International Journal for Parasitology: Parasites and Wildlife 16 (2021) 83–94

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

# Equal contributions of feline immunodeficiency virus and coinfections to morbidity in African lions

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ARTICLE INFO

Keywords:
Panthera leo
Concomitant infections
Wildlife diseases

ABSTRACT

Feline immunodeficiency virus (FIV) is a pathogenic lentivirus related to human and simian immunodeficiency viruses that has been associated with AIDS-like pathologies in domestic and wild cats, as well as in hyenas. Despite known pathologies, progressive immunosuppression and ill health effects driven by these lentiviruses in association with other secondary infections remain understudied in free-ranging species. Here, the role of coinfections by gastrointestinal parasites and tick-borne hemoparasites for FIV disease progression was explored in 195 free-ranging African lions (*Panthera leo*) living in Kruger National Park (KNP), South Africa. Using statistical methodology, we evaluated the effects of FIV on a range of health indicators to explore how direct and indirect effects of FIV and associated coinfections align to determine lion health outcomes. Findings show direct negative effects of FIV on host immunity and nutritional status, and exacerbation of aggressive behaviors, conditions which may increase exposure/susceptibility to other secondary infections. When taken together, the contribution of coinfecting parasites to morbidity in lions is of similar magnitude as direct effects of FIV infection alone, suggesting that the particular coinfection assemblage may play a role in mediating disease progression within natural lion populations.

1. Introduction

Feline immunodeficiency virus (FIV) is a single-stranded RNA lentivirus that is closely related to well-known pathogens such as human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) (Pedersen et al., 1987; Brown et al., 1994; Biek et al., 2003; Kanzaki and Looney, 2004; Hartmann, 2011). While the pathogenicity of these viruses varies widely by strain, subtype, geographic location, and degree of host-pathogen coevolution, the general hallmark of infection is a protracted decrease in $CD4^+$ T helper cell levels with resultant immunosuppression, decreased resistance to parasitic exposures, progressive weight loss, and increased mortality (VandeWoude and Apetrei, 2006; Pecon-Slattery et al., 2008; Hartmann, 2011, 2012; Troyer et al., 2011).

FIV is primarily transmitted via saliva to blood contact during aggressive encounters and has been documented in 19 wild felid species found throughout Africa; North, Central, and South America; and Asia, as well as in domestic cats and hyenas (Troyer et al., 2005; VandeWoude and Apetrei, 2006; Antunes et al., 2008). While characterization of FIV infection in most wild felids is still on-going, evidence from natural and experimental infection in domestic cats supports altered immune cell levels in some infected hosts, which mimics AIDS in humans (Hofmann-Lehmann et al., 1997; Elder et al., 1998; Hartmann, 2011). Recent studies on FIV-ple (the lion-specific strain of the virus) in African lions (*Panthera leo)* of Botswana suggest similar health effects including wide scale immunosuppression, increased liver pathologies, and declining clinical health (Kennedy-Stoskopf et al., 2003; Roelke et al., 2006, 2009). Despite these advances, potential health interactions between FIV and other parasitic coinfections in this and other species with limited access to disease control remain difficult to characterize.

As an immunosuppressive pathogen, FIV may cause significant

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https://doi.org/10.1016/j.ijppaw.2021.07.003
Received 22 February 2021; Received in revised form 12 July 2021; Accepted 12 July 2021
Available online 19 August 2021

morbidity in host species by lowering the threshold for invasion and persistence of secondary infections (Bendinelli et al., 1995; Dean et al., 1998; Hartmann, 2012). Similar to HIV in humans, destruction of immune cells and immune dysregulation may lead to increased coinfections and inflammation; disease related nutritional deficiency may limit the host's ability to compensate by increasing investment in immunity; and organ pathology may break down mechanical barriers to parasitic invasion (Bendinelli et al., 1995; Dean et al., 1998; Hartmann, 2012). In the face of these changes, alterations to sex steroids and glial cell populations of the brain may serve to upregulate aggressive behaviors, further increasing transmission potential (Fromont et al., 2003; Hawley et al., 2011; Mccaig et al., 2011; Tejerizo et al., 2012). Historically, the role of immunosuppression-driven secondary infections in disease progression of FIV and other pathogenic lentiviruses has been difficult to assess, as management of immunodeficiency virus–associated disease in model species such as humans and domestic animals typically includes preventing or treating coinfections (Bentwich et al., 2000). However, in natural animal populations not housed in the sterile indoor environment of a lab, the chronic nature of lentiviral infection is likely to increase coinfection by common parasites due to the increased window of exposure throughout an individual's lifetime (Bentwich et al., 1999; Bentwich et al., 2000; Ivan et al., 2013). Thus, understanding how parasite coinfection assemblage contributes to disease progression is necessary to understand the impact of FIV in natural host populations, as these associations are likely to play a central role in mediating health outcomes of FIV disease.

