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**REVIEW**

# A retrospective review of the histopathology of captive hornbill chicks

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**Background and objectives:** Captive rearing of chicks can be crucial to the success of management plans for endangered species of birds. This study was conducted to document lesions of hornbill chicks to provide information for pathologists and clinicians to improve rearing success in captive hornbills.

**Methods:** Clinical histories and post-mortem reports were evaluated for 40 hornbill chicks (< 3.5 months old), from three species submitted for pathological evaluation to the National Zoological Garden, South African National Biodiversity Institute between 2003 and 2017. The cause of death and histological features present were tabulated.

**Results:** In this group of chicks, 12 (30%) were a week or younger at the time of death. Bacterial infections, especially of the lung and gastro-intestinal tract, were the most common cause of death (38%). Visceral gout, renal tubular degeneration or necrosis, bacterial ventriculitis and pulmonary congestion were the most often recorded lesions. Splenic, thymic and bursal lymphoid depletion and/or necrosis were common. Chicks of all ages commonly showed hepatocyte vacuolar degeneration; and hepatic, renal and splenic haematopoiesis of no pathological significance.

**Conclusion:** This long-term survey in captive hornbill chicks provides baseline information on lesions and conditions seen in these birds and facilitated the formulation of improved captive management manuals of hornbills. Additional detailed post-mortem examinations following standardised protocols, including bacterial culture, of hornbill chicks would further improve our understanding of hornbill chick diseases.

**Keywords:** hornbill, *Bucorvus*, mortality, bacterial infection, lymphoid depletion

## Introduction

Hornbills are a family of birds (*Bucerotidae*) that share taxonomic, morphological and behavioural characteristics and play important roles in tribal cultures from South Africa to East Asia (Gonzalez et al. 2013). They are characterised by a large, long curved beak, as well as a casque (Munoz et al. 2003). The frugivorous hornbills' role as seed dispersers has contributed to the historical expansion of paleotropical forests.

The 32 species of African hornbills occupy a wide range of habitats from tropical forests to arid savannas (Munoz et al. 2003). South African hornbills include the African grey hornbill (*Lophoceros nasutus),* crowned hornbill (*Lophoceros alboterminatus),* Southern red-billed hornbill (*Tockus erythrorhynchus rufirostris),* Southern yellow-billed hornbill (*Tockus leucomelas),* trumpeter hornbill (*Bycanistes bucinator* and the Southern ground-hornbill (SGH, *Bucorvus leadbeateri) (Munoz et al. 2003; Trail 2007)*. While the populations of some species of hornbill are relatively stable, the International Union for the Conservation of Nature Red List of Endangered Species classifies the Abyssinian ground-hornbill (from Northern sub-Saharan Africa (AGH, *Bucorvus abyssinicus*), and SGH as "Vulnerable" (https://www.iucnredlist.org, accessed 12 January 2022). SGH are classified as "Endangered" in South Africa, since only 1 500–2 000 adults are estimated to occur (Engelbrecht 2007; Trail 2007; Combrink, Combrink & Botha 2017). Population risks in South Africa include anthropogenic factors such as the removal of large trees for nesting, disturbed nests, habitat alteration, poisoning, persecution, electrocution, trade and hunting for body parts used in traditional medicine (Engelbrecht 2007; Koeppel & Kemp 2015; Combrink, Combrink & Botha 2017). Population declines are exacerbated by a slow rate of reproduction and extensive habitat requirements. The South African National Biodiversity Institute's National Zoological Garden (SANBI NZG) is a member of the national SGH Action Group and maintains data and samples from this species for research. The Action Group conducts disease monitoring, harvests and hand raises very young chicks rejected by free-ranging breeding groups and re-introduces birds into areas where they have become locally extinct (Kemp et al. 2020).

Although references for the management, including hand rearing of chicks (Rehse 2014) and the medical management of ground-hornbills (Koeppel & Kemp 2022) are available, pathological lesions seen in captive hornbills are comparatively poorly documented, with no published reports of lesions in chicks. Ninety hornbill cases were recorded in the SANBI NZG wildlife disease database between 2004 and 2017. The aim of

the present study was to document the macroscopical and histological features seen in chicks ($n$ = 40) to assist pathologists interpreting such cases; and to facilitate clinical management of sick hornbill chicks.

## Materials and methods

The SANBI NZG wildlife disease database was searched for records of all captive hornbills submitted for a post-mortem evaluation by one pathologist (EPM) following the same protocol, between 2003 and 2017. In total, 90 hornbill necropsies were found with a wide variety of causes of deaths and lesions. Since age at death fell into two distinct age groups the findings in the 40 birds that were under 98 days (three months) old were classified as chicks and are the subject of this paper. Cases were provided by the Johannesburg Zoo ($n$ = 16), Mabula Ground-Hornbill Project ($n$ = 9), Monte Casino Bird Gardens ($n$ = 7), Mpumalanga Tourism and Parks Agency ($n$ = 5), NZG ($n$ = 3); and included 36 SGH, three AGH and a black and white casqued hornbill (from Central and Western Africa, BWCH, *Bycanistes subcylindricus*). The clinical history, macroscopical findings, results of additional tests (if available) and histological slides for each case were reviewed by a single pathologist (APG). The cause of death or reason for euthanasia was recorded; and morphological diagnoses were assigned for each major organ system. Birds were deemed septicaemic if heterophilic inflammation was seen in multiple organs or tissues. Gram stains and anaerobic culture were not performed due to cost considerations. Adrenal gland hypertrophy was diagnosed by subjective macroscopical assessment of gland size and dwarfing of intervening chromaffin cells by inter-renal ("cortical") cells.

## Results

The number, age, sex, species, location, origin, post-mortem date, and cause of death or euthanasia are shown in Table I. Three captive born chicks were submitted in the egg. The remaining birds ranged from 1–98 days old. Nine chicks (23%) died in the first week of life; and nine died in their second week. Of the 40 chicks, there were 14 male and six female SGH; a female BWCH, and one male and one female NGH. Sex was not recorded in 16 SHG and one NGH (43% of chicks) so no gender analysis was undertaken. Chicks were either captive bred (19/40, 48%) or harvested from the wild as very young chicks and then hand raised (16/40, 40%); origin was not recorded in five birds. Age at death was similar in both captive born and wild hatched chicks. Detailed findings including clinical context are available in Supplementary Table I.

