils were caused by HEV.

Conclusions

Alterations of haematological parameters reflected the health status of pigs of different categories on infected and on non-infected farms. Age-related changes in haematological parameters occurred in clinically healthy and in infected pigs.

Background

The measurement of haematological parameters in pigs is rarely performed. There may be 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, difficulties in blood collection and the limited availability of reference intervals required for correct interpretation of laboratory results [1, 2]. Several reference ranges of haematological parameters for pigs have been published [1, 2, 3, 4, 5, 6, 7]. However, the ranges for most haematological parameters are quite wide and vary as 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 [2, 3, 4]. All these factors must be considered when interpreting the results of haematological analyses.

There are many important reasons for determining haematological parameters in pigs. Firstly, the assessment of these parameters can be used to establish a proper diagnosis and assess not only the health status of a pig but also the health status of a herd [1, 2]. In addition, the assessment of haematological parameters can contribute to the early identification of disease or poor growth performance [1, 8] and may be highly valuable in the treatment or prognosis of many diseases [6].

The values of haematological parameters can vary considerably depending on the presence of inflammation and infection [9, 10, 11, 12]. Furthermore, several haematological parameters are strongly related to the chronic disease status. Haematological data provide information that is useful both in detection and monitoring of animals diagnosed with bacterial, viral, fungal, and parasitic infections [11]. The blood profile is also an important tool for determining the degree of inflammatory processes in pigs at slaughtering [13]. Pathogens often cause changes in white blood cell (WBC) and red blood cell (RBC) counts, haematocrit (Hct), haemoglobin concentration (Hb) and white blood cell differential count [4, 14].

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, which are either economically important for the health of pigs or constitute a potential threat to food safety, such as HEV. Infections caused by PRRSV and PCV2 only affect pigs [15, 16], but HEV is potentially fatal to human in certain populations in terms of chronic hepatitis. In addition, contaminated pork and contaminated meat products are potential sources of human infection [17]. There are few reports of haematological parameters in pigs with the presence of different viruses (PRRSV, PCV2, HEV) [10, 18, 19, 20].

The aim of the present study was to assess haematological parameters in pigs of different age categories from six farms with different health statuses.

Results

There were two farms that were free of all three pathogens (Farm 1 and Farm 3), while in the other 4 farms we found a positive result in at least one of the samples in a different age group (Table 1).

Table 1. Detection of pathogens (HEV, PCV2, PRRSV) in different age groups in oral fluid and faeces

	Farm number	5 weeks old	7 weeks old	9–10 weeks old	11 weeks old	12–13 weeks old	Fatteners	Sows
HEV	6	**pos**	**pos**	**pos**	neg	neg	neg	neg
	2	neg	neg	**pos**	neg	neg	neg	neg
PCV2	4	neg	neg	neg	**pos**	neg	neg	neg
	6	**pos**	**pos**	**pos**	neg	neg	neg	neg
PRRSV	5	neg	**pos**	**pos**	**pos**	**pos**	**pos**	neg

pos = positive

neg = negative

The results of our study showed that the category (growers, fatteners and breeding sows) on farms 1 and 3, which were without PRRSV, PCV2 and HEV, significantly affected the values of the following parameters: WBC, Hb, MCH, MCHC, PLT, percentage of neutrophils, lymphocytes and eosinophils and the absolute numbers of neutrophils, lymphocytes, monocytes, basophils and LUCs (Tables 2, 3 and 4).

Furthermore, growers were divided into age categories: 5 weeks old growers (group label 5, N=18), 7 weeks old growers (group label 7, N=19), 9–10 weeks old growers (group label 9–10, N=19), 11 weeks old growers (group label 11, N=15) and 12–13 weeks old growers (group label 12–13, N=18). Every age category of growers was also compared to fatteners and sows. Cells corresponding to statistically different pairs of age groups are shown in the supplementary files (Additional files 1, 2 and 3).

On farms 1 and 3, significantly higher WBCs were observed in the growers than in the sows (Table 2). In addition, significantly lower Hb and MCHC levels and significantly higher PLT and lymphocyte count were observed in growers than in sows and fatteners (Tables 2 and 4). 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 (Tables 3 and 4).

