AtypicaL hemograms of the commercial duck

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ABSTRACT A description of standard and atypical heterophils, lymphocytes, and 2 types of giant cells found in the circulation of 17 wk commercial ducks (N = 24) in apparent good health is the subject. Heterophils were sorted as either "classic" (HC) having red rod-shaped cytoplasmic granules, "typical" (HT) having weakly stained granules providing a reticular cytoplasmic appearance, or rarely as "variant" types (HV) having orange spherical granules. Atypical HT's and HC's were in 14 of 24 (58%) of the ducks.

Small lymphocytes (**Ls**), reactive lymphocytes and plasmacytes (**Lm**) were routinely found. Giant cells, also present, were placed with Lm or monocytes (**Mn**) depending on cytology. Two counts of 200 leukocytes gave the total white count (**TWBC**) and 2 heterophil/-lymphocyte ratios. **H/L 1** = (HT + HC +HV) / Ls; and **H/L 2** = (HT + HC + HV) / (Ls + Lm). The results showed that TWBC were normal (~ 23,000 /μL) but both H/L ratios were highly variable. HT were differentiated from HC on nuclear and cytoplasmic criteria. Many HT and HC exhibited signs of deterioration. Some giant cells likely represented developmental stages. Multiple nucleoli were evident in others suggesting polyploidy. The more common lymphoid giants were usually round whereas monocyte types were irregular. Mn types were actively phagocytic often consuming thrombocytes or rarely erythrocytes (RBC). Giant cells of either type were in 13 of 24 (54%) of the duck hemograms.

Conidiospores were detected in the blood smears of 4 ducks and bacteria in 2 with 1 duck having both.

As all ducks were in apparent good health the blood born microorganisms likely represented low grade infections. Presumably the atypical cells were a response to the presence of toxins of bacterial and fungal origin.

The presence of atypical heterophils and lymphocytes complicates interpretation of H/L ratios traditionally used to establish stress. As atypical cells can be found in the context of normal TWBC or nonstress H/L values cytological observations attain additional importance. Moreover, giant cells may be useful indicators of infection even without direct microscopic observation or isolation of the offending organisms.

Key words: duck, heterophil, lymphoid giant cell, monocytoid giant cell, hemogram

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INTRODUCTION

The hemogram or leukocyte profile is a useful tool for determining both health and stress status of avian species. Duck leukocytes, like other poultry, consist of granulocytes (heterophils, basophils, eosinophils) lymphocytes (**Lm**) and monocytes. A few descriptions of standard types are published (Hewitt, 1942, Hemm and Carlton, 1967; Tadjalli et al., 1996). The Atlas of Avian Hematology by Lucas and Jamroz (1961) includes some illustrations of duck heterophils and eosinophils. However, both he and Hewitt (1942) drew attention to existence of heterophil variants and the difficulty of differentiating heterophils from eosinophils. In chickens and ducks the cytoplasmic granules of both cells are similar, but eosinophils are scarce in these species. In contrast, turkey eosinophil granules do not usually stain with Romanowsky dyes and so are easily differentiated from heterophils. They are also more common in turkey blood (Cotter, 2018).

Among lymphocytes the small (resting, **Ls**) type occurs most often in the blood of chickens, ducks, and turkeys. Larger reactive Lm some with azurophilic cytoplasmic granules, and occasional plasmacytes may also be present. Examples of circulating plasmacytes and Mott cells (atypical plasmacytes) of chickens are given in Cotter (2015c). Examples of bone marrow plasmacytes and Mott cells of ducks are in Cotter and Bakst (2017). Circulating atypia of ducks is not described by Campbell and Ellis (2007) nor do they appear in Schalm's Veterinary Hematology 6th ed (Weiss and Wardrop, 2010). Atypical cells of all classes were detected in commercial hens (Cotter, 2015a,b) and specific pathogen free chicks (Cotter and Heller, 2016)

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whose H/L ratios were at nonstress levels with normal TWBC. Ducks were not included in either of those reports.

The purpose of the present study is to provide examples of standard and atypical heterophils and lymphocytes of commercial ducks and 2 types of giant cells. Heterophils and lymphocytes are emphasized because of the use of H/L to measure stress and determine welfare. Atypical cells as described here, called "sentinels" (Cotter, 2017a,b) are presumed to indicate a response to injury. To the author's knowledge, atypical duck cells as described are rarely seen in published literature.

