JVDI: Supplementary material

Bolfa et al. Infections and pathology of free-roaming backyard chickens on St. Kitts, West Indies

	Chickens with changes	
Organ	(no./total; %)	Lesion type and number of chickens affected
Liver	70/81; 86%	Nodular lymphoplasmacytic and histiocytic infiltration ($n = 60$)
		Lymphoid leukosis $(n = 6)^*$
		Necrotizing hepatitis $(n = 5)$
		Granulomas $(n = 5)$
		Eosinophilic and lymphoplasmacytic hepatitis $(n = 3)$
		Nodular accumulations of hemosiderin laden macrophages (Perl Prussian blue positive) $(n = 2)$ [†]
Kidney	32/81; 40%	Interstitial nephritis lymphoplasmacytic and histiocytic ($n = 29$) with tubular degeneration ($n = 5$ of 29)
		Paratanaisia bragai $(n = 3)$
		Cortical tubular degeneration and regeneration $(n = 1)$
		Lymphoid leukosis $(n = 2)^*$
		Metaplasia of renal tubular epithelium to ciliated $(n = 1)$
Lung	61/81; 75%	Pulmonary silicate aggregates $(n = 61)$
		Granulomas with no organism visibly associated on H&E, modified Ziehl–Neelsen, or Gram stain $(n = 2)$
		Lymphoid leukosis $(n = 3)^*$
		Nodules of hemosiderin-laden macrophages (Perl Prussian blue positive; $n = 2$)†
Proventriculus	12/81; 15%	Lymphoid hyperplasia ($n = 8$)
		Proventricular glands luminal ectasia with necrosis, desquamated epithelium, GALT hyperplasia and lymphoid aggregate formation $(n = 2)$
		Submucosal granuloma ($n = 1$)
		<i>Tetrameres</i> spp. $(n = 1)$
Small intestine	45/81; 56%	Luminal helminths ($n = 33$)
(duodenum and		Serosal silicate nodules ($n = 10$)
jejunum)		

Supplementary Table 1. Summary of histologic findings from 81 free-roaming feral chickens on St. Kitts, West Indies.

Large intestine	16/81; 20%	Lymphoid leukosis $(n = 8)^*$
(ceca)		Increased cellularity (mainly lymphocytes) in lamina propria with crypt hyperplasia \pm crypt abscesses ($n = 7$)
		GALT hyperplasia $(n = 6)$
		Lamina muscularis granulomas with no identified etiology using PAS, Gram (Brown & Brenn and Brown– Hopps) and acid fast stain (modified Ziehl–Neelsen; $n = 2$); parasitic tracts with sparganum (plerocercoid; $n = 2$); coccidiosis ($n = 1$)
		Luminal helminths $(n = 4)$
		Increased cellularity (mainly lymphocytes) in lamina propria ($n = 6$)
		GALT hyperplasia $(n = 2)$
		Lamina muscularis granulomas with no identified etiology $(n = 1)$
Spleen	49/81; 60%	Lymphoid hyperplasia ($n = 35$)
		Lymphoid leukosis ($n = 13$) *
		Nodules of hemosiderin-laden macrophages (Perl Prussian blue positive; $n = 2$) [†]
		Perisplenitis heterophilic and histiocytic $(n = 1)$
		Nodular accumulations of hemosiderin laden macrophages (Perl Prussian blue positive; $n = 2$)†
Gonads	7/81; 9%	Ovarian medulla lymphoplasmacytic and histiocytic infiltration $(n = 2)$
		Epididymis interstitial lymphoplasmacytic and histiocytic infiltration $(n = 2)$
		Testicular interstitial lymphoplasmacytic and histiocytic infiltration $(n = 1)$
		Seminiferous tubules within the tunica albuginea $(n = 2)$
Skin	34/81; 42%	Perivascular or interstitial inflammation, lymphocytes, plasma cells and some macrophages in most cases ± eosinophils
		Chronic $(n = 27)$
		Acute $(n = 7)$
Heart	24/81; 30%	Multifocal lymphoplasmacytic and histiocytic infiltration ($n = 16$) and eosinophils ($n = 2$)
		Focal lymphoplasmacytic and histiocytic infiltration $(n = 5)$
		Degeneration and loss of cardiomyocytes $(n = 5)$;
		Lymphoid leukosis $(n = 3)^*$
Pancreas	10/81; 12%	Multifocal lymphoplasmacytic and histiocytic infiltration $(n = 6)$
		Lymphoid leukosis $(n = 4)^*$
Brain	1/81; 1%	Accumulation in the Virchow–Robin space of round cells, compatible with lymphoid leukosis $(n = 1)^*$
Bursa of Fabricius	14/81; 17%	Bursal involution $(n = 12)$
		Lymphoid leukosis $(n = 3)^*$
		Edema and hemorrhages on the surface mucosa $(n = 1)$ §

Adrenal gland	1/81; 1%	Focal lymphoplasmacytic infiltration $(n = 1)$
Thymus	1/81; 1%	Lymphoid leukosis: cortex and medulla $(n = 1)^*$
Trachea	1/81; 1%	Lymphoid leukosis: lamina propria and submucosa $(n = 1)^*$
Skeletal muscle	1/81; 1%	Pectoral muscle degeneration and necrosis, polyphasic $(n = 1)$

No changes were seen in the esophagi of the chickens in our study.

* The morphology of the cells was uniform, consisting of large round cells (lymphoblastic), with mild anisocytosis and anisokaryosis suggestive of a lymphoid neoplasm. Mitotic figures were low, up to 1 mitosis per HPF. Sometimes, nodular proliferations in different organs were surrounded by a band of fibroblast-like cells.

[†] This change was seen in the same 2 chickens in these 3 organs.

‡ This change was seen in association with inflammatory cell infiltration.

§ Positive on serology for infectious bursal disease virus.



Supplementary Figure 1. External parasites. **A.** A body louse, presumed *Menacanthus* spp., on the feathered skin of a chicken. **B.** A shaft louse, presumed *Menopon* spp., on the feathered skin of the wing of a chicken. **Supplementary Figure 2.** In a chicken kidney, there is cortical tubular ectasia, with intratubular cellular debris and focal lymphocytic interstitial nephritis (arrow). H&E. Inset: ciliated cuboidal epithelial cells lines cystic tubules. H&E.



Supplementary Figure 3. Tubular duct in the proventriculus of a chicken, distended by an adult nematode, ~1.5 mm diameter, with a cuticle, a pseudocoelom containing eosinophilic fluid, polymyarian–coelomyarian musculature, a digestive tract lined by uninucleate cuboidal cells with abundant brown-black pigment, and eosinophilic eggs; presumably *Tetrameres* spp. H&E. Supplementary Figure 4. Intestinal cestodes from chickens. A. Multiple cestodes (*Raillietina* spp.) in the lumen of the small intestine. The scolex of an adult is seen (arrow) as well as the strobila of others. B. Multiple cestodes are free in the duodenal lumen or embedded in the mucosa. H&E. Supplementary Figure 5. In the testis of a chicken, seminiferous tubules within the tunica albuginea (arrows) are inactive, as opposed to the rest of the tubules in which spermatogenesis is active. H&E.