To investigate the role of coinfections by gastrointestinal parasites and tick-borne hemoparasites in FIV disease progression, we conducted a structured survey of 195 free-ranging lions in Kruger National Park, South Africa. Specifically, we asked: (1) what are the consequences of FIV for lion health in terms of immunity, maintenance of body condition, aggressive behaviors, biochemical homeostasis, and clinical signs of disease; (2) to what extent does FIV infection alter the richness (total number) of coinfecting parasite species; and (3) what role do coinfections play in causing morbidity in FIV disease? Key findings suggest that FIV plays a crucial role in structuring host health and susceptibility through direct pathologies, but that the contributions of coinfecting parasites may be as important or even more critical when predicting disease progression and morbidity related to FIV.

2. Materials and methods

2.1. Ethics statement

Data and samples were collected between March 2010 and September 2013 from 195 free-ranging lions as part of a larger study on lion health, behavior, and demographics (PIs Funston and Ferreira). Lion captures were conducted by South African National Parks veterinary staff under AUCC permit #FERSM5-767. Chemical immobilization protocols used for sample collection were in accordance with SANPARKS Animal Use and Care Committee (AUCC) approved guidelines, as validated by previous clinical studies in this species (Jacquier et al., 2006; Wenger et al., 2010).

2.2. Sample collection

When possible, all pride members over six months of age were immobilized with a cocktail of either butorphanol (0.31 ± 0.034 mg/kg), midazolam (0.21 ± 0.024 mg/kg), and medetomidine (0.052 ± 0.006 mg/kg); or tiletamine-zolazepam (1.8 ± 0.5 mg/kg), and medetomidine (0.07 ± 0.01 mg/kg; Kyron Laboratories (Pty) Ltd, South Africa) delivered using a CO_2 pressured dart rifle (Dan-Inject International, Skukuza, South Africa; Jacquier et al., 2006; Wenger et al., 2010). After sample collection, animals received atipamezole (0.3 ± 0.1 mg/kg; Orion Pharma, Finland) intramuscularly for reversal. During immobilization, blood was collected via venipuncture into EDTA, sodium heparin, Cyto-Chex® BCT, erythrocyte sedimentation rate (ESR) ®, and whole blood (red-topped) tubes. Feces were collected directly from the rectum. A full physical examination was performed and the following demographics recorded: approximate age, sex, and pride membership. Age was estimated using established methods for lions (Smuts et al., 1978), and sex determined via visualization of genitalia. Pride membership was provided through known pride ranges and long-term behavioral monitoring data provided by the parent study.

2.3. Health, immunity, and behavior

In order to evaluate health and immune status in FIV-positive and negative individuals, this study used a combination of physical exam parameters, blood biochemistry and endocrinology, and functional and quantitative immune assays.