MATERIALS AND METHODS

Ducks

A white Pekin meat duck commercial pure line flock, at 17 wk of age (N = 24), was grown in sex separate group housing with a controlled growth rate in preparation for entrance into the breeding phase at 20 wk. Duck welfare was monitored under the Maple Leaf Farms Trident Stewardship Program for Duck Well Being and procedures were reviewed by a PAACO certified auditor and licensed Veterinarian.

Blood and Staining

Whole blood (1−3 mL) was drawn from a leg vein into EDTA tubes. To avoid effects of storage monolayer films were made within 24 h of collection. ~3 μL blood was spread across the length of alcohol cleaned glass microscope slides and dried immediately with a hot air stream. Slides were immersed in 95% ethanol and postfixed for 10 to 15 min. Films were stained by Wright's method followed by a brief exposure to Giemsa following times and procedures recommended by the manufacturer (Sigma Chemicals, St. Louis, MO., Procedure WSGD-128) and used by Hewitt (1942). The DIFF-QUIK stain method was also used.

Standard Differential Count

Two counts of 200 leukocytes/slide (24 samples) were sorted using criteria as described by Lucas and Jamroz (1961) and Cotter (2015a). The designation "typical heterophil" (**HT**) as used here was assigned to the most frequent type seen in earlier studies (Cotter, 2015a,b,c). Classic heterophils (**HC**) resemble those most often illustrated in the literature. Rare variant heterophils (**HV**) are distinct from both HT and HV, Cotter and Heller (2016). Atypical lymphocytes were included in the "medium/large lymphocyte" group; giant cells were assigned to either the Lm or Mn group (Cotter and Buckley, 2018). Total white blood counts (**TWBC**) were determined by a modified microscopic method as described in Campbell and Ellis (p.26, 2007). Standard Differential Count (**SDC**) was determined at 40× magnification.

H/L Ratio Calculation

Division of the sum of all heterophil types by the small "resting" **Ls** gives the H/L 1; [H/L 1 = (HC + HT + HV) / Ls]. Division of the same heterophil value by the sum of all lymphocyte types, (resting, reactive, and atypical) gives the H/L 2; [H/L 2 = [HC + HT + HV) / (Ls + Lm)]. ΔH/L = H/L 1 − H/L 2.

Light Microscopy and Photomicrographs

Olympus CX-41(Olympus America, Center Valley, PA 18034-0610) equipped with Plan N 40x, 0.65 numerical aperture dry, and Plan N, 1.25 numerical aperture 100x oil objectives. All images were captured at 100× with an Infinity-2 1.4-megapixel charge-coupled device Universal Serial Bus 2.0 Camera, and processed with Infinity Analyze software (Release 5.0.3) (Lumenera, Inc., Ottawa, ON, Canada).

Statistics

One-way ANOVA, means separation tests (Tukey), and *t* tests were done using Minitab Statistical Software (Release 17 for Windows, State College, PA).

RESULTS

Atypical cells of duck blood are shown in the figures; SDC and associated H/L ratios are given in the table. Figure 1 illustrates heterophil variation (HC vs. HT) where care was taken to display examples of HT found alongside HC. Figure 1 also displays the same heterophils stained by DIFF-QUIK (panel A) and Wright-Giemsa (panel B). This approach establishes the reality of their difference and rules out stain artifacts as a cause. By both techniques standard HC nuclei and cytoplasmic granules are fully stained. Normally, standard HT nuclei are fully stained but their granules appear as outlines giving HT cytoplasm a reticular appearance. Sometimes HT granules display stained "central bodies" several of which are seen in the HT of Figure 1, panel B.

Duck and chicken HT are more variable than either HC or HV. The four illustrated in Figure 1 indicate only a portion of the range of appearance of HT nuclei and cytoplasm. Duck HT variation parallels some of what is illustrated for the chicken by Lucas (1961, p.75). The exquisite illustrations of Hewitt (1942, plate 1, p. 40) also indicate variation of duck heterophils. Moreover, he recognized the advantage of supplementation of Wright's stain with Giemsa for the study of duck leukocytes, a technique used here.

