JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(4): 386-393, December 25, 2023

Research Paper, PII: S2322455X2300041-13
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DOI: https://dx.doi.org/10.36380/jwpr.2023.41

Cytology of Turkey Blood–Reactive Hemograms and Measures of Stress

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Received: 02 October 2023
Accepted: 26 November 2023

ABSTRACT

The current study was intended to offer a cytological counterbalance to published descriptions of how processing or other procedures affect turkey welfare. Cytology represents a detailed description of morphological atypia or unusual intracellular or intercellular behavior. The study aimed to describe the variation of blood cells of commercial turkeys. Blood films were collected from 4 turkeys at four different ages of 6, 12, 17, and 19 weeks at commercial farms by a qualified veterinarian. The slides, stained by Wright-Giemsa, were photographed and interpreted off-site. Normal cells of the lymphocyte (L) and heterophil (H) series were described first, followed by examples of atypical cells of other series. These were shown with descriptions of cellularity defined as the proportion of leukocytes in each microscopic field. The results indicated examples of cells whose presence in a standard differential count (SDC) was important enough to disqualify the simple H/L ratio as a stress measure. These cells were atypical members of the lymphoid series, plasmacytes, and other cell types. Atypical granulocytes were heterophils with irregular shapes and faint nuclear staining (hypochromia). An example of a representative total white count revealed how the H/L value could depend on where the cells were counted on the slide. In conclusion, the cytology clearly shows that the presence of atypical cells in a hemogram highlights the inadequacy of relying solely on the simple H/L ratio to estimate stress status.

Keywords: Atypical cytology, Heterophil/Lymphocyte ratio, Stress, Turkey

INTRODUCTION

The information on how production procedures and other stressors affect turkeys is limited to a few studies. For instance, Scanes et al. (2020) described the effects of shackling and transportation on both the heterophil/lymphocyte (H/L) ratio and blood cortisone. The H/L ranged from 0.25 (non-stress) to 3.25 (high-stress). Information on the blood cell morphology of turkeys is also limited, lacking detailed descriptions of atypical cells. The aim of the study was to describe some of the atypical blood cells of commercial turkeys. The study was intended to offer a cytological counterbalance to published descriptions of how processing or other procedures affect turkey welfare. The cells should provide diagnostic information on inflammation and blood infections on farms.

MATERIALS AND METHOD

Animal welfare

Animal welfare and ethical issues were followed according to standard commercial production procedures under the authority of a PAACO-certified auditor and licensed veterinarian and approved by Cotter Laboratory (Approval Number 1945-015).

Blood collection and preparation of slides

Four blood samples from each farm were collected meticulously and sterilely from the ulnar vein by a licensed veterinarian at four different farm sites. Then, 1 mL of blood was drawn aseptically into a sterile 3 cc syringe. Immediately after, touch preparation smears were made by spreading one drop from the syringe (~ 3 µL) over a clean glass microscope slide. The slide was

dried in warm air. This method was used to avoid the effects of shipping and storage on hemogram quality and possible untoward effects on cytology by exposure to heparin or ethylenediaminetetraacetic acid (EDTA) anticoagulants (Campbell and Ellis, 2007). Slides were then sent to Cotter Laboratory, Arlington, MA, USA, for staining and analysis.

Turkeys

All samples in the current study came from male turkeys, commonly referred to as Toms. All were from commercial stocks, but two out of four were raised on standard conventional conditions, and two were raised on antibiotic-free (AB) facilities. They ranged in age from 6 to 19 weeks. The samples included in this manuscript were from two conventional and two antibiotic-free farms. To achieve this, blood films obtained from 4 turkeys each from flocks at 6, 12, 17, and 19 weeks of age were made directly on commercial farms by a qualified veterinarian. They were selected because of unusual cytology.

Light microscopy and photomicrographs

A light microscope (Olympus CX-41, Olympus, America) equipped with Plan N 40×, 0.65 numerical aperture dry, and Plan N, 1.25 numerical aperture 100× oil objectives was used. All images were captured at 100× with an Infinity-2 1.4-megapixel charge-coupled device Universal Serial Bus 2.0 Camera and were processed with Infinity Analyze software (Release 6.5; Lumenera, Inc., Ottawa, ON, Canada).