### *Cause of death or euthanasia*

One close to term chick was found dead in the shell (H1) and two failed to properly hatch (H2, H3). Of the chicks that hatched, bacterial infections were responsible for the death in 15 (38%) birds (H4, H11, H12, H14, H15, H18, H20–H22, H25, H27, H28, H30, H32, H36). Captive born and wild hatched chicks were equally represented. Three chicks died of aspiration pneumonia (H9, H13, H23); two of starvation (H37, H38); one each of traumatic injury (H6); visceral gout (H8); hyperthermia (H16); proventricular and gizzard impaction (H24); metabolic bone disease (H29); fungal infection (H31); acute heart failure (H34); viral pancreatitis (H33); sciatic neuritis (H35); and liver failure (H40). The cause of death could not be determined in five chicks (13%) because no significant lesions were detectable (H10, H17, H39) or autolysis precluded a diagnosis (H7, H26).

### *Histopathological findings*

The number and proportion of tissues sampled and conditions found in the hornbill chicks, as well as those tissues in which no lesions were found, are shown in Table II. Macroscopical findings were often minimal, and when present reflected the histological lesions described.

#### *Gastrointestinal tract*

Yolk sac contents were mineralised in a dehydrated chick with suspected viral pancreatitis (H33) and one that died of starvation (H38). Haematopoiesis (H3) and fat necrosis (H1) were seen in two chicks that did not hatch.

Seven chicks had ventriculitis but no gizzard impaction (Figure 1). These included a chick (H11) which had necrotising ventriculitis with intralesional rods; one (H14) with necrohaemorrhagic

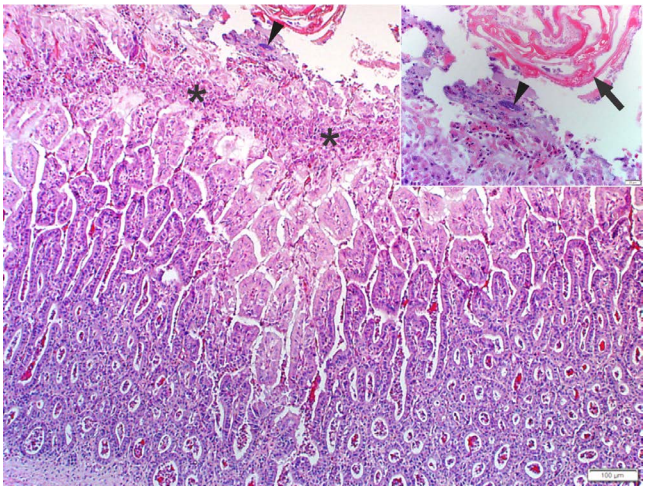

**Figure 1:** Bacterial ventriculitis in a nine-day-old Southern ground-hornbill chick (H15). Necrosis and inflammation (*) in the superficial layers is associated with colonies of bacterial cocci (arrowhead). Haematoxylin and eosin. Inset: colonies of bacteria (arrowhead) and disrupted koilin layers (arrows). Haematoxylin and eosin.

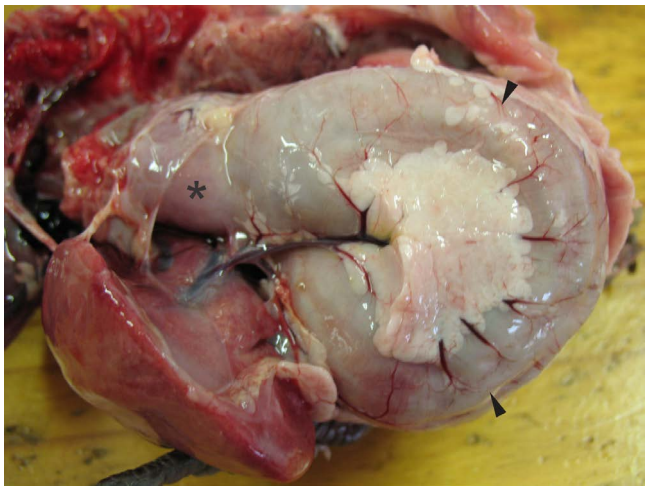

**Figure 2:** Gizzard impaction in a 21-day-old Southern ground-hornbill chick (H25). Note the distended flaccid proventriculus (*) and gizzard (arrowheads).

**Table I:** The number, age, sex, species, location, origin, date of death and cause of death or euthanasia in 40 captive hornbill chicks