2.3.1. Physical examination

Each lion was evaluated for the presence of lesions associated with FIV or regionally endemic coinfections, which included gingivitis, oral papillomas, ocular lesions, lymph node enlargement, dehydration, and hygromas (Roelke et al., 2009). Body condition was assessed by a score (BCS) ranked from one (poor) to five (ideal) using fat coverage and palpation of bony prominences over the ribs, sacrum, hips, and stomach (Ferreira and Funston, 2010). Oral papillomas were identified via visualization of large raised plaques on the gingiva, tongue, or oral mucosa, while gingivitis was identified as inflammation or ulceration of the gingiva seen along the gum line and ranked on a scale of zero (no inflammation) to four (marked inflammation and ulceration; Roelke et al., 2009). Scores of two or above merited inclusion in the gingivitis-positive category. Ocular lesions were identified using light–facilitated examination of the cornea and anterior chamber of the eye for signs of corneal ulceration or infiltrate/increased vascularization that may signify uveitis. A gross examination of each lion was used to determine the presence of dehydration (seen as sunken eyes and decreased skin turgor over the ribs), hygromas (soft fluctuant swellings over the elbow), and peripheral lymph node enlargement (palpable enlargement of one or more lymph nodes). For further evaluation, degree of systemic inflammation was determined from the number of enlarged lymph nodes ranked on a scale from negative six (all nodes reduced) to six (all nodes enlarged). This score was calculated by adding the number of enlarged lymph nodes and subtracting the number of reduced nodes detected in the submandibular, prescapular, axillary, inguinal, and popliteal regions, as well as a separate "other" category that included lymph nodes that were only rarely palpable when enlarged, such as the lateral retropharyngeal and accessory axillary.

2.3.2. Blood biochemistry and endocrinology

Blood was collected via venipuncture of the jugular or femoral veins and transported to the laboratory at ambient temperature. Serum was separated from whole blood within 8 h of collection and stored at −60 °C until analysis. Liver (alanine aminotransferase [ALT], alkaline phosphatase [ALP]) and kidney (blood urea nitrogen [BUN], creatinine [CRE]) biochemical markers, blood glucose (GLU), and total protein (TP) concentrations were assessed using the commercially available Prep II Profile (Abaxis Inc.; PN 500–7124) run on an Abaxis VS2 Vet Scan machine (Abaxis Inc., Union City, California, USA).

Leptin and ghrelin were evaluated as a measure of body condition (Kotler et al., 1984; Yamamoto et al., 1989; Freeman et al., 2004). Leptin is secreted at times of high nutrient availability due to increased body fat, while ghrelin is secreted in response to food scarcity and resulting stomach emptiness (Shibata et al., 2003; Martin et al., 2010; Depauw et al., 2012). Serum for the ghrelin assay was collected from whole blood tubes, which were transported on ice until separation. Once separated, HCl was added to acidify the sample to prevent protein breakdown and all samples were stored at −60 °C until use. Plasma for leptin was collected from heparin tubes and was also transported on ice until

storage at −60 °C. Both hormones were measured at Oregon State University using commercially available RIA kits following manufacturer instructions (Millipore Inc., Billerica, Massachusetts, USA; PN XL-85K and GHRA-88HK). The Leptin Multispecies RIA kit and Human Acylated Ghrelin RIA kit were used due to strong cross-reactivity with the corresponding feline hormones (Martin et al., 2010; Depauw et al., 2012).

2.3.3. Hematology and immunity

Immune status of lions was examined using a combination of quantitative measures including clinical hematology, flow cytometry, erythrocyte sedimentation rate, and bactericidal killing capacity. White blood cell counts were obtained by electrical impedance counting via an automated hematology analyzer (Vet ABC, Scil Animal Care Company, Gurnee, Illinois, USA) from blood collected into EDTA tubes. Differential counts of lymphocytes and granulocytes were calculated via blood smears prepared using Hemacolor Diff-Quick Staining Solution (VWR International, Radnor, Pennsylvania, USA). Packed cell volume and total solids were measured from heparinized blood spun at 15000XG in a hematocrit centrifuge (Gemmy Industrial Corporation, Taipei, Taiwan; PN HKT-400) using microcapillary tubes and a refractometer (Optika, Ponteranica, Italy; PN HR-160).