Staining

The staining process in this study was by an in-house procedure using Wright's method followed by brief exposure to Giemsa (W-G, Sigma Chemicals, St. Louis, Mo. USA, Procedure WSGD-128) done at Cotter Laboratory, Arlington MA, USA. A minimum of two counts of ~200 cells each were made at 40x magnification by sweeping across areas not thicker than monolayers. Edge-based counts were also performed when accumulations of reactive cells were noticed at low (20x) magnification. Photomicrographs of representative cells were at 100x (oil) magnification. All counts were performed by the author.

Total white blood counts

Total white blood counts (TWBC) were estimated from the standard differential counts (SDC) by applying the method of Campbell and Ellis (2007).

Heterophil lymphocyte ratios (H/L 1, H/L2, f(H/L)

H/L 1 = (HC + HT + HV) / Ls; H/L 2 = (HC + HT + HV) / (Ls + Lm); ΔH/L= H/L 1 − H/L 2; is given as real numbers. The field H/L f(H/L) is approximated by dividing the number of heterophils in a microscopic field by the number of lymphocytes and is given as a fraction.

Cellularity

Under normal quiescent (homeostatic) conditions, the composition of blood cells in mature turkeys follows specific parameters. Leukocytes (white blood cells) should make up approximately 1% of the total white blood cell count (TWBC) of mature turkeys (McGuire and Cavett, 1952; Lucas and Jamroz, 1961; Venkataratnam and Clarkson, 1962). In a thin microscopic field having space for 1-200 cells, 1 or 2 leukocytes can be expected to be found dispersed among erythrocytes (RBC). Most RBCs should be mature, fully hemoglobinized cells. Mature RBCs were recognized by their orange-yellow cytoplasm. Leukocytes should be predominated by small (D_C ~ 6 µm) lymphocytes (Ls). Thrombocytes (Th) can occupy field space, but these should not show signs of reactivity. Reactive and/or atypical cells and atypical behavior indicated that stress or frank pathology had already occurred. For the present purposes, field cellularity was given as leukocytes/number of RBC x 100. As a simplification, "cellularity" functioned as a cameo image of the entire hemogram.

Normal cells

Normal cells were defined as those not displaying atypical signs or those not participating in a behavior recognized as "reactive" or pathological. Clumping (leukergy), autophagocytosis, and zeiosis were considered examples of pathological behavior. Size was measured using both cell diameter (D_C) and nucleus diameter (D_N) to compute the respective areas (A_C and A_N). Nuclear cytoplasmic ratios (N/C) were computed by division (of A_N by A_C). In blood sampled during homeostasis, the cells of all series should display an appropriate Romanowsky-Giemsa effect or RGE; otherwise, they are considered reactive or pathologic (Wittekind, 1979).

RESULTS

Representative "normal" cells of commercial turkeys of given ages were illustrated first, followed by atypical cells. Atypia was recognized by abnormalities of cytology, staining characteristics (RGE), or behavior. The

hypothesis was that when atypical cells appeared in the SDC, their presence would alert the investigator to the likelihood that the simple H/L value would be disqualified as a stress determinator. Simply stated, the question of stress was rendered moot. The focus was on cells potentially entering the H/L calculus directly as a component of the numerator (heterophil/granulocyte series) or the denominator (lymphoid series). In other instances, atypical cells not used in the H/L computation were included since they were of sufficient importance to show that a complex hemogram exists. Reactive basophils and plasmacytes were examples of such cells.

