Comparative analysis of hematological parameters and blood compatibility in different bird species

Andreea BUTA[1], Laurenț OGNEAN[1*], Alexia HAVRET[2], Zsofia DARADICS[1], Octavia TAMAS-KRUMPE[1], Sandra SPĂTARIU[1], Cristina ȘTEFĂNUȚ[1]

[1]Physiology Department, University of Agricultural Science and Veterinary Medicine,Romania
[1]Mănăştur Street, no.3-5, Cluj-Napoca, Romania
[2]Lorrainvet, 127 ruePierre Gilles de Genes 54710 Ludres
***** Corresponding author, e-mail: lognean@yahoo.com

Abstract

Birds seem to survive and recover much faster than mammals after acute blood loss. Their ability to tolerate severe bleeding may be due to the lack of automatic responses to irreversible shocks, faster transfer of extracellular fluids into the vascular space and faster mobilization (at 12 hours) of a large number of immature erythrocytes after significant blood loss; for example, pigeons return to normal hematocrit only 7 days after 60% of the blood has been drawn, without any clear clinical signs. In this study our objectives were the comparative analysis of hematological parameters in samples from domestic birds of different species and the investigation of the possible compatibility between the blood of chickens and palmipeds. The investigations were carried out between October 2018 - May 2019 in the Physiology Laboratory of the Faculty of Veterinary Medicine, CLUJ-NAPOCA, on blood samples collected from a batch of 23 birds, composed of: 9 chickens, 5 turkeys, 4 ducks, 2 geese, 1 quail, 1 pheasant, 1 pigeon.. Blood samples were taken from the basilic vein of each bird and collected on an EDTA tube. Comparative hematological analyzes included the determination of the erythrogram parameters (total number of erythrocytes, hematocrit, hemoglobin concentration and mean erythrocyte constants) and leukogram parameters (total number of leukocytes and proportions of leukocyte subpopulations). In order to evaluate and analyze the intra- and interspecific blood compatibility, 110 Crossmatch reactions were performed, 55 for the major test and 55 for the minor test.. The hematological results, interpreted comparatively on batches and species, showed significant variations at both individual and species level. We also noticed a major inconsistency between the values of the mean erythrocyte constants and the main hematological parameters. The results regarding the evolution of intra- and interspecific blood compatibility revealed the absence of preformed isoantibodies against the various antigens of the blood group systems of the tested species. In conclusion, 14.54% positivity proportion outlined the level of the incompatibility rate for heterologous combinations.

Keywords: *birds, hematology, blood compatibility.*

Introduction

Blood groups are the result of gene expression by the presence of certain substances on the surface of erythrocytes. These substances are called erythrocyte antigens.

The existence of different erythrocyte antigens in chickens was discovered in 1924 by Landsteiner and Miller (Walford et al., 1964).

In 1930 and 1931 Todd performed isoimmunizations among the chickens in his study and obtained such a multitude of antibodies that he concluded that every chicken had a different erythrocyte antigen except close relatives (Walford et al., 1964). In addition, he proved that an individual had the same erythrocyte antigen as at least one of his parents, which means that this antigen is inherited as a dominant trait.

The first two blood group systems in chickens were discovered in 1948 by Briles, McGibbon and Irwin and were referred to as group A and B in 1950. Two other blood group systems referred to as C and D were discovered by Briles and Quinsenberry and 1951. Finally a fifth blood group system, E, was discovered by Briles (1958); C. Briles, McGibbon and Irwin (1959) (Walford et al., 1964). Independently D. G. Gilmour discovered in 1959 the systems of groups A, B, C and E but also two other systems which he calls L and N (Walford et al., 1964; Schiermann and Nordskog, 1965).

Although the majority of the 47 domestic turkeys (*Meleagris gallopavo*) were refractory to the production of detectable isoagglutinins, the isoimmunization experiment of these 47 specimens produced 18 blood typing reagents that define individual differences in the antigenic structure of turkey RBC. Corresponding to the reactions obtained with these reagents, the antigenic factors A (subtypes A1 and A2), B (B1, B2 and B3), C (C1 and C2), D, E, F (F1 and F2), G, H. I, J, K and L have been named (Law et al., 1964).