Table 2: Complete blood count parameters (mean ± SD) of sows, growers (of all ages) and fatteners

	PCR-negative farms			PCR-positive farms					
	growers (all ages) (N=89)	**fatteners (N=9)**	**sows (N=20)**	**growers (all ages) (N=181)**	**fatteners (N=39)**	**sows (N=40)**	***p-value*[1]**	***p-value*[2]**	***p-value*[3] *(G/F/S)***
WBC [10^9/L]	22.2 ± 5.8	17.0 ± 3.2	12.4 ± 1.8	19.4 ± 7.8	20.8 ± 4.5	15.8 ± 4.1	*<0.0001*	*<0.0001*	*ns/<0.01/<0.0001*
RBC [10^{12}/L]	6.25 ± 1.06	6.35 ± 1.12	5.86 ± 0.56	6.27 ± 0.81	6.96 ± 0.65	5.95 ± 0.75	*ns*	*<0.0001*	*ns/ns/ns*
Hb [g/L]	108 ± 17	122 ± 18	119 ± 10	109 ± 15	129 ± 10	123 ± 16	*<0.01*	*<0.0001*	*ns/ns/ns*
Hct [L/L]	0.376 ± 0.046	0.398 ± 0.071	0.367 ± 0.028	0.367 ± 0.057	0.408 ± 0.038	0.372 ± 0.048	*ns*	*<0.0001*	*ns/ns/ns*
MCV [fL]	60.1 ± 5.1	62.4 ± 3.2	63.1 ± 3.5	58.7 ± 4.4	59.1 ± 2.4	62.6 ± 3.4	*ns*	*<0.0001*	*<0.05/<0.05/ns*
MCH [pg]	17.4 ± 1.3	19.3 ± 1.2	20.4 ± 0.9	17.4 ± 1.4	18.6 ± 1.02	20.7 ± 0.95	*<0.0001*	*<0.0001*	*ns/ns/ns*
MCHC [g/L]	291 ± 17	309 ± 12	323 ± 13	296 ± 13	314 ± 10	323 ± 48	*<0.0001*	*<0.0001*	*<0.05/ns/ns*
PLT [10^9/L]	356 ± 133	192 ± 57	173 ± 106	406 ± 163	284 ± 102	214 ± 88	*<0.0001*	*<0.0001*	*<0.01/<0.01/ns*

ns = not significant

[1]significant differences between age categories on PCR-negative farms

[2]significant differences between age categories on PCR-positive farms

[3]significant differences between PCR-negative and PCR-positive farms in dependence on the pig category (G=growers; F=fatteners, S=sows)

On the other hand, on farms with PRRS, PCV2 and/or HEV, we found that age (i.e., breeding sows, different weeks old growers, fatteners) significantly affected the values of all observed blood parameters except the percentage of LUCs (Tables 2, 3 and 4). In sows, significantly lower WBC, RBC, absolute numbers of lymphocytes, basophils and significantly higher MCH were observed than in growers and fatteners (Tables 2 and 4). 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 2 and 3). A significantly higher Hct was observed in fatteners than in growers and sows and a significantly lower percentage of basophils was observed in sows than in fatteners (Tables 2 and 3).

Table 3: White blood differential count parameters (relative values; mean ± SD) of sows, growers and fatteners