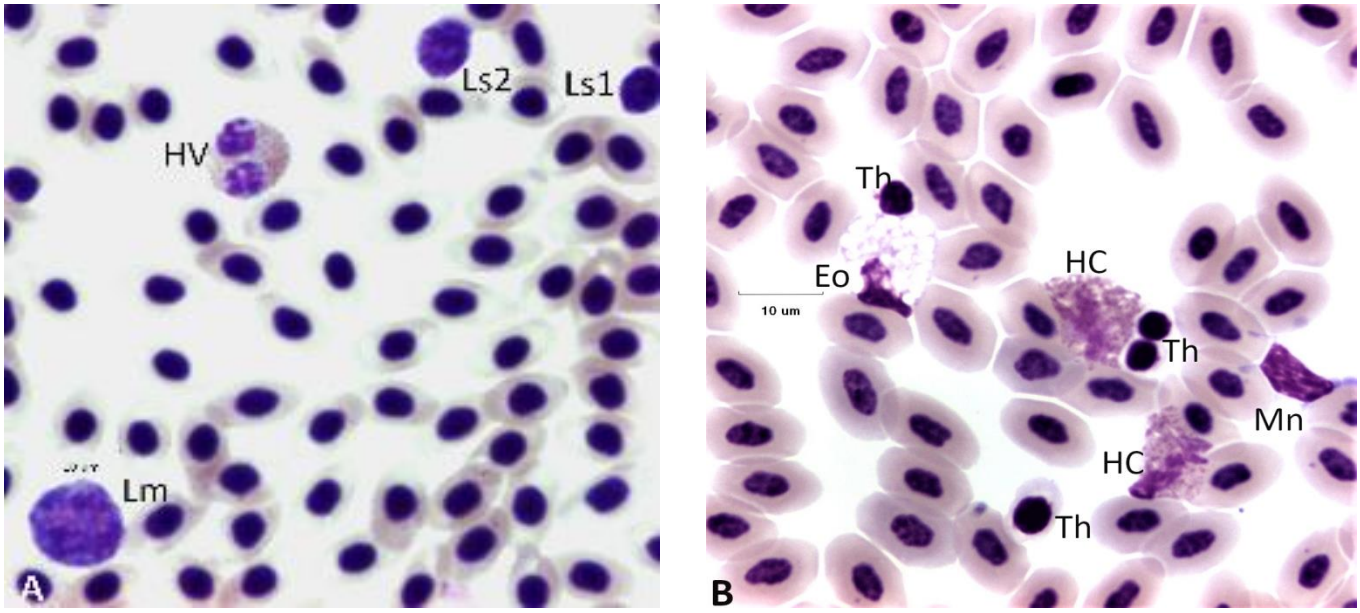

Figure 1. A: Normal cells of a 6-week Tom raised under standard conditions (HV) variant heterophil; Ls1 and 2 small resting lymphocytes, Lm medium/large lymphocyte. **B**: Reactive cells of a 19-week Tom raised on an antibiotic-free farm. Eo: Eosinophil, Th: thrombocyte; HC: Classic heterophil, Mn: Monocyte.

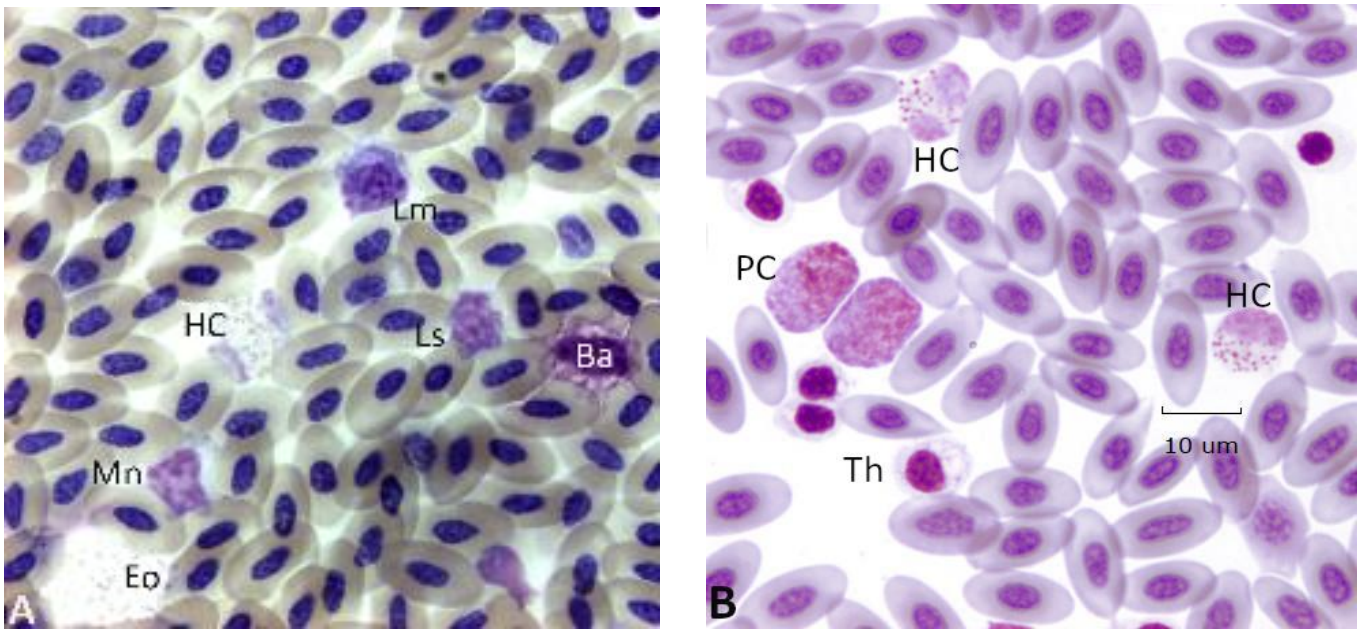

Figure 2. Cytology examples of a Tom turkey at 19-weeks of age raised on an antibiotic-free farm. **A:** A high cellularity (~6% WBC) field with small (Ls) and medium lymphocytes (Lm) a monocyte (Mn) a faint (hypochromic) heterophil (HC) a basophil (Ba) and an eosinophil (Eo). Background RBCs are unremarkable. **B**: Paired atypical plasmacytes (PC) with some Mott cell vacuole features. Reactive (swollen) thrombocytes (Th) and classic heterophils (HC) are also in the field.

