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Study Of Hematological Parameters Of Pigs Raised In The Provinces Of Chari Baguirmi, Mayo Kébbi East And N'Djamena In Chad

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Abstract:
The objective of this study is to determine the hematological parameters of pigs raised in the provinces of Chari Baguirmi, Mayo Kébbi East and N'Djamena, of which 198 farmers were interviewed. A total of 346 blood samples were collected and analyzed by the URIT automaton. R Studio software was used to analyze the data. The Kruskal-Wallis test revealed that the means of Erythrocytes were significant (P = 0.02). The means of Polynuclear Neutrophils were significant in males and females (P = 0.03). Analysis of variance (ANOVA) revealed that the means of monocytes had an impact on the age of pigs (P = 0.05). Compared with the leukocyte lines, the sex of pigs had an influence on polynuclear neutrophils (P = 0.03). Both seasons (dry and rainy) had an impact on thrombocytes (P = 0.01). This study allowed knowing the average values of the blood parameters observed in pigs raised in the province of Mayo Kébbi East, Chari Baguirmi and N'Djamena in Chad. The averages of these hematological parameters obtained in pigs raised in the three provinces are either lower or higher than the values recommended by the manufacturer and in the literature. These blood parameters will constitute a way out to solve the problems of weight gain and the quality of pork produced.

Key Word*: Pig farming, Blood parameters, Chad.*

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I. Introduction

The pig is often preferred for its rapid development, its short gestation period of 3 and a half months and its rapid growth. Pig diseases are less common than those of other animals. In addition, the pig is omnivorous and its diet is easy and less expensive. This pig diet is based on the combination of different by-products of cereal processing such as bran, artisanal draff, alcohol residues and kitchen waste, throughout the year. It has the ability to convert average quality food into good quality proteins. Pig farming is often motivated by the producer's desire to carry out a project, to adopt a production diversification strategy to combat financial and food insecurity, or by a short-term alternative. Peasant farmers do not like to slaughter their animals. Because the latter prefer to sell pigs to buy a bicycle, cattle for animal-drawn cultivation, invest in agricultural equipment, including plows, hoe, seeds and the purchase of pots for the production of local beer by women [1-4]. Pig farming is unevenly distributed throughout the national territory. Because it is particularly developed in the provinces of Logone Oriental, Mayo-Kébbi East, Mayo-Kébbi West, Moyen Chari, Tandjilé, Mandoul, Logone Occidental and Chari-Baguirmi, N'Djamena, Hadjer-Lamis and Guéra. The North of Chad, which is heavily Muslim, does not practice pig farming for religious reasons [5; 6]. On the other hand, pigs play an essential role in various ceremonies (rituals, weddings, funerals, etc.). Slaughtered or live pigs are used to recruit labor for plowing, weeding, or harvesting cotton or cereals. However, a comparative analysis of the results of the general livestock census (RGE) from 2012 to 2015 reveals an exponential increase in pig numbers. Livestock numbers have increased by: 8 for cattle; 20 for sheep; 11 for goats; 12 for camels and donkeys; 5 for horses and 88 for pigs [3; 5]. In Chad, apart from African Swine Fever, other porcine pathologies of bacterial or parasitic origin are poorly described and data on pig blood parameters are non-existent. Knowledge of porcine blood parameters raised in local conditions is important to better understand clinical suspicions made in the

field by veterinary professionals. This study, conducted using blood samples taken from apparently healthy pig farms in the provinces of Chari Baguirmi, Mayo Kébbi East and N'Djamena, aims to take stock of the indicative parameters of the Blood Count and Formula (BCF) in pigs in order to deduce the impact of a probable influence of the two main climatic seasons in the country (dry and rainy seasons) and even other factors such as breed, sex and age. The results from this study will help guide veterinary laboratories on the suspicions made by veterinary services in the field.

II. Material And Methods

Study areas

This study was conducted in four areas distributed as follows:

First area

Rigaza sub-prefecture, located 50 km from the city of Bongor. The geographic coordinates of this area produced by Global Position System (GPS) are: 10°91'604" North latitude and 15°19'604" East-West longitude.

Second zone

It is in the city of Bongor and its surroundings, capital of the province of Mayo Kébbi East, located between 235 to 240 km from the political capital N'Djamena. The geographic coordinates produced by GPS : are 10°16'29" North latitude and 15°22'39" East longitude.

Third zone

City of N'Djamena, political capital of the Republic of Chad. The geographic coordinates produced by GPS are : 12°6'47" North latitude and 15°2'57" East longitude.