	PCR-negative farms			PCR-positive farms					
	growers (all ages) (N=89)	**fatteners (N=9)**	**sows (N=20)**	**growers (all ages) (N=181)**	**fatteners (N=39)**	**sows (N=40)**	***p-value[1]***	***p-value[2]***	***p-value[3] (G/F/S)***
Neut [%]	36.7 ± 10.5	40.5 ± 11.2	43.1 ± 9.0	45.1 ± 12.0	36.5 ± 7.6	47.2 ± 8.3	*<0.001*	*<0.0001*	*<0.0001/<ns/<ns*
Lymph [%]	54.0 ± 10.4	50.3 ± 11.9	46.1 ± 7.4	45.6 ± 11.7	52.0 ± 8.8	40.6 ± 7.5	*<0.001*	*<0.0001*	*<0.0001/ns/<0.05*
Mono [%]	5.26 ± 3.72	3.79 ± 1.02	3.96 ± 0.76	4.89 ± 2.27	4.43 ± 1.71	4.21 ± 1.04	*ns*	*<0.001*	*ns/ns/ns*
Eos [%]	2.93 ± 1.75	4.38 ± 1.75	6.01 ± 3.21	3.09 ± 1.77	6.01 ± 3.05	6.94 ± 3.74	*<0.0001*	*<0.0001*	*ns/<0.05/ns*
Baso [%]	0.303 ± 0.156	0.367 ± 0.200	0.280 ± 0.174	0.418 ± 0.255	0.497 ± 0.195	0.350 ± 0.138	*ns*	*<0.0001*	*<0.0001/ns/ns*
LUC [%]	0.817 ± 0.596	0.600 ± 0.472	0.625 ± 0.324	0.810 ± 0.694	0.623 ± 0.537	0.657 ± 0.340	*<0.05*	*ns*	*ns/ns/ns*

ns = not significant

[1]significant differences between age categories on PCR-negative farms

[2]significant differences between age categories on PCR-positive farms

[3]significant differences between PCR-negative and PCR-positive farms in dependence on the pig category (G=growers; F=fatteners, S=sows)

Sows on farms with PRRSV, PCV2 and/or HEV have significantly higher WBC, absolute numbers of neutrophils, monocytes, eosinophils and a significantly lower percentage of lymphocytes than on farms without any pathogen (Tables 2, 3 and 4). Growers on farms with PRRSV, PCV2 and/or HEV have significantly higher MCHC, PLT, percentage and absolute numbers of neutrophils and basophils but lower percentage and absolute number of lymphocytes (Tables 2, 3 and 4). Fatteners on farms with PRRSV, PCV2 and/or HEV have significantly higher WBC, PLT, percentage of eosinophils, absolute numbers of monocytes, eosinophils and basophils and lower MCV than on farms without any pathogen (Tables 2, 3 and 4).

Table 4: White blood differential count parameters (absolute values; mean ± SD) of sows, growers and fatteners

	PCR-negative farms			PCR-positive farms					
	growers (all ages) (N=89)	**fatteners (N=9)**	**sows (N=20)**	**growers (all ages) (N=181)**	**fatteners (N=39)**	**sows (N=40)**	***p-value[1]***	***p-value[2]***	***p-value[3] (G/F/S)***
Neut [10^9/L]	8.35 ± 4.03	6.85 ± 2.18	5.40 ± 1.75	10.50 ± 4.99	7.55 ± 2.58	7.63 ± 3.19	*<0.0001*	*<0.0001*	*<0.001/ns/<0.001*
Lymph [10^9/L]	11.80 ± 3.21	8.59 ± 2.60	5.65 ± 1.04	10.20 ± 3.31	10.60 ± 2.45	6.22 ± 1.22	*<0.0001*	*<0.0001*	*<0.001/ns/ns*
Mono [10^9/L]	1.180 ± 0.787	0.658 ± 0.252	0.486 ± 0.098	1.150 ± 0.665	0.914 ± 0.414	0.661 ± 0.223	*<0.001*	*<0.0001*	*ns/<0.05/<0.0001*
Eos [10^9/L]	0.649 ± 0.512	0.769 ± 0.401	0.726 ± 0.337	0.687 ± 0.396	1.300 ± 0.932	1.110 ± 0.758	*ns*	*<0.0001*	*ns/<0.05/<0.01*
Baso [10^9/L]	0.069 ± 0.039	0.061 ± 0.04	0.034 ± 0.019	0.098 ± 0.068	0.101 ± 0.040	0.057 ± 0.030	*<0.001*	*<0.0001*	*<0.0001/<0.05/<0.001*
LUC [10^9/L]	0.172 ± 0.120	0.102 ± 0.071	0.078 ± 0.045	0.187 ± 0.178	0.131 ± 0.117	0.108 ± 0.073	*<0.0001*	*<0.01*	*ns/ns/ns*

ns = not significant

[1] significant differences between age categories on PCR-negative farms

[2] significant differences between age categories on PCR-positive farms

[3] significant differences between PCR-negative and PCR-positive farms in dependence on the pig category (G=growers; F=fatteners, S=sows)