Homologous transfusions (between individuals of the same species) are strongly recommended in birds (Lichtenberger Marla, 2004). If homologous transfusion is not possible, heterologous transfusion (between individuals of different species) should be taken into consideration. In both cases, minor and major compatibility tests must be performed. Since birds do not have pre-formed antibodies against blood groups, the first heterologous transfusion is usually safe. (Morrisey, 1999; Marla Lichtenberger, 2004; Matos and Morrisey, 2005). However, it has been shown that the mean half-life of donor erythrocytes is reduced by at least half in heterologous transfusions compared to homologous transfusions. For this reason it is more beneficial that the heterologous transfusion is performed between two individuals of related species (same Genus) or as a last resort between two individuals of the same Order (*Psittaciformes, Falconiformes, Columbiformes,* etc.) (Martinho, 2012).

Materials and methods

The research was carried out in the period October 2018 - May 2019 in the Animal Physiology laboratory within the Faculty of Veterinary Medicine, CLUJ-NAPOCA. The main objectives of our study were the comparative analysis of haematological parameters of different species of domestic birds, investigation of blood compatibility in the population of studied birds and establishment of the proportion of positivity in the blood compatibility test of the sample analyzed.

To conduct the investigations of our study, we collected blood from a total of 23 different specimens (n=23). The percentage distribution of the different species illustrates that the most representative species in terms of the number of subjects is the domestic hen *(Gallus domesticus)* with a percentage of 40%, which represents 9 individuals out of 23. In contrast, the pheasant (*Phasianus colchicus),* the pigeon *(Columba livia)* and the domestic quail (*Numida meleagris)* each represent only 4%; that is, one subject out of the 23 totals. The origin of the birds was different and the study was carried out in four distinct stages (Table 1). The birds introduced in this study were divided into different categories according to their species (Table 1).

Table 1.

The species and number of birds introduced into the study

Stage	Total number of specimens	Number of specimens per species	Species
1	**5**	**5**	*Gallus domesticus* (domestic hen)
2	**6**	**2**	*Meleagris gallopavo* (turkey)
		2	*Anas platyrhynchos domesticus* (duck)
		2	*Gallus domesticus* (domestic hen)
3	**7**	**3**	*Meleagris gallopavo* (turkey)
		1	*Anas platyrhynchos domesticus* (duck)
		2	*Anser cygnoides* (goose)
		1	*Gallus Domesticus* (domestic hen)

4	**5**	**1**	*Numida meleagris* (domestic quail)
		1	*Columba livia* (pigeon)
		1	*Anas platyrhynchos domesticus* (duck)
		1	*Phasianus colchicus* (pheasant)
		1	*Gallus Domesticus* (domestic hen)

Haematological examinations. For all birds introduced into our study, blood samples were collected by venipuncture from the basilic vein on an EDTA (approximately 2 ml of blood for each bird).

Determination of hematocrit (Ht). Hematocrit represents the volume, expressed as a percentage, occupied by circulating red blood cells in circulating whole blood. It is a ratio between the total volume of RBCs and the total blood volume. In birds, HT is estimated using the microhematocrit method (Ghergariu et al., 2000; Ognean and Cernea, 2006; 2011). An HT between 35% and 55% is considered physiological in most adult birds. However, an interpretation of HT should be made in comparison with the physiological norms of the species and the breed. An increase in HT may be absolute with a cellular origin or relative with a plasma origin. A decrease in HT reflects a state of anemia.

Hemoglobin dosage (Hb). This determination is difficult due to the presence of the RBC nucleus in the case of blood samples from birds (Hawkey and Samour, 1988; Samour, 2006; Campbell et al., 2007). We used the semi-automatic spectrophotometric method (Ognean and Cernea, 2011). In most vertebrates, hemoglobin is made up of four subunits each with an oxygen binding site. However, there are significant differences between the Hb of birds and that of other vertebrates. There are two types of Hb in adult birds, Hb A and Hb D. Hb A is most prevalent in bird populations and its affinity for oxygen is lower than the Hb D. This lower affinity allows easier dissociation of oxygen.

