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Comparison of blood parameters in pigs with confirmed African Swine Fever from an outbreak in Constanta County *versus* healthy pigs

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Abstract

The objective of our study was to emphasize the correlation between blood parameters and different viremia stages of African Swine Fever infection. For this purpose, blood on EDTA samples were taken for complete blood count. The first group of pigs analysed was represented by one saw, aged 18 months and 14 young feeder pigs, aged 6 months from a confirmed African Swine Fever outbreak a day before the test was conducted. The control group consisted of 5 normal pigs all aged 9 months. Our results show a corelation found between clinical signs and the blood parameters (WBC, PLT, RBC) measured using URIT-3000Plus Haematology Analyser. Of the 15 pigs confirmed with ASF, group I represented by 3 pigs were in critical condition presenting signs of fever and loss of appetite (WBC 6.3 – 7.2; PLT 16 -74; RBC 1.86 - 5.87), group II represented 5 pigs were moderately affected (WBC 8.0 – 11.9; PLT 36 - 263; RBC 5.29 – 6.48), group III represented 4 pigs were slightly affected (WBC 21.9 - 23.6; PLT 37 - 191; RBC 5.15 – 7.53). and group IV represented 3 pigs were not show-ing any signs of disease (WBC 13.4– 18.1; PLT 86 - 241; RBC 6.69 – 6.87) The study of the control group of animals that were not under known pathogen challenges showed that they had no signifi-cant differences for WBC 11.8-12.1; PLT 235- 246 and RBC 5.58- 5.64. Our results showed a sig-nificant correlation between the clinical signs of the affected pigs and the haematological values compared to the healthy pigs tested.

Keywords: African Swine Fever, viremia, complete blood count

1.Introduction

African Swine Fever (ASF) is a systemic vial disease that affects pigs and wild boars. Clinical signs are characterised as septicaemic lesions, fever, and haemorrhage. Sudden death it is noted in the supra-acute state. High temperature, low appetite, nesting, shallow breathing, refuse movement are signs noted in the acute state. ASF virus is the only member of the genus Asfavirus, in the family Asfarviridae, large-sized double – stranded DNA virus (~ 200 nm), with lipo-proteic, icosahedral shell [1]. ASF is the only DNA virus that can be considered an arbovirus since it replicates in both vertebrate hosts and arthropods [3].

The stability of the virus is a special feature: infectivity is preserved after 15 weeks in refrigerated meat, 5-6 months in processed meat and 15 years in frozen carcasses [2]. Regarding the persistence in the environment studies showed that the virus is stable at pH 4-13. The half-life of ASFV DNA was 8 to 9 days in faeces and 2 to 3 days in oral fluid at all temperature. In urine, the half-life of ASFV DNA was found to be 32.54 days at 4°C decreasing to 19.48 days at 37°C. These results indicate that ASFV in excretions may be an important route of ASFV transmission [4].

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Marked variations in the virulence of isolates: - High virulent– mortality 10-100% at 7-10 days after exposure; - Moderately virulent – Acute disease, a large percentage of pigs survive; - Weakly virulent – presents only seroconversion [5].

Epidemic cycle starts with direct contact with fomites people, vehicles, equipment and by feeding to the domestic pig with leftovers, unprocessed thermal garbage contaminated debris from pigs infected with ASF virus. Where ASF becomes endemic to doestic pigs, the virus is maintained by carrier pigs that are stamped out for preventing the spread of infection into other herds. At the necropsy there are notable enlarged spleen, bright red to black colour, friable and very enlarged and haemorrhagic gastro-enteral and renal lymph node. Diagnostic African Swine Fever should always be suspected when we have pigs with feverishness.