In the presence of the considered pathogens, several differences between age groups emerged and were shown to be significant (Tables 2, 3 and 4). For instance, in the absence of all the considered pathogens, the differences between RBC and MCH values of different age groups (i.e., sows, growers, fatteners) were not significant, but they became significant for all age-group pairs (i.e., sows-growers, sows-fatteners, growers-fatteners) if any of the pathogens was present on the farm. In the absence of all the considered pathogens, the differences between Hct, MCV, percentage of basophils and absolute number of eosinophils of different age groups (i.e., sows, growers, 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 considered pathogens, there were significant differences between percentage of lymphocytes, eosinophils, 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 are not significantly different for any of the age-group pairs if the pathogens are present on the farm. The presence of the pathogens eliminated the differences between different age groups in two haematological parameters: MCHC and absolute number of lymphocytes. Porcine Reproductive and Respiratory Virus significantly lowered percentage of lymphocytes, MCV and Hct, and increased WBC, PLT, percentage and absolute numbers of neutrophils, basophils and LUCs. Porcine Circovirus Type 2 significantly lowered percentage of lymphocytes and increased percentage and absolute numbers of neutrophils, eosinophils and basophils. Hepatitis E Virus significantly lowered MCV and percentage of lymphocytes and increased RBC, Hb, percentage and absolute number of basophils and percentage of neutrophils.

Discussion

To the authors knowledge, this is the first study that evaluated haematological parameters in pigs of different age categories; the pigs were tested by PCR for PRRSV, PCV2 and HEV. Two of the farms were PCR negative for all pathogens tested, four of them were PCR positive for PRRSV, PCV2 and HEV. Haematological reference values for different age groups of pigs have already been reported [1, 2, 3, 5, 7]; however, the authors of these studies did not perform an analysis of the pathogens.

The results of our study showed that the age on PCR-negative farms (i.e., breeding sows, different weeks old growers, fatteners) significantly affected the values of haematological parameters, which is consistent with other studies [2, 4]. When comparing the values of haematological parameters of sows from PCR-negative farms with the reference values from the literature [4, 21] most haematological parameters correspond to the reported reference ranges. However, the PLT was below the lower limit of the reference range reported in the literature [4, 21], which could be due to methodological reasons. Furthermore, the intensification of pig production and 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 [22]. 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 compared to a study carried out in five pig farms in Slovenia [2] is probably due to the use

of different haematological analysers, as well as due to differences in the sows included in these two studies (gestation period, age, different farms). WBC decreases during gestation and with age – it is higher in younger animals [4].

In our study, the values of haematological parameters for healthy growers and fatteners are consistent with those reported by Ježek et al. (2018) for growers and fatteners [2]. In our study we only found higher MCV values compared to the reported reference values [2, 4], which may be due to preanalytical issues and differences in the haematological analysers used in different studies. In addition, these values may have differed because some published reference values [21] are not specified by age, or perhaps in part because laboratory methods differed. In our study, the mean values of haematological parameters for growers and fatteners from PCR-negative farms differ from the reported reference intervals for sows [4]. In our study, the group with the highest WBC, absolute number of lymphocytes, basophils, monocytes and neutrophils were 9–10 weeks old growers, the group with the highest PLT were 7 weeks old growers, and the group with the lowest absolute number of eosinophils were 5 weeks old growers. Age significantly influenced most haematological parameters in our study, which is consistent with the results of other studies [2, 23, 24] and related to physiological changes [2, 4, 25].

When comparing the haematological parameters of sows, growers and fatteners from PCR-positive farms with the reference values from the literature [2, 4, 21], the haematological parameters correspond to the reported reference ranges. On PCR-positive farms we also found that age (i.e., breeding sows, different weeks old growers, fatteners) significantly influenced the values of all observed blood parameters except the percentage of LUCs.