Determination of the total number of red blood cells (RBC) and leukocytes (WBC). The count of RBC in s shows some differences from the count of RBC in mammals. Prochaska-modified Natt-Herrik dilution fluid is used, which protects all the figured blood elements (erythrocytes, leukocytes, platelets), being considered the standard method (Ognean and Cernea, 2011; Pierson, 2000; Campbell et al., 2007).

The determination of the erythrocyte constants was based on the use of known in the field calculation formulas (Ghergariu et al., 2000; Samour, 2006; Ognean and Cernea, 2011), these indices being important for birds, especially for the detection of nutritional origin stress.

Leukogram determination. Coloring of bird blood smears is based on the use of most Romanovsky-type stains used in mammalian smears (Wright, Gimsa, Wright-Gimsa, Leishman, Wright-Leishman, May-Grunnwald, May-Gundwald-Gimsa, DiaPanoptic etc.). For fastness reasons, in this study we used DiaQuick Panoptic staining, based on the use of 2 dyes (acidophilic and basophilic) and a fixative containing absolute methyl alcohol (Campbell, 1994).

Cross matching: cross compatibility test. This is a method of ensuring blood compatibility between the donor and the recipient by quantitatively detecting the serum level of antibodies against erythrocyte antigens. It is a reliable, fast and inexpensive method that can detect incompatibility and thus prevent transfusion accidents.

*The major cross-compatibility test***.** Major cross-compatibility represents the compatibility between the donor's red blood cell concentrate (RBC) with the serum or plasma of the recipient patient. It therefore assesses the effect of the recipient's serum antibodies on the donor's erythrocytes. The major cross-match test detects the presence of antibodies in the recipient's plasma

that could cause a hemolytic reaction upon transfusion of the donor's RBC. The major cross-compatibility test therefore consists of combining the donor RBC and the recipient's plasma (1: 4).

The minor cross-compatibility test. Minor cross-compatibility is the reverse of major cross-compatibility; it represents the compatibility between the recipient's red blood cells and the donor's plasma. It therefore assesses the effect of the donor's serum antibodies on the recipient's cells. This test is very important in the event that the donor has been previously transfused as anti-red blood cell antibodies from the recipient may be present in the donor's plasma. The minor cross-compatibility test therefore consists of combining the donor's plasma with the recipient's RBC (1: 4).

	Major compatibility test	**Minor compatibility test**
Material	• RBC from the donor • Plasma of the recipient	• GR of the recipient • Plasma of donor
Working technique	• On the slide, we put 3 µL of RBC + 12 µL of plasma • We waited 1 minute • We examined macroscopically for the absence or presence of agglutination	
Results and Interpretation	• Agglutination or hemolysis is considered a positive reaction and demonstrates blood incompatibility (Figure 1). • The absence of agglutination or hemolysis is considered a negative reaction and demonstrates blood count (Figure 1).	

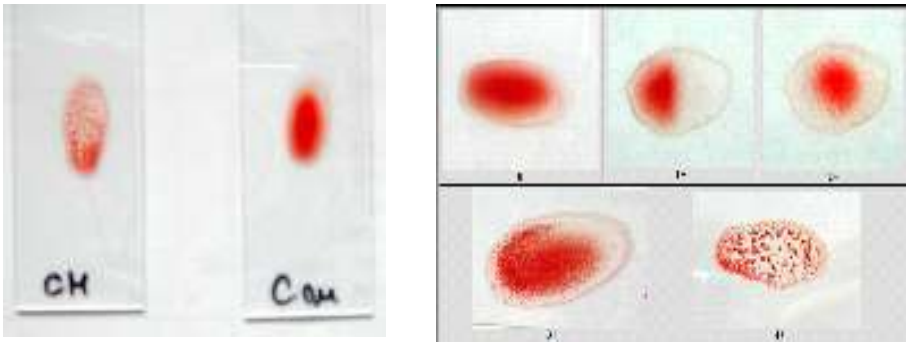


Fig. 1. Cross-match: Macroscopic aspects (Ognean and Cernea, 2011)

The verification pattern for the cross-match test was different depending on the stage of our study.