Immunolabeling and flow cytometry were used to count populations of lymphocytes including $CD4^+$ T-helper cells, $CD8^+$ cytotoxic T cells, and $CD21^+$ B cells, which are known targets of FIV infection (Roelke et al., 2006). Cells were stored as whole blood in Cyto-Chex® BCT cell preservation tubes (VWR International, Radnor, Pennsylvania, USA; PN 87005-196) for no longer than 2 weeks at 4 °C. For quantification, cells were labeled with feline-specific antibodies against CD4, CD8, or CD21 cell surface markers and allowed to interact for 15 min. Following incubation, red cells were lysed to prevent sample contamination using lysis buffer (500 mls of deionized water with 4.13g NH_4Cl, 0.5g $KHCO_3$ [Sigma Aldrich, St. Louis, Missouri, USA; PN A0171, 60339, respectively], and 0.185g Na_4EDTA [Fisher Scientific, Waltham, Maryland, USA; PN S657]). Antibodies were then conjugated to horse anti-mouse IgG-FITC over a second 15-min incubation period (Vector Labs, Burlingame, California, USA; PN FI-2000). Control tubes with unlabeled cells were also provided for proper gating of cell populations. Felid-specific antibodies and protocols for their use in lions were provided by Peter Moore at UC Davis (Leukocyte Antigen Biology Laboratory, University of California, Davis, CA, USA) and final counts for each subset of lymphoid cells were performed by faculty at Onderstepoort Veterinary Institute's Flow Cytometry Laboratory in Pretoria, South Africa.

Erythrocyte sedimentation rate (ESR), which has been used as a gauge of systemic inflammation in late stage AIDS patients, was assessed following protocols validated in humans (Ndakotsu et al., 2008). Samples were collected into citrate ESR tubes and processed immediately upon return to the lab (Greiner Bio-One, Lasec Laboratory and Scientific, Cape Town, South Africa; Vacuette ESR Systems). ESR was measured at 60 and 120 min post initiation of the assay. A bactericidal assay, which measures the ability of whole blood (cellular and protein constituents) or plasma (protein constituents) to kill bacteria, was also conducted as a measure of innate immune function using protocols established for other wildlife species (Tieleman et al., 2005; Matson et al., 2006; Beechler et al., 2012).

2.3.4. Behavioral metrics

Aggressive encounters may speed the transmission of both FIV and other parasites (Fromont et al., 2003; Hawley et al., 2011; Mccaig et al., 2011). AIDS in other species is often associated with an increase in sex steroids that promote these behaviors (Tejerizo et al., 2012). As such, wounds and testosterone levels were quantified as proxies for aggression in lions. Wounds from bites and scratches were identified visually and noted when severe enough to penetrate the skin or if there was evidence of suppurative inflammation. Testosterone was measured using a commercially available EIA kit (Cayman Chemical, Ann Arbor, Michigan, USA; PN 582701) from serum samples stored at −60 °C.

2.4. Coinfections

2.4.1. Viral diagnostics

Diagnostic virology was run on serum samples using in-house ELISAs validated for use in lions by van Vuuren et al. at the Department for Veterinary Tropical Disease, Onderstepoort Veterinary Institute in Onderstepoort, South Africa (van Vureen et al., 2003). Exposure to canine distemper, feline parvovirus, feline enteric coronavirus, and feline calicivirus was measured qualitatively using antibody-based ELISAs. Feline immunodeficiency virus was also detected using antibody-based methods, but since FIV is a life-long infection, presence of circulating antibodies was presumed indicative of active infection (Hartmann, 2011). Specificity of the FIV diagnostic is 100%, while sensitivity is lower at 78.6% (van Vureen et al., 2003).

2.4.2. Gastrointestinal parasitology

Fecal parasites were detected through the use of standardized fecal sugar flotation and centrifugation techniques (Foreyt, 2001). An approximate number of worm eggs for each species of parasite was identified visually through microscopy and quantified as the number of eggs counted per gram of feces. Parasite families identified were solely those detected using flotation methods and included nematodes, cestodes, and protozoa (for a complete list of those identified reference Table 1).

2.4.3. Blood parasitology

Hemoparasite DNA to be used for infection diagnostics was extracted from stored EDTA whole blood using a Qiagen DNeasy kit (Qiagen, Hilden, Germany; PN 69506) and run using a polymerase chain reaction (PCR) based reverse line blot. This method was chosen due to its ability to detect multiple parasites at once, as well as its capability for catch-all detection of unidentified species. The PCR membranes used for this study contained existing markers for parasites previously identified in cheetahs and lions and included *Theileria, Hepatozoon, Babesia,* and *Ehrlichia* species (for a complete list reference Table 1). All PCR diagnostics were run by M. Oosthuizen (Molecular Diagnostic Services, Department of Veterinary Tropical Diseases, Onderstepoort Veterinary Institute, Pretoria, South Africa) according to established protocols in lions (Bosman et al., 2010; Kelly et al., 2014).