As expected in our study, when comparing haematological parameters of pigs of different age categories between PCR-positive and negative farms, we found significant differences in several haematological parameters. Interestingly, we found a significantly higher WBC, absolute numbers of neutrophils, monocytes and eosinophils and a significantly lower percentage of lymphocytes in sows on PCR-positive farms compared to sows on PCR-negative farms, 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 could be due to differences in husbandry techniques, 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 [26].

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, percentage and absolute numbers of neutrophils and basophils and significantly lower percentage and absolute number of lymphocytes on PCR-positive farms, possibly due to PRRSV, PCV2 and HEV infection. All three pathogens were present in the growers. Nielson and Bøtner (1997) described transient lymphopenia in four and a half months old pigs experimentally inoculated with PRRSV [14], and Ségales et al. (2000) reported a lower percentage of lymphocytes in naturally infected animals with PCV2 compared to healthy pigs [27]. Furthermore, Halbur et al. (2002) also observed a slight increase in neutrophils, which was observed in PRRSV-infected pigs [19]. 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 seven to 13-weeks old pigs and PCV2 in five to 11-week- old pigs.

When comparing the haematological parameters of finishers on PCR-positive farms with the haematological parameters of fatteners on PCR-negative farms, WBC, PLT, percentage and absolute number of eosinophils, monocytes, and basophils on PCR-positive farms, possibly due to PRRSV, were significantly higher. PRRSV 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 [19], 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 [19]. The increased WBC on PRRSV-positive farms could be due to a secondary infection. PRRSV is able modulate or alter the immune system's ability to control other pathogens [28]. The increase in eosinophils in infected pigs is similar to the observation of Halbur and others (2002) and is most likely due to an increase in myeloid activity [19]. Eosinophilia is also frequently observed in helminth infections, which are not unusual in pigs [29, 30].

In pigs (9–10 weeks old) with all three pathogens (PRRSV, PCV2 and HEV) we found an increased percentage of neutrophils and basophils and a decreased percentage of lymphocytes, which may be related to the viral infection [21, 27]. The time between weaning and growing is particularly susceptible to infection, as maternal antibodies are withdrawn [31].

Simultaneous co-infection with PCV2 and HEV took place on Farm 6, where these two viruses were found in weaning pigs of all ages. The degree of infection by HEV on Farm 6 was high, which could be due to PCV2 coinfection that led to modification of immune system caused by the immunosuppressive effect of PCV2 [32]. In the study by Moldal et al. (2010) pigs with PCV2-associated disease (PCVAD) had significantly lower RBC, eosinophils, lymphocytes, monocytes and Hct and a significantly higher number of neutrophils than healthy pigs [33]. In our study both viruses increased percentage of neutrophils and percentage and absolute number of basophils, as well as decreased percentage of lymphocytes and HEV increased RBC and Hb in pigs. These is in contrast with the study by Adekola et al. (2019) where HEV seropositivity of the pigs was associated with a significant decrease in erythrocyte parameters (packed cell volume, Hb, RBC, MCV and MCH) [20].

We may assume that differences in health status between PCR-negative and PCR-positive farms could account for differences in the values of haematological parameters.

Conclusions

In the present study, the values of haematological parameters reflected the health status of pigs of different categories on infected and on non-infected farms. Our study showed that age-related changes in haematological parameters occurred in clinically healthy and in infected pigs. Values of several haematologic parameters differed significantly between clinically healthy and infected animals; however, haematological parameters of infected animals did not differ from published reference values. There is wide variation in reported haematological reference values for pigs due to selection of individuals, instrumentation and preanalytical factors. Therefore, it is important to establish own reference values for haematological parameters in pigs of different age and sex categories using the available haematological analyser and according to the stated preanalytical procedure.

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

Methods

Animals and farms

The study was carried out in six farms (Table 5) in Slovenia: two large one-site farms, one with 1.000 and the other with 1.800–1.900 breeding sows, one two-site farm with approximately 600 breeding sows and three small one-site farms with less than 100 breeding sows. All farms had an all-in-all-out system.