| Chick no | Age* | Sex | Species** | Location^ | Origin^^ | Date of death | Cause of death/euthanasia |
|---|---|---|---|---|---|---|---|
| H1 | 0 | - | SGH | 1 | CB | 21 Oct 13 | Dead in shell |
| H2 | 0 | - | SGH | 3 | CB | 14 Feb 13 | Dead in shell |
| H3 | 0 | - | NGH | 3 | CB | 09 Oct 14 | Hatching failure |
| H4 | 1 | - | SGH | 4 | WH | 24 Jan 12 | Bacterial septicaemia |
| H5 | 2 | male | SGH | 5 | CB | 24 Nov 05 | Dehydration |
| H6 | 2 | male | SGH | 1 | CB | 10 Sep 13 | Cerebral haemorrhage |
| H7 | 3 | - | SGH | 1 | WH | 31 Jan 14 | None possible (autolysis) |
| H8 | 4 | female | SGH | 1 | WH | 18 Feb 11 | Visceral gout |
| H9 | 4 | male | SGH | 2 | WH | 07 Dec 12 | Aspiration pneumonia |
| H10 | 4 | - | SGH | 2 | - | 12 Aug 17 | No diagnosis possible |
| H11 | 5 | - | SGH | 3 | CB | 17 Dec 12 | Bacterial ventriculitis |
| H12 | 7 | - | SGH | 4 | CB | 16 Apr 15 | Bacterial enteritis |
| H13 | 8 | - | SGH | 5 | CB | 01 Dec 08 | Aspiration pneumonia |
| H14 | 8 |  | SGH | 1 | WH | 17 Dec 14 | Bacterial ventriculitis |
| H15 | 9 | male | SGH | 1 | CB | 18 Feb 11 | Bacterial ventriculitis |
| H16 | 10 | male | SGH | 4 | WH | 16 Apr 15 | Hyperthermia |
| H17 | 10 | female | SGH | 1 | WH | 24 Nov 14 | No diagnosis possible |
| H18 | 12 | female | SGH | 1 | CB | 12 Nov 12 | Ventriculitis and cloacitis |
| H19 | 12 | male | NGH | 1 | CB | 30 Jan 13 | Hepatic necrosis |
| H20 | 13 | - | SGH | 1 | WH | 28 Dec 11 | Septicaemia |
| H21 | 14 | male | SGH | 2 | WH | 08 Apr 15 | Ventriculitis, enteritis |
| H22 | 16 | female | SGH | 3 | WH | 28 Dec 12 | *Enterococcus faecalis* septicaemia |
| H23 | 19 | male | SGH | 3 | CB | 06 Dec 10 | Aspiration pneumonia and septicaemia |
| H24 | 21 | female | SGH | 5 | WH | 27 Jan 03 | Proventriculus-gizzard impaction |
| H25 | 21 | - | SGH | 1 | CB | 25 Oct 07 | *E. coli* septicaemia |
| H26 | 23 | - | SGH | 2 | WH | 27 Jan 09 | None possible (autolysis) |
| H27 | 25 | male | SGH | 2 | CB | 07 Jan 08 | *E. coli* septicaemia |
| H28 | 27 | male | SGH | 2 | WH | 04 Feb 09 | Bacterial ventriculitis, rickets |
| H29 | 33 | - | SGH | 1 | - | 04 Feb 09 | Metabolic bone disease |
| H30 | 34 | male | SGH | 2 | CB | 02 Nov 07 | *E coli* septicaemia |
| H31 | 34 | male | SGH | 3 | - | 17 Jan 14 | Fungal enteritis and pancreatitis |
| H32 | 35 | female | SGH | 2 | - | 21 Jan 08 | Salmonellosis |
| H33 | 39 | male | SGH | 1 | CB | 03 Feb 14 | Pancreatic necrosis |
| H34 | 84 | male | SGH | 3 | CB | 24 Mar 14 | Acute heart failure |
| H35 | 98 | male | SGH | 2 | WH | 04 Apr 11 | Sciatic neuritis |
| H36 | neonate | - | SGH | 1 | WH | 31 Jan 14 | Bacterial ventriculitis |
| H37 | juvenile | - | SGH | 4 | WH | 16 Apr 15 | Starvation |
| H38 | Juvenile | - | SGH | 4 | - | 25 Aug 17 | Starvation |
| H39 | juvenile | female | NGH | 1 | CB | 03 Feb 14 | None possible |
| H40 | juvenile | female | BWCH | 1 | CB | 24 Feb 09 | Liver failure |

*age in days after hatching; 0, dead in shell
** SGH, Southern ground-hornbill; NGH, Northern ground-hornbill; BWCH, black and white casqued hornbill
^1, Johannesburg Zoo; 2, Ground-Hornbill Project; , 3, Monte Casino Bird Gardens; 4, Mpumalanga Tourism and Parks Agency; 5, National Zoological Gardens
^^WH, wild harvested; CB = captive-bred, -, unknown

**Table II:** The number and proportion of tissues sampled and conditions found in 40 captive hornbill chicks

| System | Tissue sampled | Condition | *N* | %* |
|---|---|---|---|---|
| Gastrointestinal | **Yolk sac** | NSF** | 8 | 66 |
| | *N = 12 (30%)* | Mineralisation (H33, H38) | 2 | 17 |
| | | Haematopoiesis (H3) | 1 | 8 |
| | | Fat necrosis (H1) | 1 | 8 |
| | **Oesophagus** *N = 19 (48%)* | NSF | 19 | 100 |
| | Proventriculus | NSF | 22 | 96 |
| | *N = 23 (58%)* | Proventriculitis (H28) | 1 | 4 |
| | **Gizzard** | NSF | 23 | 64 |
| | *N = 36 (90%)* | Ventriculitis (H11, H14, H15, H18, H21, H28, H36) | 7 | 19 |
| | | Gizzard impaction (H20, H24, H28, H38) | 4 | 11 |
| | | Koilin oedema (H25, H34, H35) | 3 | 8 |
| | | Mineralised koilin (H21, H39) | 2 | 6 |
| | | Koilin erosion (H38) | 1 | 3 |
| | **Intestine** | NSF | 29 | 80 |
| | *N = 36 (90%)* | Enteritis (H12, H20, H21, H25, H31) | 5 | 14 |
| | | Intestinal villous atrophy (H28) | 1 | 3 |
| | | Intestinal epithelial hyperplasia, cryptosporidiosis (H30) | 1 | 3 |
| | **Liver** | NSF | 1 | 3 |
| | *N = 36 (90%)* | Vacuolar change (H1-4, H5, H8, H11, H13-18, H20, H21, H24, H31, H36, H39) | 19 | 53 |
| | | Haematopoiesis (H2, H3, H8, H13-18, H20, H21, H24, H31, H36, H39) | 16 | 44 |
| | | Congestion (H27, H28, H32, H34) | 4 | 8 |
| | | Cholestasis (H38, H40) | 2 | 6 |
| | | Hepatocellular atrophy (H28, H40) | 2 | 6 |
| | | Hepatitis (H20, H32) | 2 | 6 |
| | | Hepatocellular necrosis (H19, H22) | 2 | 6 |
| | **Pancreas** | NSF | 13 | 62 |
| | *N = 2 1 (54%)* | Atrophy (H20, H22, H25, H30) | 4 | 19 |
| | | Pancreatitis (H31, 33) | 2 | 10 |
| | | Haematopoiesis (H23) | 1 | 5 |
| | | Congestion (H34) | 1 | 5 |
| Respiratory | **Trachea** *N = 13 (33%)* | NSF | 13 | 100 |
| | **Lung** | NSF | 17 | 47 |
| | *N* = 36 (90%) | Congestion (H15, H22, H24, H27, H29, H32, H38) | 7 | 19 |
| | | Oedema (H8, H20, H22, H29, H32) | 5 | 14 |
| | | Atelectasis (H2, H5, H21, H32) | 4 | 11 |
| | | Air sacculitis (H4, H15, H20, H32) | 4 | 11 |
| | | Aspiration pneumonia (H9, H13, H23) | 3 | 8 |
| | | Granulomatous pneumonia (H30) | 1 | 3 |
| | | Interstitial pneumonia (H39) | 1 | 3 |
| | | Haemorrhage (H6) | 1 | 3 |
| Cardiovascular | **Heart** | NSF | 32 | 84 |
| | *N = 38 (95%)* | Epicarditis and/or pericarditis (H22, H30, H32) | 3 | 8 |
| | | Myocardial necrosis (H33) | 1 | 3 |
| | | Myocardial haemorrhage, mild, focal, acute (H3) | 1 | 3 |
| | | Myocardial fibrosis (H32), | 1 | 3 |
| | **Aorta** *N = 4 (10%)* | NSF | 4 | 100 |
| Lympho-haemopoietic | **Spleen** | NSF | 7 | 23 |