Staining methods employing Petrunkevitch fluid as a fixative cause the granules to dissolve so chicken and turkey HC can resemble HT, as are illustrated in Lucas (1961, p80, p207). Since fully stained HC of Figure 1 appears in close proximity to HT dissolution of granules is an inadequate explanation of their differences. HT bear some resemblance to the faintly stained cell

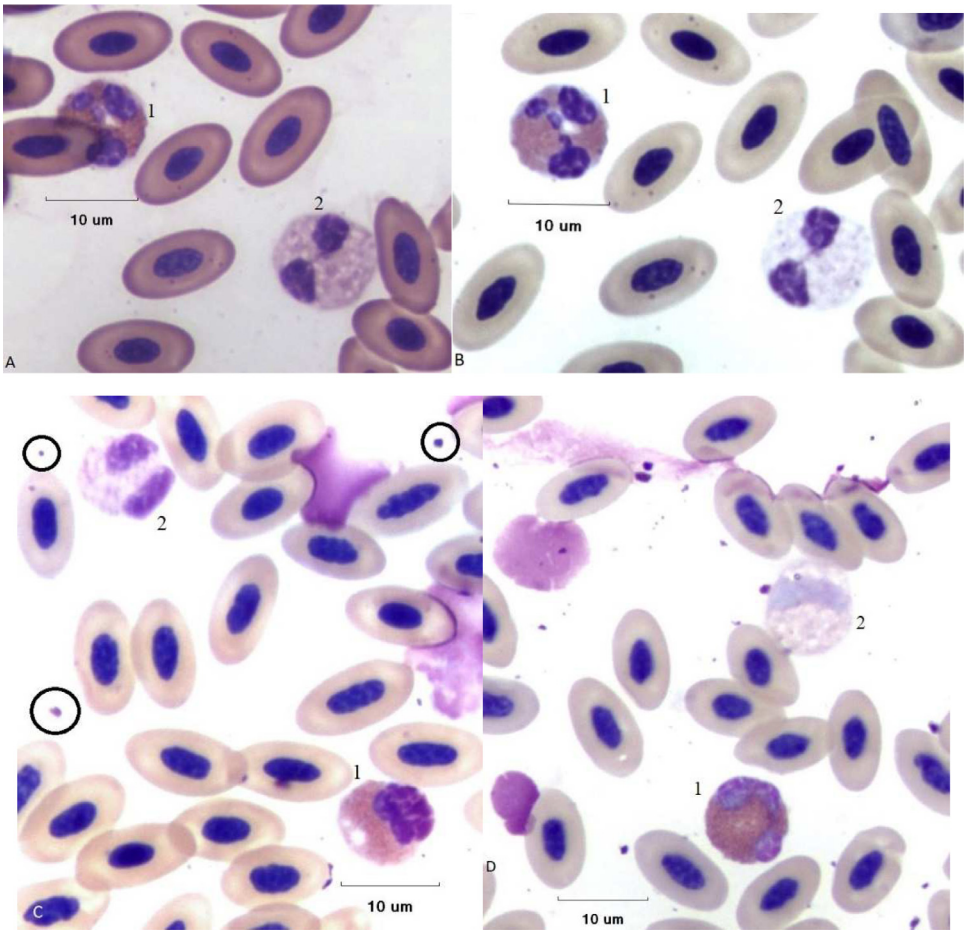

Figure 1. (A) Classic heterophil (HC, 1) stained with DIFF-QICK has a three-lobe nucleus. The cytoplasm displays fully stained red granules. Two irregular shaped "toxic" vacuoles are intermingled within the nuclear lobes. The two-lobed nucleus of the typical heterophil (HT, 2) is fully stained in contrast with its cytoplasmic granules. (B) The appearance of the same cells as in panel A after decolorization and restraining with Wright-Giemsa. Several RBC were lost during the restraining procedure. (C) An HT with two stained nuclear lobes (2) a fully stained HC with two toxic cytoplasmic vacuoles (1). Encapsulated bacteria are encircled. (D) An HT with both a weakly stained nucleus and cytoplasm (2) and a fully-stained HC (1).

illustrated by Hewitt (1942, plate 1, cell no. 6) described as a "Heterophile" (sic) using Giemsa's stain.