2. Materials and method

2.1 Materials

8 ml EDTA tubs and 6 ml vials containing no anticoagulant for whole blood (plasma and serum) were used to collect blood form the jugular vein form 15 pigs at the time they were stamped out in accordance to European and national law's regarding the welfare and care of animals, form a farm that was confirmed as an outbreak a day before the experiment was conducted by using Real Time PCR method, genome detection of African Swine Fever virus. The control group representing 5 pigs from another farm that were free of any known disease. To analyse the samples, we used a haematology analyser URIT-3000Plus and ELISA reader- Ledetect 96 Led Based & Channel Microplate Reader for detection of anti-virus antibodies African Swine Fever by immunoenzymatically technique (ELISA).

The first group of pigs analysed was represented by one saw, age 18 months and 14 young feeder pigs, age 6 months, accommodated in 4 separated pens, from a confirmed African Swine Fever outbreak.

The second group of 5 normal pigs all aged 9 months.

2.2. Methods

2.2.1.The anticoagulated blood samples were analysed within 6 hours of collection using an automated veterinary haematology analyser (URIT-3000Plus) manufacturer Urit Medical Electronics to determine total white blood cells (WBC), mean platelets volume MPV, red blood cells RBC, Haemoglobin concentration HGB, haematocrit (relative volume of erythrocytes) HCT, mean corpuscular volume MCV, mean corpuscular haemoglobin MCH, mean corpuscular haemoglobin concentration MCHC, red blood cells distribution width repeat precision RDW_CV. All the results were compared to calibrated URIT-3000Plus analyser references for Sus scrofa domestica.

2.2.2 The plasma and serum samples were analysed for detection of anti-virus antibodies African Swine Fever by immunoenzymatically technique (ELISA) using Kit ID VET and for the reading of the plates was used and ELISA reader- Ledetect 96 Led Based & Channel Microplate Reader.

Table 1. Comparison of blood parameters in group I represented by 3 pigs that were in critical con-dition presenting signs of fever and loss of appetite, from a confirmed African Swine Fever outbreak in Constanta County.

Parameters analysed[1]	Range	Saw	Pig 1	Pig 2
WBC count, x10^9/L	11.0 - 22.0	6.7	6.3	7.2
Platelets, x10^9/L	200 - 700	74	33	16
MPV, fL	6.0 – 12.0	7.6	7.4	7.4
RBC x10^12/L	5.00 – 9.50	1.86	5.24	5.87
HGB, g/Dl	9.9 – 16.5	2.8	7.4	10.5
HCT, %	32.0 – 50.0	10.8	30.9	42.0
MCV, fL	51.0 – 68.0	58.5	59.0	71.6
MCH, pg	17.0 – 22.0	15.0	14.1	17.8
MCHC, g/Dl	30.0 – 38.0	25.9	23.9	25.0
RDW_CV, %	14.0 – 19.0	16.1	13.7	13.8

Table 2. Comparison of blood parameters in group II represented 5 pigs that were reluctant to move, and the blood parameters are moderately affected from a confirmed African Swine Fever outbreak in Constanta County.

Parameters analysed[1]	Range	Pig 3	Pig 4	Pig 5	Pig 6	Pig 7
WBC count, x10^9/L	11.0 - 22.0	8.0	8.3	10.0	11.2	11.9
Platelets, x10^9/L	200 – 700	111	28	263	36	53
MPV, fL	6.0 – 12.0	6.7	8.0	8.6	7.0	7.6
RBC x10^12/L	5.00 – 9.50	6.48	5.68	5.29	5.33	5.54
HGB, g/Dl	9.9 – 16.5	9.3	7.8	7.8	7.6	8.0
HCT, %	32.0 – 50.0	38.2	32.2	31.7	30.7	33.3
MCV, fL	51.0 – 68.0	59.1	56.8	60.1	57.6	60.2
MCH, pg	17.0 – 22.0	14.3	13.7	14.7	14.2	14.4
MCHC, g/Dl	30.0 – 38.0	24.3	24.2	24.6	24.7	24.0
RDW_CV, %	14.0 – 19.0	14.4	15.0	13.4	14.8	14.1

Table 3. Comparison of blood parameters in group III represented 4 pigs were not showing any clini-cal signs instead the WBC count is over the normal range from a confirmed African Swine Fever outbreak in Constanta County.