Fourth zone

The cities of Mandalia and Mailao are respectively sub-prefecture and prefecture in the province of Chari Baguirmi. The geographical coordinates of this zone carried out by GPS are: 11°43’37’’ latitude North and 15°14’52’’ longitude East for the prefecture of Mandalia; 8°31’0’’ latitude North and 15°46’0’’ longitude East for the sub-prefecture of Mailao.

These four zones correspond to pig farming areas and the most important in Chad and are represented in Figure 1.

Data collection technique

The collection of epidemiological data and samples was carried out from September 2021 to September 2022. Interviews were conducted with each farmer before taking the various samples. The blood sample of 2 to 4 mL per animal was taken by puncture of the saphenous vein using a Venoject,® Vacutainers® needle by a member of the team in a tube containing ethylenediaminetetraacetic acid (EDTA) from 5:30 to 10:00. For each sample, we recorded the geographical information (provinces, departments, sub-prefectures, prefecture, cities, cantons, districts, neighborhoods and villages), the socio-demographic characteristics of the animals (sex, ages, breed and origin of the animal) and extrinsic factors (season, type of breeding and soil types).

Laboratory sample analysis technique

Whole blood (2 to 4 mL) collected in the tube containing EDTA was used to determine the NFS. The latter was determined by a hematology machine branded "URIT-3000Vet-Plus" inside which was packaged four of the following manufactured reagents:

• **Lytic Reagent-URIT®** L 21 of batch number 632003434 and whose expiration date was set at 06/29/2023 and intended for URIT machines whose function is to measure blood cells;

• **Detergent-URIT®** of batch number 641905681; whose expiration date was set at 12/27/2023 with the function; to clean the compartments of the automaton involved in the reaction (composed of 1.5% Sodium Chloride, 0.1% anhydrous sodium sulfate, 0.1% sulfating agent);

• **Diluent-URIT®** of lot number 631903744; whose expiration date has been set at 16/12/2023; it contains sulfate chlorite, preservation solution, anticoagulant and a buffer solution,

• **Probe Cleaner-URIT®** of lot number 632003192; whose expiration date has been set at 17/06/2023; it is used to clean the pipes of the automaton after the end of the analyses and also for the daily maintenance of the device (contains 20% sodium hypochlorite).

With the machine powered on and switched on, wait five minutes for an automatic update of all the hematological parameters to be measured in the required intervals before starting the BCF analysis program which is done specifically by choosing the "Animal" parameter initially programmed by the manufacturer. After

five minutes, the porcine species must be chosen and then the sample contained in the EDTA tube must be mixed well so that the tip of the aspirator needle of the device can properly bathe in the blood contained in the EDTA tube to facilitate its aspiration. Once the sample is introduced, the trigger must be pressed to start the operation until you hear a beep signaling the end of aspiration. Then remove the sample and wait 3 to 7 minutes for the results to be displayed on the machine screen. The results of the parameters evaluated are displayed on the machine screen with reference values as well as the curves corresponding to the evaluation of each parameter present in the blood analyzed. For the present study, the expected results in terms of mean values concerned the following parameters:

• **Red blood cells**: Red blood cells (erythrocytes), Hemoglobin, Hematocrit, Mean Globular Volume (MGV), Mean Corpuscular Hemoglobin Tenor (MCHT), Mean corpuscular hemoglobin concentration (MCHC) and Reticulocytes,

• **White blood cells**: White blood cells, neutrophils, eosinophil, basophils, lymphocytes and monocytes;

• **Platelets** are evaluated in a single result.

Statistical Analysis

The collected data were entered into an Excel spreadsheet and then converted into CSV and exported into R Studio software version 4.0.4.2021 for statistical analysis.

In order to assess the effect of seasonality, race and sex on the investigated parameters and taking into account the non-normality of the sample distribution, the Wilcoxon or Kruskal Wallis tests were applied according to the observed trends. The significance level was set at P <5%. One-way analysis of variance (ANOVA) was used to compare the means of blood parameters between the different age groups.

III. Result

Repair according to the sample collection areas:

The census of pig farms was carried out in four areas and allowed to have the result presented in Table 1. A total of 492 pig farmers were interviewed among which 386 farmers had agreed to participate in the study and 106 had refused to participate in the study.

During the data collection and sampling, 188 registered farms no longer had animals among the 386 who had previously agreed to participate in the study. The collection of information and blood samples on live animals was actually carried out in 198 farms.