Heterophils of male ostriches resembling HT and HC were illustrated in Tadjalli et al. (2013) and judging by their microphotographs HT were the more frequent type. In this study HT comprised only 30% (851/2870) of heterophils, however.

HV and eosinophils were scarce in this study (Cotter, personal observation) so that descriptions will be reserved to occasions when they are more frequent.

Lymphocyte variation is the subject of Figure 2. With nuclear/cytoplasmic ratios of about 1 and diameters of ~ 6 μM the Ls of ducks resemble those of chickens and turkeys and are not shown. Medium to large size Lm including reactive types (panel D) has lower nuclear/cytoplasmic ratios. Plasma cells (panel A) display distinct Golgi's. Cells with more elaborately developed endoplasmic reticulum (panel B, C) are also included in the Lm group. These cells share some features with Mott cells of duck bone marrow (Cotter and Bakst, 2017). Presumptive precursors to these lymphoid giant cells, possible equivalents to Türk irritation cells (proplasmacytes) of mammals (Spriggs and Jerome, 1967) were described briefly (Cotter, 2017b).

The size contrast between reactive lymphocytes (Figure 2, panel D, top left) and giant lymphoid type cells is shown in panel D, bottom right. A group of 5 thrombocytes with irregular cytoplasmic edges (panel C) appear similar to the aggregated thrombocytes seen in a duck suffering from acorn toxicosis and yeast (*Candida*) infection (Murray, 2011).

Giant Lm were more common in SDC's than Mn types, but both are large enough to be located at 10× magnification. Giant cell shapes were considered as elliptical although Mn types were more irregular. The long and short axis of 48 giants, measured with a micrometer, were used to estimate areas. At 427.6 μm^2, Lm areas (N = 30) were significantly smaller than the 617.5 μm^2 areas of Mn giants (N = 18; One-way ANOVA, $F_{(1,46)} = 14.6$, $P = 0.0$). For comparison purposes, the areas of standard size leukocytes (10 HC and 10 HT) averaged 65.9 and 75.3 μm^2, respectively.

Additional examples of giant cells are given in Figure 3. A giant phagocytic monocytoid cell in the process of consuming an atypical thrombocyte is in a field with a reactive lymphocyte (panel A). A free atypical thrombocyte with scanty cytoplasm has an irregular edge is also in that panel. A giant cell, possibly a transitional type with