For step 1, we examined the blood compatibility between the 5 hens (Table 2). For this check, we made 20 combinations as follows: 10 combinations for major compatibility and 10 combinations for minor compatibility.

Table 2.
Cross reactions embodiment of the step 1

		Recipients				
	Identification	**1**	**2**	**3**	**4**	**5**
Donors	**1**		X	X	X	X

	2			X	X	X
	3				X	X
	4					X
	5					

For step 2, we examined the blood compatibility between the two turkeys, between the two turkeys and the two ducks, between turkey 1 and the two hens (Table 3). For this assay, we made 14 combinations as follows: 7 combinations for major compatibility and 7 combinations for minor compatibility. The amount of blood that we collected from all the individuals was insufficient; thus we could not verify the compatibility between turkey 2 and the two hens, duck 1 & 2 and the two hens and also between the two hens.

Table 3.

Cross reactions embodiment of the step 2

		Recipients					
	Identification	**Turkey 1**	**Turkey 2**	**Duck 1**	**Duck 2**	**Hen 1**	**Hen 2**
Donors	**Turkey 1**		x	x	x	x	x
	Turkey 2			x	x		
	Duck 1						
	Duck 2						
	Hen 1						
	Hen 2						

For step 3, we investigated the blood compatibility between the two turkeys, between the two turkeys and the two ducks and between turkey 1 and the two hens (Table 4).

For this check, we made 36 combinations as follows: 18 combinations for major compatibility and 18 combinations for minor compatibility. For step 3, we did not check the compatibility between the two geese, between goose 1 and the hen and finally between the goose 2 and the hen.

Table 4.

Cross reactions embodiment of the step 3

		Recipients						
	Identification	**Turkey1**	**Turkey2**	**Turkey3**	**Goose1 (m)**	**Goose 1 (f)**	**Duck**	**Hen**
Donors	**Turkey 1**		X	X	X	X		X
	Turkey 2			X	X	X		X
	Turkey 3				X	X		X
	Goose 1 (m)							
	Goose 1 (f)							
	Duck	X	X	X	X	X		X
	Hen							

*For step 4***,** we investigated all possible combinations of blood compatibility between quail, pigeon, duck, pheasant and hen (Table 5). For this study, we made 40 combinations as follows: 20 combinations for major compatibility and 20 combinations for minor compatibility.

Table 5.
Cross reactions embodiment of the step 4

		Recipients				
	Identification	**Quail**	**Pigeon**	**Duck**	**Pheasant**	**Hen**
Donors	Quail		X	X	X	X
	Pigeon	X		X	X	X
	Duck	X	X		X	X
	Pheasant	X	X	X		X
	Hen	X	X	X	X	

Within our study, the total number of combinations that we made between the individuals in our study is 55, which means we performed a total of 110 cross-match reactions (major compatibility and minor compatibility).

Results and discutions

Comparative analysis of haematological parameters During our study we observed significant variations in HT between our birds. Stage 1 hens have an Ht that falls within the physiological values for the species; while that of the hens in step 2 is lower than normal. The HT for all stage 2 birds is below standard and has much lower values than for the other two stages. Within step 3, there are also significant variations between the HT of gallinaceae and that of palmipeds.

Comparative analysis of Hb (g / dl) reveals values within broadly similar margins for all gallinaceans among themselves and for all palmipeds among themselves. The palmipeds introduced in our study nevertheless have higher Hb values than those of the gallinacea. The Hb values of all the hens in the study are physiological. For all the ducks in our study, Hb had values above the physiological upper limit.

The total RBC count (10^{12} / L) of stage 1 birds was found to be significantly higher overall than that of stage 2 and stage 3 birds. Goose 1 from stage 3 was the individual with the lowest total RBC count in our entire study, while hen 1 from stage 1 is the individual with the lowest total RBC count. The total RBC count of all palmipeds in our study was below physiological norms and was found to be lower than that of the gallinaceae in our study; With the exception of turkey 1 from step 2. Hens 1 and 5 from step 1 are the only gallinacea with a total number of RBCs within physiological standards. Finally, it appears that overall all the individuals in our study have a total number of RBCs lower than normal.