| System | Tissue sampled | Condition | *N* | %* |
|---|---|---|---|---|
| Lympho-haemopoietic | *N = 31 (78%)* | Haematopoiesis (H3, H6, H9, H14, 16, H20, H22, H29, H31, H39) | 10 | 32 |
| | | Lymphoid atrophy (H3, H8, H11, H13, H15, H32) | 6 | 19 |
| | | Splenitis (H15, H23, H25, H27, H30, H32) | 6 | 19 |
| | | Reticuloendothelial hyperplasia (H20, H21, H29, H32) | 4 | 13 |
| | | Follicular lymphoid hyperplasia (H14, H40) | 2 | 6 |
| | | Lymphocyte necrosis (H11, H18) | 2 | 6 |
| | | Congestion (H24, H35) | 2 | 6 |
| | | Splenic haemosiderosis (J40) | 1 | 3 |
| | **Thymus** | NSF | 9 | 64 |
| | *N = 14 (35%)* | Thymic atrophy (H28, H35, H36) | 3 | 21 |
| | | Thymic lymphoid necrosis (H18, H20, H28) | 3 | 21 |
| | **Bursa of Fabricius** | NSF | 10 | 43 |
| | *N = 23 (58%)* | Lymphoid atrophy (H3, H10, H22, H23, H25, H27, H28, H32, H34, H36) | 10 | 43 |
| | | Lymphoid necrosis (H2, H3, H10, H11, H18, H22) | 6 | 26 |
| | | Bursitis (H3, H25) | 2 | 9 |
| | **Bone marrow** *N = 4 (10%)* | NSF | 4 | 100 |
| Urinary | **Kidney** | NSF | 8 | 22 |
| | *N = 36 (90%)* | Haematopoiesis (H4, H8, H9, H11, H14, H17-19, H22, H27, H28, H31, H33, H36, H39) | 15 | 42 |
| | | Visceral gout (H15, H20, H22, H23, H28, H30, H32, H37, H40) | 9 | 25 |
| | | Tubular degeneration and/or necrosis (H6, H24, H25, H29, H31, H33, H39, H40) | 8 | 22 |
| | | Nephritis (H15, H25) | 3 | 8 |
| | | Intratubular mineralisation (H19, H21) | 2 | 6 |
| | | Glomerulonephritis (H32) | 1 | 3 |
| | | Congestion (H34) | 1 | 3 |
| | | Haemorrhage (H6) | 1 | 3 |
| Nervous | **Brain** | NSF | 23 | 92 |
| | *N = 25 (63%)* | Meningeal haemorrhage (H6) | 1 | 4 |
| | | Encephalitis (H27) | 1 | 4 |
| | ***Sciatic nerve*** | NSF | 6 | 86 |
| | *N = 7 (18%)* | Neuritis (H35) | 1 | 14 |
| Integumentary | **Skin** | NSF | 21 | 84 |
| | *N = 25 (63%)* | Dermatitis (H3, H5, H22) | 3 | 12 |
| Endocrine | **Adrenal gland** | NSF | 10 | 59 |
| | *N = 17 (43%)* | Adrenal gland hyperplasia (H9, H20, H21, H22, H25, H27, H30) | 7 | 41 |
| | **Thyroid gland** | NSF | 10 | 90 |
| | *N = 11 (28%)* | Thyroiditis (H34) | 1 | 10 |
| | **Parathyroid gland** | NSF | 3 | 33 |
| | *N = 9 (23%)* | Parathyroid gland hyperplasia (H18, H22, H25, H30, H34, H35) | 6 | 67 |
| Musculoskeletal | **Muscle** | NSF | 14 | 88 |
| | *N = 16 (40%)* | Skeletal muscle degeneration and necrosis (H29, H35) | 2 | 13 |
| | **Bone** | NSF | 5 | 45 |
| | *N = 11 (28%)* | Rickets (H27-30, H32) | 5 | 45 |
| | | Fibrous osteodystrophy (H29) | 1 | 9 |
| Reproductive | Testis *N = 3 (8%)* | NSF | 3 | 100 |
| | Ovary *N = 2 (5%)* | NSF | 2 | 100 |

*Percentage is based on number of birds in which the organs were sampled. Percentages don't add up to 100 due to rounding, or if more than one lesion is present in the same organ/bird.

ventriculitis; and a chick with necrotising ventriculitis with intralesional rods (H28) from which a sparse mixed bacterial growth, chiefly rough *E. coli*, was isolated from the liver. Two chicks (H15, H36) had heterophilic ventriculitis; H18 had heterophilic ventriculitis as well as necrohaemorrhagic heterophilic and lymphoplasmacytic cloacal and bursal inflammation. One chick (H21) had heterophilic ventriculitis and enteritis.

Marked gizzard impaction with undigested food was seen in four chicks (Figure 2, Table II). H20 also had necrotising heterophilic enteritis and septicaemia. A specific pathogen was not isolated. One chick (H28) had necrotising proventriculitis and ventriculitis with intralesional rods. H38 had moderate superficial gizzard erosions. H24 had concurrent renal and hepatic degeneration and necrosis.

Mild koilin oedema was seen in three birds: H25, a chick with bacterial septicaemia and intra-koilin bacterial rods; and in H34, which died of acute heart failure and had intra-koilin bacterial cocci. One chick had koilin oedema and mineralisation (H39). Mineralisation of the koilin layer was also seen in a chick with ventriculitis (H21).

Necrotising and or heterophilic enteritis was seen in five chicks, including a chick (H12) with intralesional rods, a septicaemic chick (H20) and one with ventriculitis (H21). A septicaemic chick (H25) had cloacal inflammation as well as necrotising enteritis, with bacterial rods in many tissues. A heavy growth of *E. coli* was isolated from the liver. A chick (H31) with necrohaemorrhagic and heterophilic fungal enteritis also had fungal pancreatitis. Suspected viral pancreatic necrosis was seen in one chick (H33). H28, which died of bacterial proventriculitis and ventriculitis, also had atrophic enteritis and rickets. Intestinal hyperplasia associated with Cryptosporidia was seen in a septicaemic chick (H30).