Table 1: Summary of the census of pig farms on the three study sites.

Study sites	Accepted farms	Rejected farms	Total
Rigaza sub-prefecture	76	36	112
Bongor and surrounding areas	200	28	228
N'Djamena	71	19	90
Mandalia and Mailao	39	23	62
Total	**386**	106	**492**

Samples were taken from 346 pigs aged 2 to 48 months at the farms targeted during the census, i.e. a ratio of 1.74 animals per farm. The distribution of animals according to breed, age group and sex is presented in Table 2. However, of the 346 animals surveyed, 61 were recorded in the dry season and 286 in the rainy season.

The sampled animals were divided into five age classes, all sexes combined. Animals aged 0 to 10 months are the most sampled, followed by those aged 11 to 20 months; then those aged 21 to 30 months. Depending on the breed, it was noted that the local breed was the most frequent with 219 animals recorded or a percentage of 63.29%, followed respectively by the improved breed with 78 animals or 22.54% and the hybrid breed which had 49 individuals or a percentage of 14.18%. In general, females were the most frequent in our study sample with a size of 279 animals or 80.63% of the study sample.

Evaluation of the effect of seasonality on blood parameters of pigs

The distribution of the sample representing the study population was not homogeneous, the Wilcoxon test was used. This test revealed the differences between the means of blood parameters such as lymphocytes, neutrophils, MCHC; MCHT and platelet count quantified in dry and rainy season in pigs were statistically significant at P < 5% (Table 2).

Table 2: Repair of the surveyed sample according to age classes, breed and sex.

Class Ages Total	**Distribution by breed of pigs**						**Total**
	Local		**Improved**		**Hybrid**		
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	
0 – 10 months	34	79	9	21	10	12	165
11 – 20 months	8	38	3	11	2	10	72

21 – 30 months	0	40	1	24	0	12	77
31 – 40 months	0	20	0	7	0	2	29
+ 40 months	0	0	0	2	0	1	3
Total	**42**	**177**	**13**	**65**	**12**	**37**	
	219		**78**		**49**		**346**
Distribution by season of pigs							
Blood parameter	**Dry**		**Rainy**		**P-value**		**Reference values**
Averages of parameters blood							
White Blood Cells	51589.59		47507.66		0.99		11-22. 10^3
Lymphocytes	32.21311		38.58596		0.004		4290-13640
Polynuclear Netrophils	2368.033		2780.972		0.002		28-47
Polynuclear Eosinophils	566.918		587.7333		0.23		55-2420
Monocytes	798.6885		789.0772		0.08		220-2200
Reticulocytes	3.508197		8.133333		0.11		0-1
Red blood cells	17125951		17529944		0.12		5-8.10^6
Hemoglobin	10.00164		10.85895		0.10		100-160
Hematocrit	33.42623		35.34386		0.10		32-50
MGV	48.72131		49.38947		0.49		50-68
MCHT	16.52459		18.23158		0.02		17-21
MCHC	29.03279		30.73684		0.02		30-34
IDR	14.29508		14.23158		0.94		11 - 15
Platelets	1346109		986133.8		0.01*****		3.25.10^5 -7.15.10^5

Distribution according to the effect of breed on blood parameters of pigs

Regarding the evaluation of the effect of breed on the variability of the blood parameters studied, the Kruskal-Wallis test revealed that at least one of the parameters does not have the same central tendency as the other parameters (Erythrocytes, P = 0.02769 (Table 3).

Table 3: Distribution according to the effect of breed on the blood parameters studied.

Breed of pigs				**P-value**	**Reference values**
Blood parameter	**Local**	**Improved**	**Hybrid**		
	Blood parameter means				
White Blood Cells	48809.77	47512.74	46602.73	0.85	11-22. 10^3
Lymphocytes	36.71111	37.04167	41.53061	0.35	4290-13640
Polynuclear Netrophils	2771.276	2645.181	2510.959	0.69	28-47
Polynuclear Eosinophils	508.466	650.8056	833.1224	0.24	55-2420
Monocytes	858.2444	636.9028	707.0408	0.38	220-2200
Reticulocytes	7.404444	9.569444	3.612245	0.16	0-1
Red blood cells	16070944	18629989	22110111	0.027	5-8.10^6
Hemoglobin	10.54622	10.7	11.46122	0.21	100-160
Hematocrit	34.64	34.52778	37.38776	0.31	32-50
MGV	49.41778	49.15278	48.77551	0.83	50-68
MCHT	18.19556	17.4583	17.40816	0.73	17-21
MCHC	30.34667	30.02778	31.44898	0.57	30-34
IDR	14.25333	14.25	14.18367	0.99	11 - 15
Platelets	1195895	862793.2	652311	0.08	325 000-715 000