The comparative analysis of MCV (fl) shows large variations within stage 3 birds. Indeed, step 3 illustrates that palmipeds have a significantly higher MCV than gallinacea.

Stage 3 birds, except Turkey 1 and Turkey 2, all have higher than normal MCV. On the other hand, the differences in MCV values are less marked between gallinacea and palmipeds for stage 2. turkey 1 from stage 3 is the gallinaceous with the highest MCV while the duck from stage 3 is the palmiped with the highest MCV. The MCH (pg) values of almost all the birds introduced in our study are above the physiological values of the species. Only hen 1 from stage 1 and all turkeys in our study had physiological MCH values. Male goose and all ducks have significantly higher MCH values than other specimens. Stage 2 turkeys 1 and 2 had a higher MCH than all the hens in our study. With the exception of turkey 1 and turkey 3 from step 3, all MCHC (g / L) values were found to be greater than physiological values. The comparative analysis of MCHC values shows much higher values for stage 2 birds than for stage 1 and 3 birds; these values being clearly higher than the physiological values. In human literature (Healthline Media UK Ltd, Brighton, UK.), elevated MCHC is often associated with conditions where Hb is more concentrated in red

blood cells or with conditions where red cells are more fragile. This increased fragility of the red blood cells leads to vascular hemolysis with dissemination of the Hb outside the RBCs (Reavill and Joseph, 2002).

The comparative analysis of total leukocytes reveals very varied values between the different stages of our study but also between different individuals and species. Overall all the individuals presented leukocytosis with the exception of the hen from stage 3. We observed an excessively higher level of leukocytes than the other individuals for duck 1 from stage 2 and for turkey 1 from stage 3. Stage 1 hens have a physiological heterophile percentage, as do the geese from stage 3. Thus there is no significantly different distribution in heterophile percentage between gallinacea and palmipeds. The percentage of heterophiles of the birds in stage 2 is generally within the same range of values and reveals an overall heterophilia of the group. Analysis of the percentage of eosinophil reveals values that are well above the standards for stage 2 ducks. By comparing the values of step 1 (normal) with those of geese in step 3 we notice that there is no difference between the standards of gallinacea and those of palmipeds. In our study, we observed that only one basophil in all of our 23 blood smears. The comparative analysis of the lymphocyte level reveals very low values for all the individuals from stage 2 but also for the geese from stage 3. Stage 1 hens and stage 3 chickens show similar variations. The greatest difference is observed for the values of ducks between those of step 1 and that of step 3. Analysis of the percentage of monocyte revealed that all of the individuals introduced into our study presented monocytosis. However, compared to other individuals, hen 2 from stage 1 and ducks from stage 2 show a more moderate increase.

Analysis of blood compatibility. The cross-compatibilities reactions performed in step 1 between the hens at the university did not show any agglutination and therefore were all found to be negative.

Recipient hens do not have pre-formed circulating antibodies directed against RBCs of donor hens and the donors do not have pre-formed circulating antibodies against RBCs of recipient hens. In other words, the chickens at our university all exhibit blood compatibility with each other.

The combinations made within step 2 showed the highest percentage of positive reactions each in a major cross match. Of the 7 combinations investigated, 5 came out positive (positivity of 71.42%).