### *Liver*

Chicks of all ages had hepatocyte cytoplasmic microvesicular vacuolar change (19/36, 53%) and haematopoiesis (16/36, 47%, Figure 3). Hepatic congestion was recorded in four chicks: a septicaemic chick with encephalitis (H27); a chick with ventriculitis and rickets (H28); a chick with necrotising heterophilic hepatitis due to salmonellosis (H32); and a chick with acute heart failure (H34). H20, a septicaemic chick also had necrotising heterophilic hepatitis. Hepatocyte degeneration occurred in a chick with ventriculitis (H11) and one with gizzard impaction (H24). Hepatic cholestasis was present in a chick that died of starvation (H38) and in another chick with hepatocellular atrophy (H40). Hepatocellular atrophy was also found in a chick with ventriculitis (H28). In H19, hepatic necrosis caused death but an aetiology was not determined. H22, a septicaemic chick, had periacinar hepatocellular necrosis. A heavy growth of *Enterococcus faecalis* was isolated from the liver.

### *Pancreas*

Zymogen cell atrophy was recorded in four chicks with bacterial septicaemia (H20, H22, H25, H30). A dehydrated emaciated chick with fungal enteritis (H31) had heterophilic necrotising pancreatitis with intralesional yeasts, as well as lymphocytic and

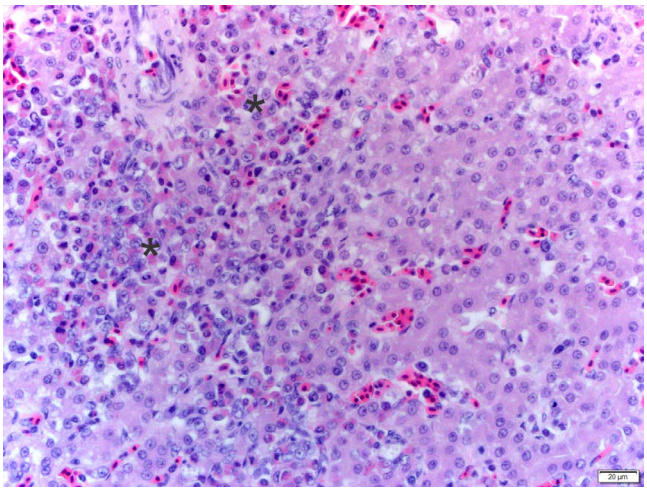

**Figure 3:** Hepatic haematopoiesis in a 34-day-old Southern ground-hornbill chick (H31). Note clusters of haematopoietic cells, mainly developing granulocytes (*), around hepatic portal areas. Haematoxylin and eosin.

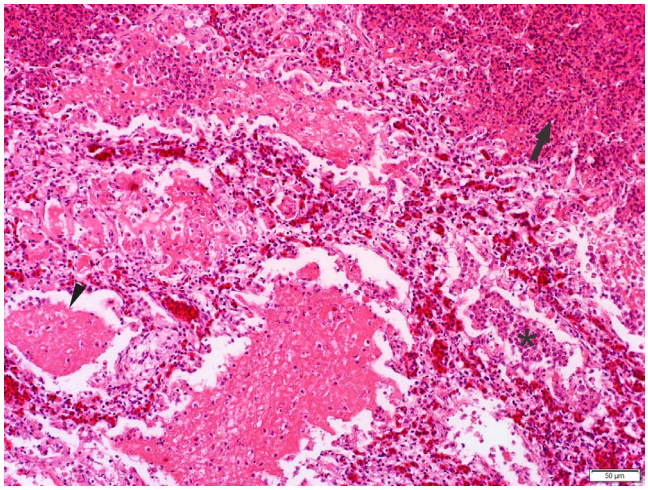

**Figure 4:** Aspiration pneumonia in an eight-day-old Southern ground-hornbill chick (H13). Heterophils (*) and amorphous eosinophilic food material (arrowhead) greatly expand parabronchi. The lungs show moderate hyperaemia as well as foci of caseous necrosis (arrow). Haematoxylin and eosin.

heterophilic, perivascular steatitis and vasculitis in surrounding adipose tissue. H33 had marked necrotising and heterophilic pancreatitis. A systemic viral infection was suspected but no viral particles were found on transmission electron microscopy of the pancreas. Pancreatic haematopoiesis was seen in a chick with aspiration pneumonia and septicaemia (H23). H34 died suddenly with no apparent cause, and showed only widespread tissue congestion, including the pancreas.

### *Respiratory system*

Nine chicks had lung and/or air sac inflammation. Pulmonary congestion with variable oedema was seen in chicks that died of septicaemia (H22, H27), salmonellosis (H32), proventricular and gizzard impaction (H24), ventriculitis (H15), metabolic bone disease (H29), and a chick that died of starvation (H38). Pulmonary oedema alone was present in a chick with visceral gout (H8) and one with septicaemia (H20). Four chicks showed pulmonary atelectasis: one that did not hatch (H2); a dehydrated

inappetent chick (H5); a chick (H21) with ventriculitis and enteritis and one with salmonellosis (H32).

Three chicks had aspiration pneumonia with food material in the airways (Figure 4). Lymphoplasmacytic and histiocytic interstitial pneumonia with type II pneumocyte hyperplasia was found in H9. A heavy mixed growth of bacteria was isolated from the lung. H13 had fibrinonecrotic and heterophilic aspiration pneumonia with intra-lesional coccobacilli. Heterophilic aspiration pneumonia and fibrinous air sacculitis were present in H23. *E. coli* and *Citrobacter workmanii* were isolated from the liver.

One septicaemic chick (H30) had fibrinonecrotic to granulomatous bronchopneumonia with intralesional rods. A heavy growth of *E. coli* was isolated from the liver. H39 had mild heterophilic and lymphocytic interstitial pneumonia. One chick (H6) with a head injury had pulmonary haemorrhage.

Three septicaemic chicks had heterophilic air sacculitis but not pneumonia, including a chick with bacterial ventriculitis and visceral gout (H15). An *Enterococcus* sp. was isolated from the lung. A septicaemic chick from which no bacteria were isolated (H20), and one (H32) from which *Salmonella typhimurium* was isolated also had air sacculitis. In addition, one harvested chick without septicaemia (H4) had heterophilic air sacculitis.