Table 1
Coinfecting parasite species identified in African lions from Kruger National Park, South Africa.

COINFECTIONS		
Hemoparasites	Gastrointestinal parasites	Viruses
Ehrlichia/Anaplasma spp.	Ascarid spp.	Canine distemper virus
Babesia felis	Tapeworm spp.	Feline calicivirus
Babesia leo	Hookworm spp.	Feline enteric coronavirus
Babesia microti	Whipworm spp.	Feline parvovirus
Babesia lengua	Coccidia spp.	Feline immunodeficiency virus
Babesia rossi	*Toxoplasma gondii*	
Babesia vogeli		
Babesia canis		
Hepatozoon spp.	***Parasite abundance***	***Parasite richness***
Theileria annae	Worm eggs per gram of feces	Number of parasite species per host
Theileria bicornis		

[a] Viral measures are based on seroprevalence (exposure) and do not denote active infection.

2.5. Data analysis

2.5.1. Statistical analysis

Direct associations between FIV and nutritional status, biochemical and clinical health, inflammation and immunity, aggression, and coinfection were explored using a combination of linear mixed (LMM) and generalized linear mixed (GLMM) models with varying error and function link structures as dictated by the data. First, to examine the direct associations between FIV infection and nutritional status, models with response variables for nutritional metrics (leptin, ghrelin, BUN, and hydration status), were compared against fixed effects for FIV status, age, sex, and body condition, as well as two-way interactions between age and FIV, sex and FIV, and a random effect accounting for pride membership. Inclusion of this last variable was necessitated by the fact that animals from the same pride are not fully independent (Bolker et al., 2009) due to commonalities in disease exposures, prey availability, and relatedness. Similar models were used to explore associations between FIV status and clinical or biochemical health, and included response variables for physical exam parameters (body condition score, gingivitis, oral papillomas, ocular lesions, lymph node enlargement, dehydration, and hygromas) or biochemical measures (ALT, ALP, BUN, CRE, GLU, TP), paired against the previously mentioned fixed and random effects. Models to test whether or not aggression increases in FIV positive lions also followed this format and included the same independent variables compared against the number of bites, scratches, suppurative wounds, and circulating levels of testosterone detected via physical examination or EIA methods. An increase in any of these variables was seen as a proxy for increased aggression. Finally, evaluation of coinfection prevalence in FIV-positive and FIV-negative animals was evaluated individually for each parasite of interest (Table 1), as well as in combination for parasite richness (the number of parasite species per host) and abundance (the number of eggs/oocysts per gram of feces) compared against all independent variables of interest.

While models for nutrition, aggression, coinfection, and health status followed similar formats, models for immune parameters (hematology, packed cell volume, total solids, ESR, and bactericidal ability) required the inclusion of an additional parameter to account for wound-derived systemic inflammation, which correlates significantly with circulating numbers of immune cells. Thus, models exploring the direct effects of FIV on immune dysregulation examined singular associations between each immune parameter of interest paired individually against independent variables for age, sex, FIV status, body condition, suppurative wounds, two-way interactions between FIV and age/sex, and a random effect for pride status. Within models for bactericidal ability, this last variable (pride status) was replaced by a random effect for "batch", which accounted for variability in bacterial plating between days (1–2 prides were captured per day).

All model assumptions were evaluated independently after exploring normality (via the Shapiro-Wilk's Test), residual patterns, and data overdispersion (R Foundation for Statistical Computing, 2016; Kleiber and Zeileis, 2008), and models fit using either LMMs or GLMMs using the nlme and lme4 packages, respectively (Bates et al., 2014; Pinheiro et al., 2015). Model selection was based on stepwise comparison of AIC values provided by the stepAIC function in the MASS package for LMMs (Venables and Ripley, 2002), or the dredge function in the MuMIn package for GLMMs (Bartoń, 2017). GLMMs were fit with a gaussian distribution and log link for continuous data; and a binomial distribution and logit link for binary data (Bartoń, 2017). Data was backtransformed for presentation. Final LMMs were modeled using REML (Pinheiro et al., 2015). As overdispersion was a common problem in count data, and means of Poisson modeled variables were not prohibitively small (>5), discrete count variables were modeled using penalized quasi-likelihood GLMMs in the MASS package (Venables and Ripley, 2002) and backwards selection via Wald T statistics provided by the aod package (Lesnoff and Lancelot, 2012). Samples with zero values were removed to avoid zero inflation.