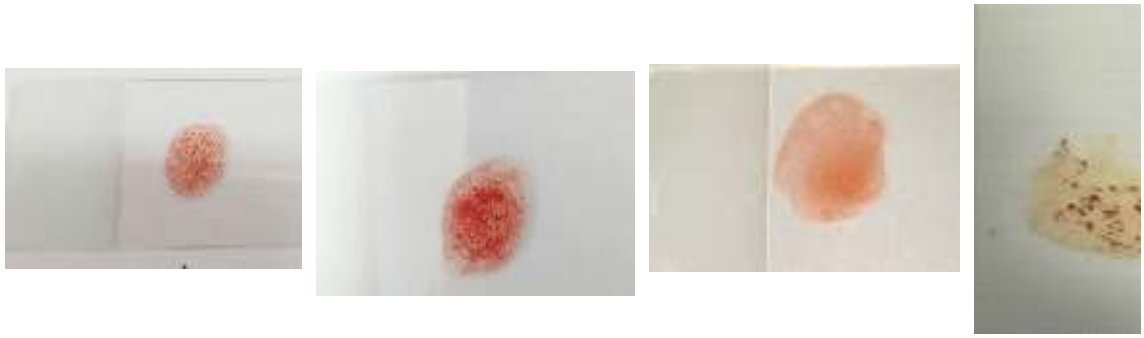
When combining turkey 1 / duck 2, we obtained a positive reaction with moderate agglutination during the major cross match. This shows the existence of anti-turkey RBC antibodies in the circulating blood of the duck. In contrast, the absence of agglutination during the minor cross-match demonstrates the absence of serum antibodies in turkey plasma to duck RBC. When combining turkey 1 / hens (1 and 2), we obtained a positive reaction with strong agglutination during the major cross match. This shows the existence in the circulating blood of the hens of anti-RBC antibodies in turkeys. In contrast, the absence of agglutination during the minor cross-match demonstrates the absence of serum antibodies in the turkey plasma directed against the RBC of the hens. When combining turkey 2 / ducks (1 and 2), we obtained a positive reaction with moderate agglutination during the major cross match. This shows the existence in the circulating blood of ducks of anti-turkey RBC antibodies. In contrast, the absence of agglutination during the minor cross match demonstrates the absence of serum antibodies in turkey plasma directed against duck RBC.

The combinations made in step 3 revealed two positive reactions. Of the 18 combinations investigated, 2 combinations came out positive in the major cross match (positivity of 11.11%). When combining turkey 1 / hen, we obtained a positive reaction with moderate agglutination during the major cross match. This shows the existence in the circulating blood of the hen of anti-turkey

RBC antibodies. In contrast, the absence of agglutination during the minor cross-match demonstrates the absence of serum antibodies in the turkey plasma directed against the RBC of the hen. In the duck / hen combination, we obtained a positive reaction with strong agglutination during the major cross match. This shows the existence in the circulating blood of the hen of anti-duck RBC antibodies. In contrast, the absence of agglutination during the minor cross-match demonstrates the absence of serum antibodies in the duck plasma directed against the RBC of the hen.

The combinations made in step 4 revealed a positive reaction (with agglutination) during a major cross-match. This represents a positivity of 5% out of the 20 combinations investigated. In the quail / pigeon combination we got a positive reaction with very strong clumping during the major cross-match. This shows the existence in the circulating blood of the pigeon of anti-quail RBC antibodies. In contrast, the lack of agglutination during the minor cross match demonstrates the absence of serum antibodies in the quail plasma to the pigeon's RBC.

At the level of all the combinations performed in our study (n = 55), we had only 8 positive combinations (14.54%).We observed that all the positive reactions took place during the major cross- match but with different agglutination intensity (Figure 2).



A **B** **C** **D**

Fig. 2 - Different agglutination intensity: A – turkey – hen; B – duck – hen; C- turkey – duck; D – quail - pigeon

Conclusions

1. The comparative analysis of haematological parameters revealed variations for each parameter; both individually and in terms of belonging to a phyllogenetic group (ducks showed the highest values for erythrocyte parameters overall).
2. Despite erythrocyte and leukocyte parameters generally outside physiological ranges, clinical examination of each animal did not suggest anything abnormal. Thus, the birds appear clinically resistant and the clinical manifestation of the hematological changes therefore implies extremely reduced and / or increased values.
3. The homologous combinations made between the hens at our university (step 1) did not reveal any incompatibility; just like the homologous combinations of turkeys (stages 2 and 3). It can thus be assumed that individuals of the same species do not have performed antibodies to antigens of

different blood groups of that species. In other words, regardless of their belonging to a blood group system, individuals of the same species should not be incompatible within the first transfusion.
4. There is no positive correlation between blood compatibility and belonging to the same order (here *Gallinaceae* and *palmipeds*). Indeed, we obtained compatibility between turkeys and geese; while the turkey / duck, turkey / hen, and duck / hen combinations have demonstrated varying degrees of incompatibility.

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