### *Cardiovascular system*

Three septicaemic chicks had visceral or parietal heterophilic or fibrinonecrotic pericarditis (H22, H30, H32). Moderate perivascular myocardial fibrosis was present in one of these chicks (H32). Mild myocardial haemorrhage was noted in a chick (H3) that did not hatch. A chick (H33) with pancreatic necrosis had mild multifocal myocardial necrosis. One chick (H34) was diagnosed with acute heart failure since it had widespread tissue congestion but no other life-threatening lesions.

### *Lympho-haemopoietic system*

Splenic haematopoiesis was present in seven chicks with inflammatory disease, including those with dermatitis (H3); aspiration pneumonia (H9); bacterial ventriculitis (H14); septicaemia (H20, H22); fungal pancreatitis and enteritis (H31); and a chick in which the cause of death could not be established (H39). Haematopoiesis was also present in three chicks without inflammatory disease, including those that suffered from traumatic head injury (H6); sudden death with no detectable cause (H17); and metabolic bone disease (H29).

Splenic lymphoid depletion was present in two chicks with ventriculitis (H11, H15), and in chicks with visceral gout (H8), aspiration pneumonia (H13) and salmonellosis (H32). One chick (H3) also had splenic lymphoid atrophy. Fibrinonecrotic and/or heterophilic splenitis was seen in a chick with bacterial ventriculitis (H15), one with aspiration pneumonia (H23), and four with septicaemia (H25, H27, H30, H32). Two septicaemic chicks (H20, H32), one with bacterial ventriculitis and enteritis (H21), and one with metabolic bone disease (H29) had splenic reticuloendothelial hyperplasia. H14 (a chick with ventriculitis) and H40 (a chick with liver failure) had splenic follicular lymphoid hyperplasia. H40 also had splenic haemosiderosis. Splenic lymphocyte necrosis was seen in two chicks with ventriculitis (H11, H18). Splenic congestion was present in two chicks (H34, H35).

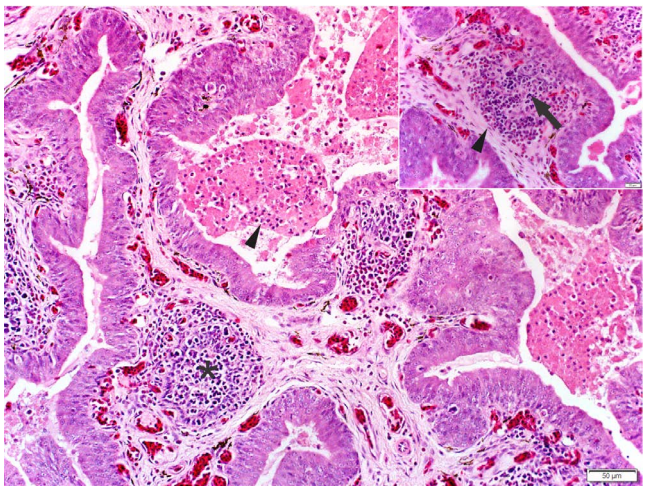

**Figure 5:** Lymphoid necrosis, atrophy and heterophilic inflammation in the bursa of Fabricius from a 21-day-old Southern ground-hornbill chick. Bursal lymphoid tissue (*) is depleted, with necrotic debris in the lumen (arrowhead). Haematoxylin and eosin. Inset: heterophils in the submucosa (arrowhead) and necrotic lymphocytes (arrow). Haematoxylin and eosin.

Thymic lymphoid atrophy and necrosis was present in two chicks with ventriculitis (H28, H36), and one with sciatic neuritis (H35). Two chicks with ventriculitis (H18, H28) and a septicaemic chick (H20) had thymic lymphoid necrosis.

Lymphoid atrophy of the bursa of Fabricius was present in a chick that failed to hatch (H3), one in which the cause of death could not be established (H10), four septicaemic chicks (H22, H23, H25, H27), two chicks with ventriculitis (H28, H36), one with salmonellosis (H32) and one with acute heart failure (H34). Lymphoid necrosis in the bursa was present in H2 and H3 (chicks that were hatching failures), H10 (a chick with mild traumatic injury but no other lesions), H11 and H18 (chicks with ventriculitis) and H22 (a septicaemic chick). H3 and H25, which had *E. coli* septicaemia, had heterophilic bursitis (Figure 5).

### *Urinary system*

Haematopoiesis was seen in chicks of all ages. Renal gout was present in two chicks with ventriculitis (H15, H28), three with septicaemia (H20, H22, H30, H32), one with aspiration pneumonia (H23), one that starved (H37) and one with liver failure (H40). H40 also had renal tubular degeneration and necrosis. Renal tubular degeneration and/or necrosis unrelated to visceral gout was seen in chicks with a head injury (H6), gizzard impaction (H24), metabolic bone disease (H29), fungal enteritis and pancreatitis (H31), pancreatic necrosis (H33) and a chick of uncertain cause of death (H39). Heterophilic interstitial nephritis was present in a chick (H15) with ventriculitis and another with *E. coli* septicaemia (H25). Only two chicks had small numbers of intra-tubular mineralised foci: H19 (with hepatic necrosis) and H21 (with ventriculitis and enteritis). Glomerulonephritis was present in a chick with salmonellosis (H32). The kidney was congested in a chick with widespread tissue congestion due to heart failure (H34).

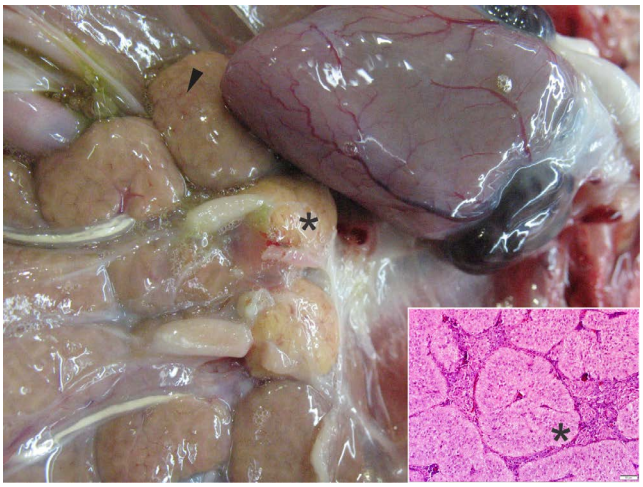

**Figure 6:** Inter-renal cell hypertrophy in a 21-day-old Southern ground-hornbill chick (H25). Note the enlarged adrenal gland (*) adjacent to the cranial portion of the kidney (arrowhead). Haematoxylin and eosin. Inset: Inter-renal cell lobules (*) dwarf intervening chromaffin cells.