Distribution according to the effect of sex on blood parameters of pigs

According to the effect of sex on the blood parameters studied, the Wilcoxon test was performed. The means of Polymorphonuclear Neutrophils were significant in males and females (P = 0.03) but the differences in the means observed between males and females were not statistically significant for the other parameters studied (P > 5%) (Table 4).

Table 4: Effect of sex on blood parameters of pigs.

Pig sex			**P-value**	**Reference values**
Paramètre sanguin	**Male**	**Female**		
	Blood parameter			
White Blood Cells	52179.25	47278.28	0.24	11-22. 10^6
Lymphocytes	35.34328	37.97133	0.30	4290-13640
Polynuclear Netrophils	2400.388	2782.082	0.03	28-47
Polynuclear Eosinophils	562	589.362	0.34	55-2420
Monocytes	449.9254	872.6237	0.81	220-2200
Reticulocytes	8.895522	6.939068	0.08	0-1
Red blood cells	19396299	16993423	0.87	5-8.10^6
Hemoglobin	10.36716	10.78961	0.46	100-160
Hematocrit	33.9403	35.26165	0.36	32-50
MGV	49.53731	49.20789	0.73	50-68

MCHT	17.98507	17.91756	0.65	17-21
MCHC	30.22388	30.48746	0.56	30-34
IDR	14.95522	14.07168	0.13	11 - 15
Platelets	1459045	951271.4	0.49	325 000-715 000

Evaluation of the effect of age on blood parameters of pigs

According to the age groups, the analysis of variance (ANOVA) was used to know if the age of the pigs would have an impact on the different blood parameters of the animals. This analysis of variance (ANOVA) had revealed a significant mean of monocyte (P = 0.05) but on the other hand for the other blood parameters considered ANOVA had revealed no significant difference (P > 5%) (Table 5).

Table 5: Studies of blood parameters according to age.

Parameters Blood	**Age group: in months**					
	0 -10 months	**11-20** months	**21-30** months	**31-40** months	**+ 40** months	**P-value**
White Blood Cells	51006.39	50364.03	37250.57	59830.69	13666.67	0.29
Lymphocytes	38.25455	35.875	39.11688	31.24138	49.66667	0.51
Polynuclear Netrophils	2652.455	2634.181	2742.429	3179.276	2115	0.43
Polynuclear Eosinophils	635.3273	727.0972	434.2078	319.7931	732.6667	0.06
Monocytes	635.3273	727.0972	434.2078	398.7241	1208.333	0.05
Reticulocytes	6.490909	13.80556	4.25974	4.413793	3.666667	0.64
Red blood cells	15819867	20847267	19515530	12042759	25833333	0.69
Hemoglobin	10.62788	10.8125	11.12208	9.806897	10.66667	0.91
Hematocrit	34.47273	34.95833	37.06494	33.03448	31.66667	0.66
MGV	49.29091	50.33333	48.38961	48.2069	55.66667	0.73
MCHT	17.86061	18.59722	17.54545	17.7931	17	0.74
MCHC	30.26667	31.52778	30.97403	27.2069	31	0.36
IDR	1233624	982183.9	633626.5	1317189	635957.7	0.14

IV. Discussion

In this study 492 farmers were interviewed, 386 agreed to participate in the present study with a high participation in the prefecture of Bongor with 200 breeders or a rate of 51.81%, followed respectively by the sub-prefecture of Rigaza with 76 breeders with a threshold of 19.71%, the city of N'Djamena with 71 breeders or a proportion of 18.41% and in the prefecture of Mandélia and sub-prefecture of Mailao with 39 pig breeders or a rate of 10.10%. This result shows a significant participation of breeders in the province of Mayo Kébbi East represented by the city of Bongor and the sub-prefecture of Rigaza as well as in the city of N'Djamena. These areas represent a high concentration area of pig farmers in Chad and reflect the importance of pig farming at these sites directly bordering the far north of Cameroon due to the high demand for pork from Cameroon [7].