In the case of flow cytometric cell counts, sample size was prohibitively small for FIV-negative individuals (n = 4). Thus, relationships between FIV status and $CD4^+$, $CD8^+$, and $CD21^+$ cell counts were examined using t-tests (R Foundation for Statistical Computing, 2016) independent of other fixed effects. To account for confounding variables, cell counts were also modeled against demographic parameters for age and sex using generalized linear models with a Quasipoisson distribution to account for overdispersion as indicated by the dispersiontest function in the AER package (Kleiber and Zeileis, 2008; R Foundation for Statistical Computing, 2016). Wound status, body condition score, and pride were not included as fixed effects in the full model to avoid further data partitioning. For quality control, animals with $CD4^+$, $CD8^+$, or $CD21^+$ cell counts significantly greater than the gated T/B cell catch-all measure were also dropped from the final analysis. Model selection was conducted via manual backwards selection using AIC values and p-values via the drop1 function (Kleiber and Zeileis, 2008).

2.5.2. Path analysis

Partial Least Squares Path Models (PLS-PM) are very useful in establishing causal relationships in observational studies on natural processes (Serrano et al., 2014), including the impact of diseases (Fan et al., 2016). Two types of relationships are described in PLS-PM. The first one involves relationships between the latent variables (e.g. a combination of physiological parameters defining an abstract concept such as "health status"), while the other considers the links between the latent variables (LVs) and its own block of manifest variables (MVs). These MVs are variables directly measured in the field or in the laboratory (e.g. body condition or leptin concentration). The PLS-PM approach does not require strong assumptions with respect to the distributions of the MVs, the sample size, or the measurement scale (Wold, 1985).

For our purposes, we have defined five blocks of LVs in our path model: FIV infectious status (0 for FIV-free and 1 for FIV-infected lions), immune response (IMM), hemoparasite infections (Hemoparasites), helminth infections (Helminths), and morbidity (Morbidity). Because of its importance for our research purposes, FIV infectious status was considered a latent variable with a single manifest variable (see examples in Aleuy et al., 2020; Serrano et al., 2014). Descriptive statistics for the set of manifest variables defining each block are shown in Table 2.

Additional manifest variables describing immune response or health status that were initially included in our path model, but were finally excluded because of their low contribution to their own LVs, are also shown in Table 2. Other viral (e.g., FCV, FPV, CDV) infections, as well as Toxoplasma gondii, detected in our lions were initially considered for being included in independent LVs, but finally excluded for not contributing to the path model due to low co-occurrence and overall prevalence in the population. Noting that old lions were more prone to be infected with FIV (mean age of FIV-infected lions = 5.5 yrs whereas age of FIV-free lions = 3.9 yrs; $t = -4.5$; $df = 131.8$; $p < 0.01$) the age of animals was also included as a LV.

When specifying the conceptual model, it was assumed that FIV and IMM should have both a direct and indirect impact (through Helminths and Hemoparasites) on Morbidity. The vector of outer weights (Wj) associated at each block of MVs was obtained as the vector of the regression coefficients in the multiple regression of the inner estimate of the LVs on their associated MVs (Tenenhaus et al., 2005). Each outer weight can be considered as a proxy for the importance of each MV in the construction of the LV. The path coefficients (β) were obtained by least squares regression of connected LV scores, and they were interpreted as standard regression coefficients (Vinzi et al., 2010). Finally, once the full model including all possible relationships between LVs (Table 2) was fitted, a model simplification was performed by removing those MVs uncorrelated with their own LVs and later those relationships between LVs with the lowest R2 values. The fit of the final model was measured by the goodness-of-fit index (Tenenhaus et al., 2004). Model parameters and fit indices were validated by bootstrapping. All the

Table 2
Description of latent and manifest variables used to fit a partial least squares path model in order to examine the relationships between FIV infection, immune status, coinfection with helminths or hemoparasites, and morbidity in 195 lions from Kruger National Park, South Africa.