### *Central nervous system*

Meningeal haemorrhage was present in a chick with traumatic head injury (H6). H27 was a blind septicaemic chick with necrohaemorrhagic and heterophilic encephalitis. H35 had lymphoplasmacytic neuritis with axonal degeneration. No pathogens were isolated from the spleen on bacterial culture.

### *Skin*

One chick (H3) that had voided waste in the egg also had diffuse cutaneous oedema with heterophilic dermatitis. No bacterial pathogens were isolated from the yolk sac. H5 had focal mild necrotising dermatitis. A chick with *E. faecalis* septicaemia (H22) had diffuse heterophilic dermatitis with intralesional cocci.

### *Endocrine system*

Seven chicks showed adrenocortical (inter-renal) cell hypertrophy (Figure 6): three chicks with *E. coli* infections (H25, H27, H30), a chick with aspiration pneumonia (H9), one with septicaemia (H20), a chick with ventriculitis (H21) and one with *E. faecalis* septicaemia (H22).

Lymphocytic thyroiditis was present in a chick (H34) with acute heart failure. Parathyroid hyperplasia was seen in six cases, including a chick with ventriculitis (H18), two chicks with *E. coli* septicaemia (H25, H30) and one each with *E. faecalis* septicaemia (H22), sciatic neuritis (H33) and acute heart failure (H34).

### *Musculoskeletal system*

A chick with metabolic bone disease (H29) had acute skeletal muscle necrosis of the pectoral muscle, and one with sciatic neuritis (H35) had mild skeletal muscle degeneration. Five chicks had metabolic bone disease. Rickets was present in two chicks with *E. coli* septicaemia (H27, H30), one with ventriculitis (H28), and one with salmonellosis (H32). H29 had multiple deformed soft bones as a result of a combination of rickets and fibrous osteodystrophy.

## Discussion

Hornbill chicks are altricial (Kemp & Kemp 1980) and are therefore unable to thermoregulate or feed themselves and have a poorly developed immune system at hatching (Flammer & Clubb 1994). Medical problems are common in chicks in the first week of life (Flammer & Clubb 1994; Meyer 2016). In this series 46% of chicks died in the first two weeks. Causes of death in very young chicks included late incubation or death during hatching. Since the shells were normal, disease in the hornbill hens was likely not the cause (R.E. Schmidt, D.R. Reavill & Phalen 2003a). The exact cause of death in chicks that failed to hatch could not be determined. Closer supervision of hatching, training on proper egg turning techniques, and maintenance of correct incubator temperature and humidity should be employed to ensure chicks are strong enough to hatch (Joyner 1994; R.E. Schmidt, D.R. Reavill & Phalen 2003a; Meyer 2016). The importance of incubator humidity and handling during or after the hatching phase is illustrated by very young chicks with cutaneous oedema, dehydration, dermatitis, and traumatic injury (R.E. Schmidt, D.R. Reavill & Phalen 2003a).

In both captive bred and wild harvested chicks, non-infectious conditions noted in the first 11 days of age included dehydration (H4, H5, H8), and hyperthermia (H10). Since it is easier to foster eggs than chicks (Flammer & Clubb 1994), and since the second chick in SGH nests never survive (Kemp & Kemp 1980), candling of the eggs to ensure that chicks are harvested on day one is recommended to ensure the best possible chance of survival. The dehydration, visceral gout, hepatocellular, and pancreatic atrophy seen in this series could be indications of anorexia in weak or sick chicks (Koeppel & Kemp 2022). Pulmonary atelectasis could be due to insufficient respiratory effort due to weakness.

The most common cause of death of the chicks of all ages in this study was bacterial infection (15/40, 38%). Necropsies on hornbill chicks should therefore routinely include culture of the spleen and/or lung as well as any macroscopically visible lesions. Proper hygiene and prompt recognition and treatment of infections are essential for hand raising hornbills (Flammer & Clubb 1994; Galama, King & Brouwer 2002; Meyer 2016). Diagnosis of septicaemia was based on the presence of heterophilic inflammation in multiple organs, particularly the respiratory and gastrointestinal systems and the spleen (Table II). In only eight of these cases, bacteria were histologically present in the inflammatory lesions. In five chicks with intra-lesional bacteria, samples were not submitted for culture due to the absence of macroscopically visible signs of infection. Heterophilic inflammation was presumed to be due to bacterial infection in three chicks, although bacteria were not seen histologically and samples were not submitted for culture. Culture results were likely affected by ante-mortem antibacterial therapy.

Three potentially pathogenic bacteria were cultured from five chicks: *E. faecalis*, *E. coli* and *S. typhimurium*. Only *S. typhimurium* is a primary pathogen (Koeppel & Kemp 2022); *Enterococcus spp*. and *E. coli* are opportunistic pathogens. The gastrointestinal microbiome of hornbills is not well documented (Sun et al. 2019). *Campylobacter* may be a contaminant as it was isolated from the brain and intestine of a chick with gizzard impaction,

although the sections of the brain and intestine did not have any compatible lesions. *Citrobacter workmanii* was isolated from the liver of a chick with aspiration pneumonia, and was considered a contaminant or opportunistic pathogen.

Environmental or management factors that could make chicks susceptible to opportunistic infection include a poorly developed neonatal immune system in altricial chicks, stress and other factors causing immune suppression in hand reared birds, hygiene, and improper environmental temperature and humidity (Flammer & Clubb 1994; Galama, King & Brouwer 2002; Koeppel & Kemp 2022). All seven chicks with inter-renal cell hyperplasia had bacterial infections, suggesting that they were stressed. Both harvested and captive born chicks were affected. Cool environmental temperatures predispose passerine chicks to salmonellosis (Trupkiewicz, Garner & Juan-Sallés 2018), so incubator temperatures should be carefully monitored. Chicks may be exposed to pathogens in food and on food preparation utensils and personnel, so careful cleaning can limit the risk associated with these sources (Flammer & Clubb 1994).

Lymphoid atrophy or necrosis of the bursa of Fabricius, spleen and thymus were relatively common. The true prevalence of these lesions and their relationship to normal age-related changes (R.E. Schmidt, D.R. Reavill & Phalen 2003b) is uncertain because these organs were not always sampled and the number of birds examined was small. Splenic lymphoid atrophy and bursal lymphoid atrophy and necrosis occurred in birds of all ages, suggesting that they were not primarily age-related. However, thymic lymphoid atrophy and necrosis was only seen in chicks older than 12 days; and splenic reticuloendothelial cell hyperplasia was only seen in birds older than 13 days so these could be age-related changes.