The averages of the blood parameters obtained according to the two main seasons (dry and rainy) highlighted the significant differences in red blood cells as regards the MCHC which is 29.03 g/100ml in the dry season and 30.73 g/100ml in the rainy season. On the other hand, the average of the MCHT is 16.52pg in the dry season and 18.23pg in the rainy season. These parameters, also called erythrocyte index, both represent the amount of hemoglobin contained in the red blood cell and are obtained by making the ratio between Hemoglobin and Hematocrit. The decrease in MCHC and MCHT generally induces normocytic anemia. The two relatively low values in the dry season as indicated by the results of the present study, show a decrease in the amount of hemoglobin thus reflecting anemia in the animals due to lack of food during the dry season. These low values of the means of MCHC and MCHT could well be the direct consequences of the high number of piglets (165 / 346) observed in our study sample. According to [8] the piglets will suffer from mammary anemia due to the considerable decrease in the quantity of milk produced by the sows in the dry season due to the simple fact that the piglets are born with limited iron reserves, while the colostrum and the sow's milk are relatively poor in iron. These assertions will be able to support the results obtained in this study [8]. The average hemoglobin values recorded in the dry and rainy seasons are respectively 10.00 g/L and 10.85 g/L even though the calibrated interval for the device is set between 100 g/L and 160 g/L. The average hemoglobin values are lower than the norm. Two reasons can explain the low rate in this study. First, the animals live in stray and are malnourished and underfed. Second, the pig farmers interviewed say they do not have sufficient means to buy food supplements such as amino acids, minerals and vitamins for their animals. According to the age groups, the analysis of variance reveals a significant impact of age on the average monocytes. These results can be explained by the fact that the majority of pigs included in this study lived in stray so it is possible that these animals were infected by bacteria, parasites or a virus. In case of parasitic, bacterial and viral infection, monocytes are the first blood cells to intervene. For [9], monocytes are blood cells with a pro-inflammatory tendency, with a high capacity to synthesize pro-inflammatory cytokines after stimulation by a

lipopolysaccharide (notably TNF-α and IL-1) and the possibility of presenting antigens to T lymphocytes. Monocytes are able to synthesize an anti-inflammatory cytokine, IL-10. They have a greater expression of CD14 compared to CD163+ [9].

The mean values of erythrocytes according to the three pig breeds are significant (P = 0.02). Two reasons could explain these results. First, hybrid and improved pig breeds do not live in straying. These two breeds live in piggeries built of durable materials and these animals are raised in piggeries that meet hygiene standards. Even the floor of the piggery is made of concrete. Secondly, hybrid and improved breed animals benefit from a controlled and balanced diet. But on the other hand, local breed animals live in wandering and only find food during the rainy season when there is an abundance of plant-based foods and water. This is certainly what could explain these results.

Compared to leukocyte lines, the sex of pigs has a significant influence on neutrophil polynuclear cells (P = 0.03). This significance is due to the simple fact that local breed pigs live in stray and the hybrid breed is either in confinement or on stakes and are often exposed to microorganisms (parasites, bacteria, fungi and viruses). This exposure to microorganisms involves neutrophil polynuclear cells which are phagocytic cells. As phagocytic cells, these neutrophils play a vital role in the host defense mechanisms (pigs). In this study, the two seasons (dry and rainy) had a significant impact only on thrombocytes (P = 0.01) but not on other blood parameters. Generally, the increase in platelets is due to a reaction or condition related to bleeding, iron deficiency, infection or inflammatory disease. In the case of this present study, iron deficiency is due to the scarcity of food or the unavailability of the food ration in the dry season coupled with the after-effects of microbial infections contracted in the rainy seasons. This is certainly what could be at the origin of the increase in thrombocytes in pigs in this study.

V. Conclusion

This study made it possible to know the average values of the blood parameters observed in pigs raised in the provinces of Mayo Kébbi East, Chari Baguirmi and N'Djamena in Chad. The averages of these hematological parameters obtained in pigs raised in the three provinces are either lower or higher than the values recommended by the manufacturer and in the literature. The different averages of the blood parameters had an impact on the socio-demographic characteristics of the pigs. Erythrocytes have an impact on the breed, neutrophils and monocytes have an impact on the sex of animals. These results also represent a milestone in the field of hematology and provide the scientific community with reliable and usable data allowing them to improve pig breeds. Knowledge of these hematological parameters will contribute to improving pig farming in these three provinces. These blood parameters will be a way out to solve the problems of weight gain and the quality of meat produced.

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Conflicts Of Interest

All authors declare no conflict of interest related to this study.

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