Examples from a 6-week-old male (Tom) raised in a conventional facility are presented in Figure 1. Panel A. (100x) had four normal cells located in a single field (cellularity 7%; f(H/L) ~ 1/3): Ls1 and Ls2 were small "resting" lymphocytes (D_C Ls1, 6.8 µm; D_C Ls2, 8 µm; N/C's ~1), which were likely T-cells; and Lm (D_C 13.7 µm) was likely a B-cell. A variant heterophil (HV), sometimes called by mistake an eosinophil (D_C 11.6 µm) was standard-sized (A_C ~100 µm^2). Its orange cytoplasmic granules were spherical and smaller than those of turkey eosinophils, which often stained weakly with W-G alone. Figure 1B shows an (Eo) whose cytoplasmic granules are barely stained by W-G, normal for a turkey Eo, and is attached to a small "reactive" (Th). Classic heterophils were attached to Th, or RBCs were irregularly shaped. A resting Th was at the bottom middle of the field, and a monocyte (Mn), whose cytoplasm projected filopodia from a stretched nucleus (zeiosis), was in the middle right. Approximately 1/3 of the RBCs in this field were late polychromatic types (pRBC) clustered in the bottom hemisphere. Figure 1B (100x) shows two forms of atypia. First were HCs displaying distorted shapes, and an Mn whose cytoplasm was reduced to filopodia projecting from the nucleus, and an Eo whose cytoplasmic granules lack staining was normal and not an artifact. The behavioral issue of Th attachment to HCs and the Eo was superimposed on the atypical cytology. Moreover, the aggregation of pRBC was remarkable.

Examples from a 19-week-old male (Tom) raised in an AB-free facility are in Figure 2. Panel A (100x) had a high cellularity (~6% WBC) field with 2 two lymphocytes, and the topmost was large enough to be a B-cell (A_C 61 µm^2; N/C 0.53). The Lm may have transitioned into a plasmacyte as its cytoplasm appeared secretory. An Mn, an HC with a faintly stained nucleus (hypochromia), a reactive Ba, and an Eo with normal (weakly stained) cytoplasmic granules were on the lower left. The background RBCs were mature cells (full Hb) and otherwise unremarkable. Figure 2B (100x). An opposing pair of primitive (developmental) plasmacytes with Mott cytoplasmic features (Russell bodies, RB) were found (Cotter and Bakst, 2017). A polyploid (Th) was recognized by its ~2x nuclear size, and it also had a swollen cytoplasmic vacuole. Two classic heterophils (HC) were also present. All were in the blood of a 17-week-old Tom raised on an AB-free farm.

Figure 3 shows examples from a 17-week Tom raised in a conventional facility (100x). A field containing a normal small lymphocyte (Ls, D_C 6.2 µm, A_C ~ 30 µm^2; N/C ~ 1), a giant Mott plasmacyte (*Lm, D_C 11.6 µm, A_C ~ 106 µm^2; N/C ~ 0.4) and a thrombocyte were found. The cytoplasm of the plasmacyte was fenestrated by small vacuoles, Russell bodies (RB), with diameters < 0.5 µm. Some vacuoles displayed a faint pink tinge, and others were clear. The pink RB likely contained defective IgA, allowing this cell to be subcategorized as a "flame cell." A few Dutcher bodies could be seen in the nucleus (RB equivalents). The combination of pink and clear vacuoles suggests that this cell produces at least two (defective) Ig isotypes. Although plasmacytes are rare in normal circulation, a solitary cell, in the absence of other atypia, is insufficient for disqualification. However, not only was this plasmacyte a giant cell, but it appeared to be a very rare multiple isotype secretor. Defective plasmacytes (Mott cells) were more frequent under stress. The lysed RBC nuclear remnants and an encapsulated diplococcus (arrow) should not be left without notice. Additional examples of atypia from the same turkey are in Figure 3, Panel B. A large PC was accompanied by a giant amitotic RBC at the separation stage. After division, each of the daughter RBCs would be of unequal size. The smaller member of the pair will become a "pyrenocyte." A resting lymphocyte (Ls) was also in the field. Bacteria were scattered throughout the field, and a nuclear and the presence of a remnant added to evidence of the toxic environment.