Latent Variable	Manifest Variable (Units)	Descriptive statistics according to FIV infection status		Reference Range
		Free	Infected	
Feline Immunodeficiency Virus (FIV)	0 = FIV-free	27.20%	72.80%	–
	1 = FIV-infected	n = 53	n = 142	
Age (Age)	Years	3.9	5.5	–
Immune response (IMM)	Leukocytes (cells/ul)	17305.2 ± 1505.9 (5000–35050)	14200.8 ± 547.9 (5350–29000)	7139–31045
	Neutrophils (cells/ul)	10414.3 ± 970.4 (386.6–22256.3)	8654.7 ± 466.3 (583.3–28040.1)	3893–18545
	Lymphocytes (cells/ul)	2731.1 ± 308.4 (31.4–5390)	1726 ± 109.1 (22.7–4011)	761–7220
	Monocytes (cells/ul)	928.7 ± 139.4 (20.9–2430)	636 ± 62.5 (0–2804)	153–3606
	Eosinophils (cells/ul)	1709.4 ± 299.2 (73.2–7495)	1049.6 ± 98.4 (0–3985)	217–4015
	Basophils (cells/ul)*	37.8 ± 22.5 (0–350.5)	30.8 ± 9.49 (0–318)	0–1
	Packed cell volume (%)*	38 ± 0.9 (27.3–45)	38.2 ± 0.5 (20–51)	33.7–46.7
	Total solids (g/dl)*	8.4 ± 0.2 (8.0–9.5)	8.4 ± 0.1 (6.9–10.4)	7.5–9.1
	CD4 (cells/10000)	513.3 ± 17.9 (375–658)	412.5 ± 16.4 (77–978)	242–745
	CD21 (cells/10000)	434.7 ± 16.6 (223–542)	339.29 ± 16.7 (16–855)	119–1874
	CD8 (cells/10000)	277.7 ± 13.7 (100–401)	255.6 ± 10.6 (20–709)	51–1140
	ESR120 (units at 120 min)	49.3 ± 3.1 (8–104)	55.5 ± 2.3 (1–126)	7.8–107.6
	BKaWB (BKA)	35.7 ± 3.25 (2.5–59.2)	22.8 ± 1.5 (0–57.6)	–
	BKaPlasma (BKA)	15.2 ± 2.4 (0–53.9)	20.6 ± 1.2 (0–65.2)	–
Hemoparasite infections (Hemoparasites)	**Richness** (No. species/lion host)	2.4 ± 0.18 (0–4)	3.1 ± 0.10 (0–5)	–
Helminth infections (Helminths)	**Burden (eggs/gram of feces)**	4 ± 1.81 (0–81)	25 ± 6.6 (0–700)	–
	Richness (No. taxa/lion host)	0.34 ± 0.08 (0–2)	0.83 ± 0.08 (0–3)	–
Health impairment (HealthIMP)	**Emaciation (- log BCS)**	−1.14 ± 0.003 (−1.2 to −1.08)	−1.13 ± 0.002 (−1.2 to −1.04)	–
	ALT (U/L)	23.3 ± 1.5 (4–54)	39.2 ± 1.9 (4–133)	6.6–91.4
	ALP (U/L)	30.2 ± 3.6 (4–66)	32.1 ± 3.5 (4–129)	6.8–86.0
	GLU (mg/dl)	92.3 ± 5.9 (29–272)	96.2 ± 2.9 (9.8–244)	42.6–151.0
	BUN (md/dl)	48.1 ± 2.6 (19–96)	44.9 ± 1.42 (21–113)	8.3–78.5
	TP (g/dl)	10.1 ± 0.2 (7.5–14.9)	10.1 ± 0.11 (7.2–15.1)	–
	CRE (mg/dl)	1.5 ± 0.1 (0.1–3.2)	1.8 ± 0.14 (0.1–12.5)	0.8–2.6
	GHR (pg/ml)	21.3 ± 7.3 (10.5–35.2)	40.8 ± 37.3 (9.5–178.6)	11.4–37.5
	Lept (ng/ml)	3.1 ± 0.6 (0.07–27.1)	2.2 ± 0.3 (0.15–20.3)	0.3–4.8
Aggression*	Testosterone (ng/ml)*	12.1 ± 5.0 (0.6–91.8)	17.8 ± 3.9 (3.8–171.9)	0.06–10.5