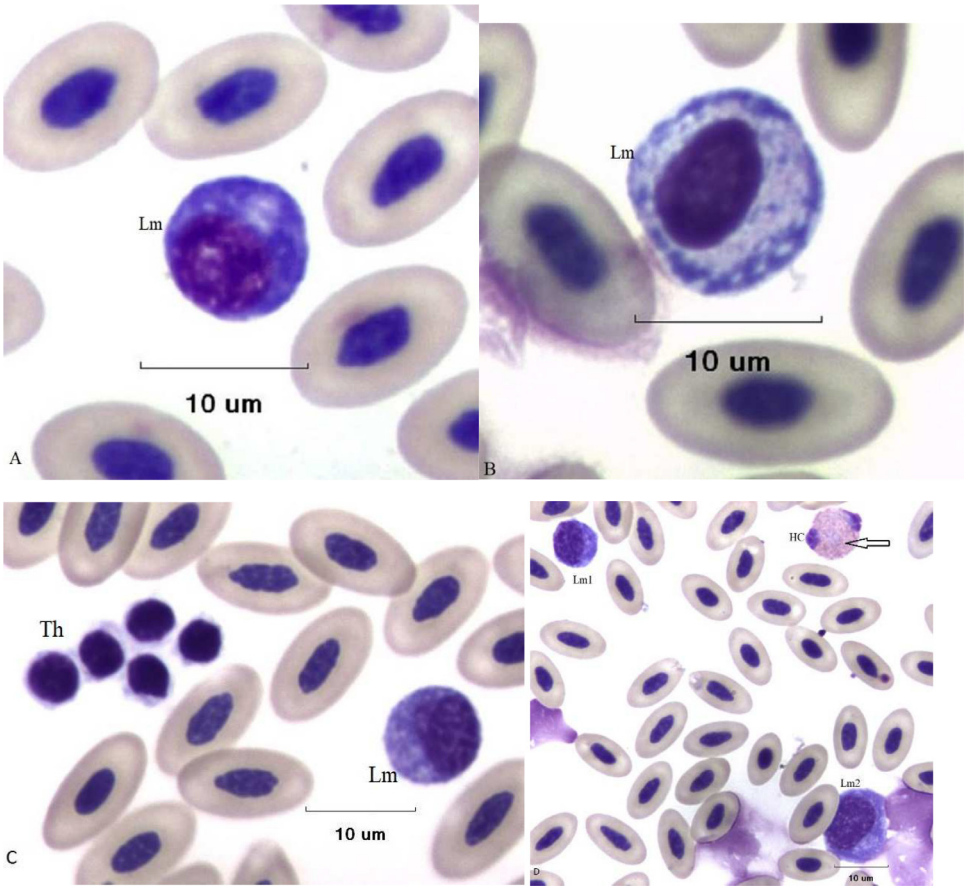

Figure 2. (A) Plasmacyte (Lm) with an eccentric nucleus, a conspicuous Golgi and several nearby small cytoplasmic vacuoles. (B) A large plasmacyte with diffuse circumnuclear cytoplasmic vacuoles some of which are faintly pink. (C) Plasmacyte (Lm) with eccentric nucleus and dispersed irregular cytoplasmic vacuoles (right). An aggregate composed of five atypical thrombocytes (Th). (D) Standard size plasmacyte (Lm1), giant plasmacyte (Lm2) classic heterophil (HC) with a very faint central nuclear lobe (arrow).

multiple nucleoli (panel B) is likely polyploid. A monocytoid giant cell displays a pseudopod and multiple cytoplasmic vacuoles (panel C). Differentiation of lymphoid from monocytoid giants is sometimes a challenge. Assignments were aided by illustrations of human monocyte variants appearing in Goasguen et al. (2009).

Evidence of fungal infection is in Figure 3 panel D. A solitary conidiospore is attached at the edge of a deteriorating HT whose granules are barely discernable.

The SDC data of the Table 1 indicate TWBC were in a range considered normal in ducks (Hewitt, 1942; Hemm and Carlton, 1967) and all gender differences were nonsignificant. Conversely both H/L 1 and H/L 2 ratios tended to be higher than those thought to indicate nonstress, a situation that parallels a study of caged hens (Cotter, 2015b). Higher numbers of Lm in the SDC magnifies the H/L 1- H/L 2 difference, ΔH/L. When ΔH/L > 0.1, atypical cells become an important part of the blood picture (Cotter and Heller, 2016) and their presence should be considered when interpreting H/L ratios.

Heterophil variants were found in all ducks, however the flock average for all heterophil types was close to what has been reported for ducks (Hewitt, 1942; Magath and Higgins, 1934). Interestingly, the latter authors described a type of heterophil (presumably an HT) having cytoplasmic granules so weakly stained it could be called a "neutrophil". Their observations are consistent with Hewitt's (1942, plate 1, cell no. 6) illustration, and the differences between HC and HT shown in Figure 1.

Giant cells were found in hen blood in the company of polymicrobial bacteremia and fungemia (Cotter, 2015a). Most were monocytoid, some phagocytic, and others contained cytoplasmic granules. Bacterial and fungal levels were much higher in those hens than found in this study. However, ducklings are highly sensitive to aflatoxin B1 (Chen, et al., 2014) supporting the possibility of toxin involvement.

DISCUSSION

Although this study is small the demonstration of distinct duck heterophil types is important for several reasons. First, is an immediate effect on computation of H/L ratios. A low (nonstress) H/L value using all available heterophils, including atypical types, cannot have the same interpretation as a similar value using only fully stained (standard) types.