Examples of cells from a second 19-week-old male, Tom, raised in an AB-free farm, can be seen in Figure 4 (100x). Panel A. A highly cellular field (cellularity, 8%) contained a reactive dendritic-type basophil (Ba), a pair of monocytes (Mn) whose large nuclei were at least tetraploid. Both Mn radiated pseudopods that extended to nearby RBCs. A thrombocyte, attached to the central Mn, was at an early reactive stage and partially phagocytized by the Mn. An HC and a Th were on a background of mature RBCs. Cell-associated bacteria (CAB) were located by arrows (Panel B). An atypical PC had an irregular cell membrane, and its cytoplasm differentiated into blue ectoplasm (external) and clear endoplasm regions. A (toxic) RBC was in the process of separation into an (anuclear) erythroplastid (ep) and a nucleated pyrenocyte (py).

A field displaying multiple atypia from a 12-week-old Tom is depicted in Figure 5. The PC, a large plasmacytoid cell (A_C ~222 µm^2; [perimeter] P_C ~ 53 µm; N/C ~ 0.58), had characteristic features. The eccentric nucleus with condensed chromatin was arranged as a cart-wheel, and a nucleolus was seen at 11 o'clock. The light

blue cytoplasm was fenestrated by many small diameter (~ 0.3 µm) clear vacuoles. A small cytoplasmic bleb was located at 12 o'clock and was likely to be shed as an apoptotic body. The large nuclei of RBCs 1 and 3 (A_N 44 μm^2 and 38 μm^2, respectively) were likely polyploid; the cytoplasm displayed an RGE appropriate for mature RBCs. These contrast with the faintly stained (diploid) nuclei of cell two types. The cytoplasm of RBCs 1 and 3 were not fully hemoglobinized. A giant (A_C 380 μm^2, P_C 69 µm) atypical cell (G) at the right has both granulocytic and histiocytic features. A nucleus was not obvious, and the cytoplasm contained a central pair of deep blue granules, not likely the products of phagocytosis. The remnants of a phagocytosed thrombocyte containing a phagocytosed bacterium are attached to (G) at 1 o'clock. This cell displayed a type of "second-order" phagocytosis, a term first coined to describe reactive bone marrow cells of lame ducklings. Panel B. An aseptate fungal hyphae, possibly a *Mucor sp*., had formed a coil with a pair of Th and RBCs, it was found in the same sample as in panel A.

To conserve space, a single representative standard differential count for 6 weeks. Tom was raised on a conventional farm as indicated in Table 1. Four individual counts of ~200 cells were used to compute values for the TWBC and the H/L statistics. Three of four cell counts were done in the mid (monolayer) regions of the slide (counts 1-3) and another in an area thinly populated with cells (count 4).