Table 5
Number of blood samples for haematological analyses taken per farm

Farm	Sows	Growers 5 weeks old	Growers 7 weeks old	Growers 9–10 weeks old	Growers 11 weeks old	Growers 12–13 weeks old	Fatteners	Total (farm)
1	10	9	10	10	8	9	9	65
2	10	10	10	10	9	10	9	68
3	10	9	9	9	7	9	0	53
4	10	10	10	10	10	10	10	70
5	10	10	10	9	9	10	10	68
6	10	7	0	10	0	9	10	46
Total/age group	60	55	49	58	43	57	48	370

On all farms the animals were divided into 3 main groups, including growers, fatteners and breeding pregnant sows. The group of growers was further subdivided by age to: 5 weeks old, 7 weeks old, 9–10 weeks old, 11 weeks old and 12–13 weeks old growers. Thus, we had 7 age groups of pigs, including 5 age groups of growers, 1 group of fatteners and 1 group 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. The clinical examination of the herd was carried out on farm site visit. The animals were clinically healthy.

We decided to test for the presence of PRRSV, PCV2 and HEV because they are the most common pathogens on pig farms, especially PCV2 and HEV often occur subclinically [32, 35, 36, 37]. However, we have not taken any on-site preventive measures against the pathogens observed in this study.

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 in accordingly to the recommendations of the National Research Council category (2012) [38].

Samples

Individual blood samples (Table 1) for determining complete blood count (CBC) and white blood cell differential count (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 OF and faeces samples were collected to determine the health status of the herd and to confirm the presence of PRRSV, HEV and PCV2. The group OF and faeces samples were collected as described in the study by Plut et al. (2020) [39]. A total of 36 OF samples and 36 faeces samples 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 [39].

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 utilizes 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 LUC category consists of a heterogeneous population of all large cells that fail to exhibit any peroxidase activity (atypical lymphocytes, immature granulocytes and blasts).

Statistical analysis

Statistical analyses were performed using the software package R [40]. Firstly, we analysed the haematological parameters using unbalanced one-way ANOVA with the null hypothesis stating that all groups of pigs (classified by age) have the same mean parameter values. The results of a one-way ANOVA can be considered reliable if the following assumptions are met: the parameter values being tested are independent within and among the groups, the groups associated with each mean in the test are normally distributed and there is equal within-group variance across the groups associated with each mean in the test (homogeneity of variance). 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 [41]. 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. Rejecting the null hypothesis is taken to mean that the differences in the mean haematological parameter values between groups of pigs are unlikely to be due to random chance. To find pairs of groups with significantly different means we used Tukey's honestly significant difference test (HSD). Since the null hypothesis for Tukey's test states that the means being compared are from the same population, rejecting the null hypothesis (with significance level 0.05) is taken to mean that the difference in the mean haematological values between groups of pigs are unlikely to be due to random chance. 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.

Secondly, 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) have the same mean parameter values. Thirdly, we analysed the samples using unbalanced one-way factorial ANOVA with the null hypothesis stating that all groups of pigs (classified by age and presence of pathogens) have the same mean blood parameter values. The groups of pigs were all possible combinations of the 2 factor levels (age and presence of pathogens). 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.

Abbreviations

Baso: basophils

CBC: determining complete blood count

Eos: eosinophils

Hb: haemoglobin concentration

Hct: haematocrit

HEV: Hepatitis E Virus

HSD: Tukey's honestly significant difference test

LUC: large unstained cells

MCH: mean corpuscular haemoglobin

MCHC: mean corpuscular haemoglobin concentration

MCV: mean corpuscular volume

Mono: monocytes

Neut: neutrophils

OF: oral fluid

PCV2: Porcine Circovirus Type 2

PCVAD: PCV2-associated disease

PLT: platelet count

Lymph: lymphocytes

PRRSV: Porcine Reproductive and Respiratory Virus

RBC: red blood cell counts

WBC: white blood cell counts

WCDC: white blood cell differential count

Declarations

Ethical approval and consent to participate

Blood samples, oral fluid (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.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

IGO contributed to field sampling, interpreted results, and prepared the manuscript. ANS performed laboratory tests and reviewing the manuscript. JP contributed to field sampling, and performed laboratory tests. MH provided the statistical analysis of results. MS contributed to idea and experimental design, and coordinated the experiment and field sampling. All authors have contributed to the conceptualisation of the work. All authors read and approved the manuscript before submission.

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