Parameters analysed [1]	Range	Pig 11	Pig 12	Pig 13	Pig 14
WBC count, x10^9/L	11.0 - 22.0	21.9	23.6	23.6	22.8
Platelets, x10^9/L	200 - 700	191	37	57	52
MPV, fL	6.0 – 12.0	7.1	8.8	6.8	7.9
RBC x10^12/L	5.00 – 9.50	5.15	7.53	6.98	5.20
HGB, g/Dl	9.9 – 16.5	7.9	12.6	9.7	11.6
HCT, %	32.0 – 50.0	32.0	48.6	39.2	47.8
MCV, fL	51.0 – 68.0	62.3	64.6	56.3	62.9
MCH, pg	17.0 – 22.0	15.3	16.7	13.8	15.9
MCHC, g/Dl	30.0 – 38.0	24.6	25.9	24.7	24.1
RDW_CV, %	14.0 – 19.0	15.1	13.9	15.1	14.

Table 4. Comparison of blood parameters in group IV represented 3 pigs that were not showing any signs of disease and the WBC count are in the normal range from a confirmed African Swine Fever outbreak in Constanta County.

Parameters analysed [1]	Range	Pig 8	Pig 9	Pig 10
WBC count, x10^9/L	11.0 - 22.0	13.4	15.8	18.1
Platelets, x10^9/L	200 - 700	210	86	241
MPV, fL	6.0 – 12.0	7.8	8.1	7.6
RBC x10^12/L	5.00 – 9.50	6.69	6.95	6.87
HGB, g/Dl	9.9 – 16.5	9.4	11.2	9.9
HCT, %	32.0 – 50.0	38.9	44.3	40.4
MCV, fL	51.0 – 68.0	58.2	63.8	58.9
MCH, pg	17.0 – 22.0	14.0	16.1	14.4
MCHC, g/Dl	30.0 – 38.0	24.1	25.2	24.5
RDW_CV, %	14.0 – 19.0	15.4	16.2	15.2

3. Results and discussion

We investigate the changes in blood parameters of the 15 pigs confirmed with ASF using the Real Time PCR method that were divided in to IV groups according to the different viraemia stages.

We have conducted a haematology test where we gather results to show the different viremia stages of ASF by analysing the WBC count that rapidly increases staring with 0-1dpv (day post onset of

viraemia) and sharply decline at 2- 6 dpv (day post onset of viraemia) [6].

We have conducted the ELISA test on all 15 pigs. The results were negative. The reason we used ELISA test was to demonstrate that the pigs form the outbreak were in the viraemia stage and to exclude the possibility of passing more than 14 days of infection for the specific ASF antibodies to be detected.

Conclusions

The study conducted on blood samples showed a long infectious period to develop pathological processes that causes a dropped level of WBC and the fact that the examined isolates were not showing haemorrhagic syndrome (like cyanosis or haemorrhages of the skin and ears or bloody diarrhoea) were not observed during this experiment the RBC and MPV are marginally low or in normal range. For this reason, blood parameters such as RBC and MPV parameters are not reliable to diagnose the ASF at the viraemia stage, instead we can rely on WBC count. The viral infection between the host and the pathogen and the spared of ASF virus to other hosts it can be evaluated by looking at the Table 1; 2; 3; 4 compering the blood parameters to the progression of infection to the entire body followed by the replication and then shedding of ASF virus within secretion, excretion followed by transmission to other susceptible individuals.

Depending on how susceptible the host is and the virulence of the ASF virus we concluded that the blood parameters of the saw is showing a late stage of infection, probably at 12 -14th dpi, compared to feeder pigs where the blood parameters showed that they were at the initial stage of viraemia period.

Our results showed a significant correlation between the clinical signs and the haematological values of the affected pigs compared to the healthy pigs tested representant by group of animals that were not under known pathogen challenges showed that they had no significant changes of blood parameters.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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