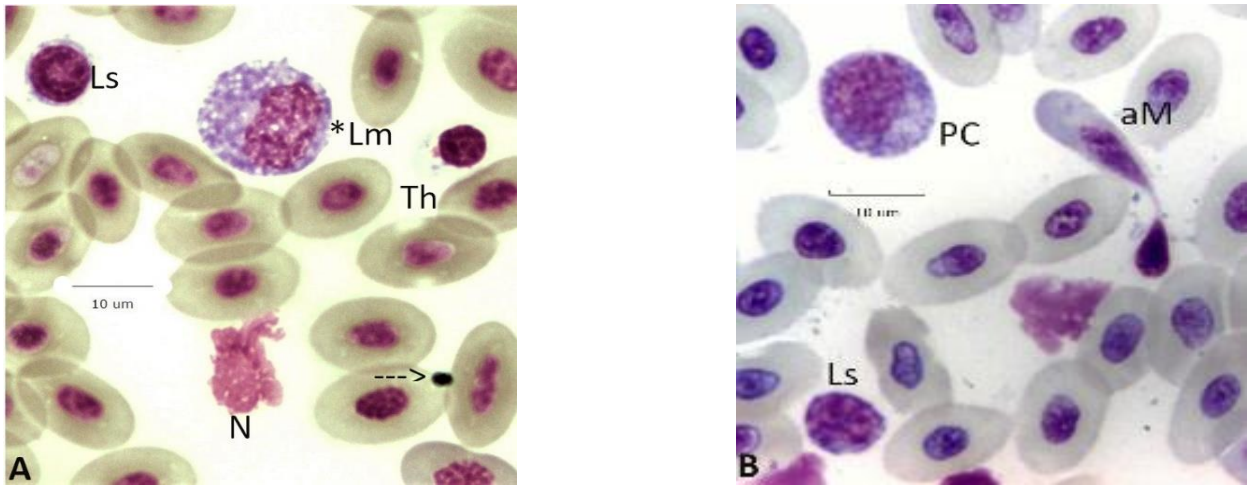

Figure 3. Reactive blood cells of a 17-week Tom raised at a conventional site located in Zeeland Mi, USA. **A:** Normal small lymphocyte (Ls) Mott plasmacyte (*Lm) and nuclear remnants of a lysed RBC (N) a thrombocyte (Th) and an encapsulated diplococcus is located by the arrow. **B:** Large plasmacyte (PC), a small lymphocyte (Ls), and a giant (tetraploid) amitotic RBC (aM) at the separation stage.

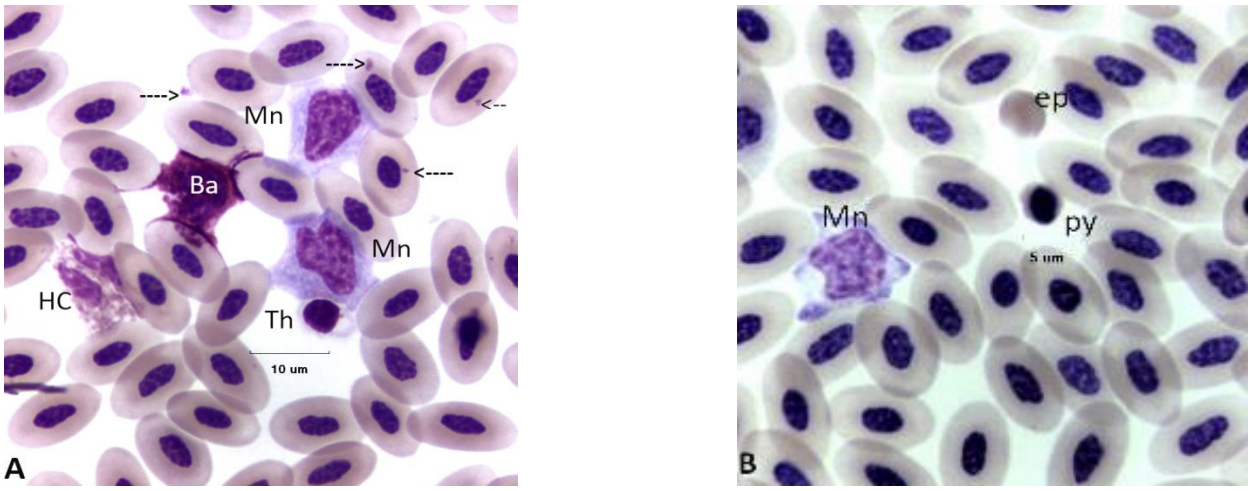

Figure 4. Cytology examples from a Tom turkey at 19 weeks of age raised on an antibiotic-free farm site located near Zeeland, MI, USA. **A:** The field contains a dendritic basophil (Ba), a pair of monocytes (Mn1 and Mn2), a toxic heterophil (*HC), and a partially phagocytized thrombocyte (Th). The background cells are mature RBCs. Surface bacteria are located by arrows. **B:** A large reactive monocyte (Mn), and an erythroplastid/pyrenocyte pair (ep/py) are nearby.