Heterophils whose nuclei are incompletely stained or whose cytoplasmic granules are nondistinct represent true atypia and should not be dismissed as simple artifacts. This is obviously true when an atypical cell appears in proximity to fully stained types. Davis and Maney (2008) who reviewed the H/L and its relation

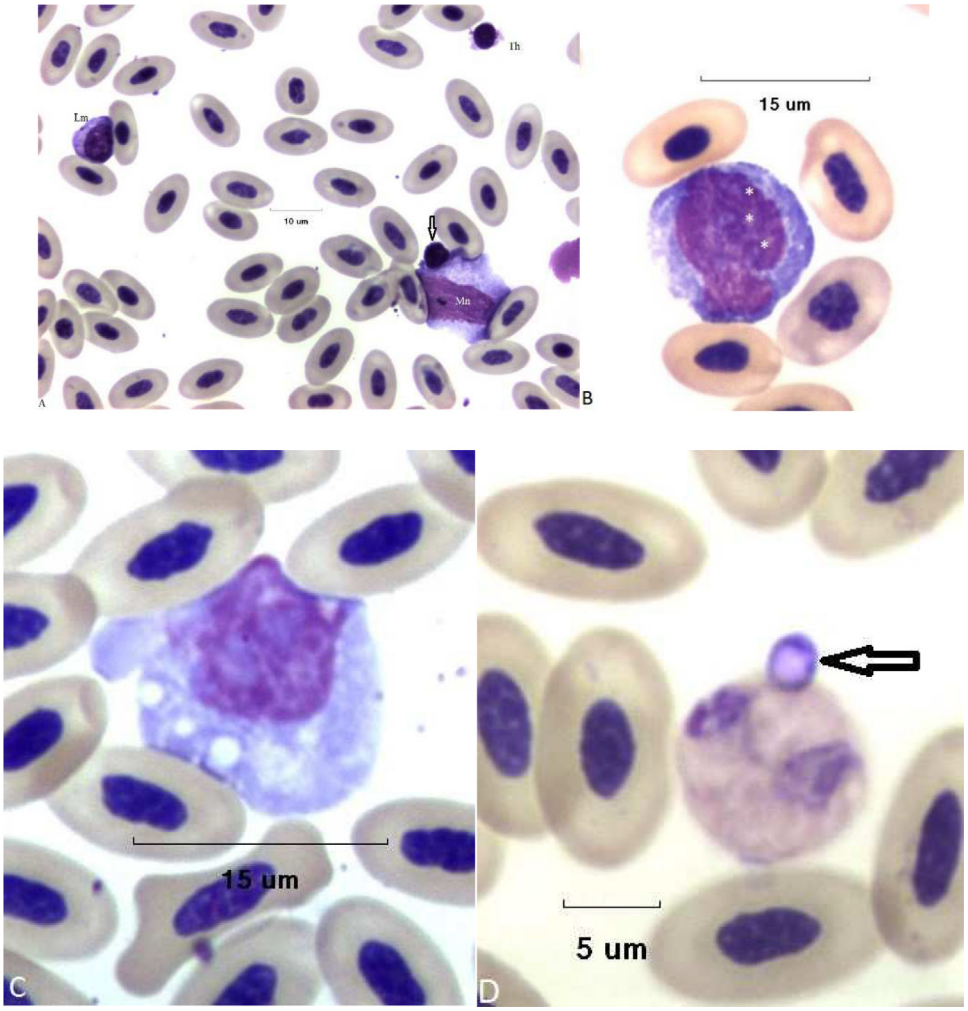

Figure 3. (A) Cells: A phagocytic monocytoid giant (Mn) in the process of consuming an atypical thrombocyte (arrow). A reactive lymphocyte (Lm). An atypical thrombocyte (Th) with an irregular cytoplasmic edge. (B) A lymphoid giant cell with a large nucleus containing multiple nucleoli (*). (C) A monocytoid giant cell displays a pseudopod and multiple cytoplasmic vacuoles is directly above an irregular shaped RBC. (D) A heterophil with a poorly stained nucleus and deteriorating cytoplasmic granules has a conidiospore attached to its surface (arrow).

to glucocorticoid hormones in a wide variety of vertebrates concluded that high values of each were correlated. Surprisingly the nucleus of the cell used by these authors to illustrate an avian heterophil was so poorly stained it would have been included here with atypical cells.

When reactive lymphocytes and plasmacytes are included in the H/L the result may be meaningless. Moreover, as some giant cells are members of the lymphocyte series, their presence in the SDC is a further complication. In addition, H/L values, no matter how they are computed, must be integrated with the TWBC. In poultry a TWBC above 50k generally indicates leukocytosis, and above 100k, a leukemoid reaction has occurred. Either implies stress or frank disease. The question of relation of H/L's and leukopenia has not yet been addressed. A low H/L must accompany a normal TWBC for it to be a legitimate indication of nonstress.