Stress due to poor hand-rearing techniques, disturbed resting periods between feeds, acute feed restriction or fasting can cause immune suppression and increased susceptibility to disease (Flammer & Clubb 1994; R.E. Schmidt, D.R. Reavill & Phalen 2003b). Splenic and thymic lymphoid atrophy and necrosis only occurred in chicks with inflammation in other organs. A four-day-old chick had both bursal lymphoid atrophy and necrosis, but no other lesions. In nine other chicks with bursal lymphoid atrophy, inflammatory conditions were present. Bursal lymphoid necrosis was present in a chick that failed to hatch; as well as in four other chicks with inflammatory disease. It is therefore possible that lymphoid atrophy and necrosis were associated with increased disease susceptibility.

Apart from age and stress, malnutrition and toxicity can affect the immune system, including calorie, vitamin A or zinc deficient diets (Cui et al. 2004), exposure to aflatoxins or lead and treatment with anthelmintics (R.E. Schmidt, D.R. Reavill & Phalen 2003b). Anthelmintics were not used in the chicks in this study. Exposure to lead occurs in SGH (Koeppel & Kemp 2015) but none of the chicks in this series hatched from parent birds with confirmed lead toxicity. Bacterial ventriculitis was relatively common (19%) as well as gizzard impaction (11%), so particular attention should be paid to feeding regimens of captive hornbill chicks (Meyer 2016). Possible causes of ventriculitis in chicks include traumatic injury to the koilin or mucosa by feeding tubes (R. Schmidt, Reavill & Phalen 2003c), inappropriate food temperature (Flammer & Clubb 1994) and immune suppression. No evidence of conditions such as ventricular nematodes, fungal infections or neurological changes was present; and the typical changes of zinc toxicity were absent. Inclusion of tough indigestible material in the diet (feathers/bones/skin or beaks from day old chicks) and large dry food items should be monitored since ingested fibrous material, sand, rocks, rubbish has been recorded as a cause of gizzard impaction in rheas (Flammer & Clubb 1994; Reissig & Robles 2001) and ingested baling string in layer chickens (Schlegel & Brash 2015).

Given that three birds had aspiration pneumonia, chick hand feeding best-practice protocols need to be followed. Feeding excessively large meals or feeding when there is no feeding response can predispose chicks to regurgitation and aspiration (Meyer 2016). Reduced gastrointestinal motility, leading to regurgitation or proventricular and gizzard impaction, may be reduced in weak or dehydrated birds. Gram-negative bacterial infections, hypothermia and ingestion of cold, poorly digestible or too large food items, or ingested bedding material may also play a role (Flammer & Clubb 1994).

Two chicks had soft deformed bones and metabolic bone disease. One also had bacterial ventriculitis and atrophic enteritis, so it is unknown whether maldigestion and/or malabsorption or imbalanced dietary calcium and phosphorus, were responsible for the bone disease in this chick. Choosing the correct substrate, feeding tube size and adequate exposure to sunlight are important to prevent leg deformities in growing chicks (Rehse 2014). Inclusion of the parathyroid gland for histological examination is useful for pathologists to better evaluate the pathogenesis of metabolic bone disease. Gizzard mineralisation may be due to excessive dietary calcium (R. Schmidt, Reavill & Phalen 2003c). As it is difficult to reproduce natural hornbill diets, which consist of intact or regurgitated food items (Galama, King & Brouwer 2002; Gamble 2015), nutritional disease in captive birds is not unexpected (Koeppel & Kemp 2022). Bone was only collected in a small proportion (28%) of chicks, so the true prevalence of bone disease is unknown.

The only non-bacterial infectious cause of death was the case of fungal enteritis and pancreatitis. Fungal infections may have similar predisposing causes as bacterial infections. Viral infections were suspected in a chick with hepatic necrosis (with no bacteria present), one with atrophic enteritis and one with lymphocytic sciatic neuritis but this could not be confirmed. In this study, therefore, bacterial infections were the most common infectious disease.

Common non-specific findings included tissue congestion; yolk sac, hepatic, renal and splenic haematopoiesis; and hepatic vacuolar change. The cause of tissue congestion was not always certain but septicaemia or acute heart failure may have contributed. The yolk is the primary source of haematopoietic cells (Stark 2020), which spread to the liver and other organs (Wong & Cavey 1993; R.E. Schmidt, D.R. Reavill & Phalen 2003b), so their presence in these organs is likely normal in juvenile hornbills including those up to three-months-old. Although less common, haematopoiesis in the pancreas of a septicaemic chick

with aspiration pneumonia may be a result of the increased requirement for inflammatory cells (Asakura & Rudnicki 2002).

The cause of the hepatic vacuolar change could not always be determined: lipidosis and or glycogen accumulation could have been responsible. Over-feeding as well as dietary fibre, vitamin and mineral deficiencies can result in hepatic lipidosis (Flammer & Clubb 1994). However, the degree of vacuolar change was usually mild and the condition was seen in chicks up to 34-days-old (past the age by which yolk fats are resorbed), so this may be a common insignificant feature in hornbill chicks. The cause of renal tubular degeneration and/or necrosis that was not related gout, may have been due to acute heart failure.

As this was a retrospective study, lesion prevalence may not be a true reflection of the prevalence of conditions in all juvenile hornbills especially for sciatic nerve, bone marrow and repro tract, which were rarely sampled. Similarly, as only two NGH and one BWCH were sampled, this paper can make no conclusions about the prevalence of the various conditions in these species.

## Conclusion

In these hornbill chicks, the first weeks of life were critical to survival. Bacterial infections, commonly of the respiratory and gastro-intestinal tracts, are significant causes of death. Managing factors that could influence susceptibility to infection are likely crucial in the rearing of hornbill chicks. The success of the captive breeding and re-introduction programme of the Mabula Ground-Hornbill Project is partially dependent on the captive rearing of wild-harvested or captive-bred Southern ground-hornbill chicks. This long-term detailed investigation of mortalities facilitated the ongoing refinement of rearing techniques, that may be applied to other hornbill species. The findings of this investigation were incorporated in the latest SGH rearing manual (Rehse 2014) and a textbook on ground-hornbill disease (Koeppel & Kemp 2022).

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The authors declare that no competing interests exist.

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