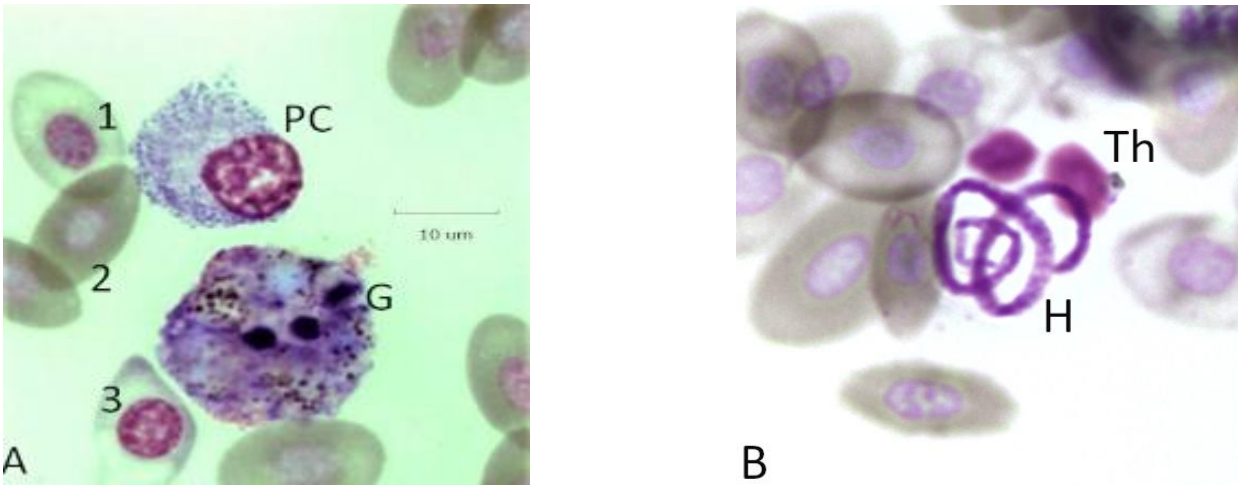

Figure 5. A: A field displaying multiple atypia from a 12-week Tom raised on a conventional farm, near Zeeland MI, USA. PC is a very large cell (A_C ~222 μm^2; P_C ~ 53µm; N/C ~ 0.58) with plasmacytoid features. The eccentric nucleus has condensed chromatin arranged as a cart-wheel and a nucleolus is seen at 11 o'clock. The light blue cytoplasm is fenestrated by fine vacuoles. The nuclei of RBCs 1 and 3 (A_N 44 and 38 μm^2, respectively) are polyploid, and RBC2 is diploid (A_N ~ 20 μm^2). A giant atypical cell (G; A_C 380 μm^2, P_C 69 µm) at the right has both granulocytic and histiocytic features. **B:** An aseptate fungal hyphae (H), possibly a *Mucor* sp. has formed a coil with a pair of thrombocytes (Th) and RBCs; all were found in the same sample as in Figure 1A.

Table 1. A representative standard differential count (%) and H/L 1, H/L 2 ratios based on four individual 2 × 200 cell counts from 3 mid-slide areas (with the average) and one thin slide area of a 6-week Tom raised on a conventional farm.

Ct	Area	HT	HV	HC	Ls	Lm	NK	Bst	Mn	Ba	Eo	TWBC (K)	H/L 1	H/L 2
1	Mid	75	0	0	79	39	0	0	0	10	0	50	0.95	0.64
2	Mid	75	0	0	62	46	0	3	1	18	2	50	1.21	0.69
3	Mid	76	0	0	62	46	0	3	1	18	2	50	1.23	0.7
Ave Ct 1-3 (%)	Mid	36.5	0.0	0.0	32.7	21.1	0.0	1.0	0.3	7.4	0.6	50	1.1	0.7
4	Thin	26.4	0.0	0.0	51.2	14.4	0.0	0.0	1.0	7.0	0.0	15	0.51	0.4

H: Heterophil (HC, classic, HV, variant, HT, typical), Ls: Small lymphocyte ~6 µm diameter, Lm: Medium, & Large, lymphocyte (diameter 8-10 µm), NK: Natural killer, Bst: Granulocyte blast, Mn: Monocyte, Ba: Basophil, Eo: Eosinophil, TWBC(K): Total white blood cells per cubic mL in thousands (K). H/L 1 = (HC + HT + HV) / Ls; H/L 2 = (HC + HT + HV) / (Ls + Lm); Average of Mid Counts 1-3 in (%)

DISCUSSION

The purpose of this manuscript was two-fold. First, it was designed to supplement scarce information on turkey hematology and (abnormal) cytology. Second, it draws attention to the theory and practice of using an SDC derivative statistic, the H/L alone, as a stress measure. The H/Ls are often used without reference to TWBCs or descriptions of abnormal cells. This aspect has been partially addressed by some of the observations already made on chickens, ducks, and turkeys (Cotter, 2023; Davis et al., 2008). Atypia described in some of those studies parallel the examples described here. Starting with examples of "normal" cells (Figure 1), the question of lymphocyte category is raised. How many Lm's (presumptive B-cells) can be used in the denominator before a hemogram is declared reactive? If a reactive hemogram exists, is there any merit remaining in a derivative statistic, the simple H/L? How many cells (TWBC) are allowed before leukocytosis or a leukemoid reaction is declared? The opposite also applies. The question of hemograms with too few white cells, panleukopenia, sorely needs to be addressed. In this regard, Girish et al. (2008) indicated turkeys (Commercial Strain H) exposed to *Fusarium* mycotoxin experienced a decline in TWBC during an 8-week experimental period. Examination of their data suggests that both the Fusarium exposed and control animals were suffering from panleukopenia as their TWBCs were less than 1/3 of turkeys' normal levels. Is this peculiar to Commercial

Strain H turkeys, or was there some systematic error in hematological procedures?