Secondly, multiple heterophil types impacts the expanded use of H/L ratios to nontraditional fields as ecology and evolution. As examples, Minias 2019) used meta-analysis of H/L values in over 250 bird species to examine evolutionary associations with ecological and life-history traits. Pap et al. (2015) used SDC data, based on 50 cell counts, from 105 species of European birds to investigate the relation between innate immunity and life history. Given the potential for between species and within sample variation seen in poultry (Lucas and Jamroz, 1961, p214-220) acceptance of the conclusions of these studies should be a matter of caution.

Lastly, Scanes (2016) in a meta-analysis on the relation of H/L to hormone levels in determining stress in chickens emphasized the importance of assay validation and laboratory standardization. This author agrees and suggests that the validation principle be extended to include cytology.

CONCLUSIONS

Several distinct types of heterophils occur in the hemogram of apparently healthy ducks. They are similar to types described in chickens and ostriches. Called classic (HC) and typical (HT) types they are differentiated on distinct cytology. Both were in hemograms with variable H/L ratios and TWBC at normal levels. Additional

Table 1. Average of differential counts (2 × 200 cells) as a percent of TWBC and heterophil/lymphocyte ratios for text figures.

Fig(panel)	Sex	HT	HV	HC	Ls	Lm	Mn	Ba	Eo	TWBC(K)	H/L 1	H/L 2	Δ H/L
1A, 1B	H[1]	12.3	0.0	5.8	10.7	68.1	1.4	1.4	0.0	20.0	2.2	0.2	2.0
1C, 3C	H	21.3	0.2	29.5	26.0	18.3	2.7	2.0	0.0	20.0	2.0	1.2	0.8
1D	H	6.9	0.5	18.4	45.7	26.8	0.7	1.0	0.0	16.0	0.6	0.4	0.2
2A, 3B	D	14.1	0.4	23.9	35.8	22.6	1.7	1.5	0.0	18.0	1.3	0.8	0.5
2B, 2C	D	6.9	0.5	18.4	45.7	26.8	0.7	1.0	0.0	16.0	0.6	0.4	0.2
2D	D	2.2	0.0	24.8	50.1	21.1	0.0	1.7	0.0	16.0	0.5	0.4	0.2
3A	H	17.0	0.0	4.4	41.7	13.3	19.7	3.9	0.0	30.0	0.5	0.4	0.1
3D	H	13.5	0.0	8.4	50.7	24.4	0.7	2.2	0.0	30.0	0.4	0.3	0.1
	Flock[2]	8.7	0.1	20.6	36.3	28.6	2.4	2.1	0.1	23.3	1.0	0.5	0.5

[1]Sex: H, hen, D, drake. Cells: H, heterophil (HC, classic, HV, variant, HT, typical) Ls small lymphocyte ~6 μM diameter, Lm medium, large (diameter 8−10 μM) and L giants (diameter 10−20 μM) Mn, monocyte including Mn giants, Ba, basophil, Eo, eosinophil. H/L ratio calculations are described in methods. TWBC (K), total white blood cells per cubic μL in thousands (K). H/L 1 = (HC + HT + HV) / Ls; H/L 2 = (HC + HT + HV) / (Ls + Lm); ΔH/L= H/L1 − H/L2.

[2]All *t* tests for gender differences were N.S.

atypical heterophils, recognizable by incomplete nuclear or cytoplasmic granule staining, were also common. These are shown to be deteriorating types, not technical artifacts. In summary it is recommended that atypical heterophils should not be included with normal heterophils in H/L calculations because their presence in the hemogram indicates dyscrasia, stress or disease.

Lymphocyte atypia in the form of large and giant reactive types resembling proplasmacytes (Türk irritation cells, Spriggs and Jerrome, 1967) or Mott cells occur as well. These are seen along with giant monocytes having a capacity for phagocytosis of thrombocytes or erythrocytes. As is the case with heterophils, reactive/atypical lymphocytes should not be included in H/L calculations.

Cells as described here may be present in blood in response to toxins released by bacteria or fungi during low grade infections.

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DISCLOSURES

The author has nothing to declare.

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