Additional examples of apparent leukopenia are in dos Santos Schmidt et al. (2009) and Azeez et al. (2011), who described highly unusual hemograms in Bronze and Nigerian turkeys, respectively. In both studies, normal hematocrits of ~ 40% were reported. This was accompanied by total RBC counts of ~ 1-2 x 10^6/µL, values well below what would be expected to support the reported hematocrits. A similar situation was reported by Chowdhury et al. (2005), indicating normal values for hemoglobin and hematocrit in control 4-week-old Commercial Strain H turkeys, coupled with a total RBC value ~ 1.5 x 10^6/µL, a value half of what is needed to support the reported hematocrit and hemoglobin values. None of these publications includes a single photo of cells. This lack of cell detail also extends to Scanes et al. (2020). It is clear from these examples that the available literature on the hematology of the turkey has insufficient cytologic detail.

One egregious example of poor hematology appears in Huff et al. (2010), where H/L ratios calculated using the data in Table 1 turn out negative.

The author acknowledges some limitations in the current study. The most important was the lack of consensus on how an H/L ratio should be computed. A partial solution was to recognize that atypical heterophils and lymphocytes should not be included in the computations. Finding these cells indicates the existence of a complex hemogram and implies stress exists. Moreover, heterophils were not a single series, a fact easily discerned by examination of figures available in Lucas and Jamroz (1961). A previous study indicated that there is as much variation in the turkey and duck heterophils as for chickens (Cotter, 2021). The same statements are true for lymphocytes, particularly members of the plasmacyte series.

Davison et al. (1983) used the granulocyte/lymphocyte (G/H) ratio as the stress measure in its original concept. This means basophils and developmental cells might have been included. An excess of basophils (basophiliosis) has been described in chickens. Basophiliosis may be a direct indication of bacteremia/fungemia. How could such a hemogram not indicate that microbiological stress is already in progress?

If stress activates the adrenal cortical axis and a demand is placed on the supply of heterophils, the new recruits to the circulating population should be "band" types (young cells), a circumstance rarely considered. If the circulating population is of the "right-shift" (aged) type (Lucas and Jamroz, 1961), then this circumstance alone challenges the utility of the H/L (Cotter, 2021). Lastly, many of the cells described here were found in the blood of turkeys whose ages are close to those described by Scanes et al. (2020). The turkeys of this study were raised either on conventional or antibiotic-free farms. Therefore, at least some of the atypical cells described here might be expected to have been in the samples described by Scanes et al. (2020) and presumably were ignored. Thus, their reported H/L values may be weak proxies for the true blood pictures of their turkeys.

CONCLUSION

In conclusion, the present observations add important information not widely available elsewhere concerning the cytological variation of blood cells in the turkey. They supply crucial observations missing in most publications on this species. Atypia complicates the interpretation of the simple H/L ratio as a stress measure. In the author's opinion, there is no good reason not to provide photos of representative cells and include the TWBC, along with the derivative H/L, in experiments on questions of stress in turkeys. This should be true where stress is either of direct interest or is included as a part of future studies of the physiology of this species.

DECLARATION

Acknowledgments

The author is indebted to D. Pyle, DVM, Zeeland, MI, 49464, USA, who provided the samples. The author also acknowledges the contributions of reviewers of an earlier version of this manuscript who suggested improvements.

Funding

This study received no funding from public or non-profit organizations.

Ethical consideration

The sole author has ensured that the research adheres to ethical principles such as avoiding plagiarism, obtaining consent to publish, avoiding misconduct, preventing data fabrication or falsification, refraining from double publication or submission, and avoiding redundancy.

Competing interests

The author declares that there is no competing interest with any financial organization regarding the materials discussed in the manuscript.

Availability of data and materials

The data for this study are carefully selected examples from a library of ~400 photomicrographs of turkey blood smears.

Authors' contributions

Paul F. Cotter, the sole author, conceived, wrote, and revised the manuscript.

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