[a]Values in the table describe the sample mean ± standard error, range minimum, and range maximum for each manifest variable included in the full path model. Asterisked parameters were measured for the purposes of this study, but were not included as manifest variables in the path analysis. Manifest variables marked in bold represent those that made a statistically significant contribution towards predicting the outcome of their respective latent variable. Only those values were retained for the final path analysis. Reference intervals for wild lions are provided for comparison and were generated using clinically healthy animals from the same sample population (Broughton et al., 2017).

variables have been standardized (mean = 0, var = 1) for our PLS-PM purposes. The final sample size in our PLS-PM was 106 lions once excluding individuals with missing information in our manifest variables. This statistical procedure was performed using the package 'plspm' version 0.4.736 (Sanchez et al., 2017) of the statistical software R version 4.1.0. (R Foundation for Statistical Computing, 2021).

3. Results

FIV infection was common in KNP lions, affecting 142 of the total 195 sampled (72.8%; 95% CI = 66.5–79.1%). Prevalence of FIV infection was similar among male (75.6%; 95% CI = 65.8–85.3%) and female (70.2%; 95% CI = 62.1–78.3%) lions and between park regions, but increased with host age ($n = 195$; $\beta = 1.29$; *p-value* < 0.01; for a complete demographic breakdown reference Fig. 1). Overall, results of this study showed negative effects of FIV infection on host nutrient balance, clinical health, and immunocompetence, as well as alterations to endocrine and behavioral parameters that may reflect increased aggression in FIV-positive hosts.

3.1. FIV infected lions are malnourished

Lions infected with FIV showed higher levels of serum ghrelin ($n = 194$; $\beta = 3.40$; *p-value* < 0.01), indicating low frequency of feeding; lower levels of leptin ($n = 194$; $\beta = 0.23$; *p-value* < 0.01), indicating decreased fat reserves; and reduced blood urea nitrogen ($n = 193$; $\beta = 0.88$; *p-value* = 0.04), reflecting reduced protein intake. Together, these findings suggest impaired resource acquisition or retention in FIV-positive hosts (Fig. 2a; Table 2 and S1Table).

3.2. FIV infected lions show biochemical alterations and clinical signs suggestive of feline AIDS

FIV infection was associated with an increase in liver enzymes (ALP and ALT; $n = 193$; $\beta = 1.89$ and 1.65; *p-values* < 0.01 and 0.01, respectively; Table 2 and **S1 Table**); an increased prevalence of ocular lesions ($n = 184$; $\beta = 10.19$; *p-value* < 0.01); and increases in gingivitis with age ($n = 181$; $\beta = 1.63$; *p-value* = 0.04). Contrastingly, FIV infection was associated with a lower prevalence of oral papillomas ($n = 180$; $\beta = 0.50$; *p-value* = 0.04; Fig. 2a; **S1 Table**).

3.3. FIV alters inflammation and immunity

FIV-positive lions showed an increase in erythrocyte sedimentation rate ($n = 100$; $\beta = 3.10$; *p-value* = 0.02); and higher total solids ($n = 94$; $\beta = 1.04$; *p-value* = 0.05), suggesting elevated protein levels. Infected animals also showed a reduction in total lymphocyte count ($n = 90$; $\beta = 0.58$; *p-value* = 0.04; Fig. 2a–b; Table 2 and **S2 Table**). While no difference was detected in flow cytometric counts for $CD4^+$, $CD8^+$, and $CD21^+$ cells based solely on FIV status ($n = 57$; $t = 1.69$, 0.52, and −0.94; $p = 0.17$, 0.63, and 0.41, respectively; Table 2 and **S3 Table**), $CD4^+$ and $CD8^+$ helper cell populations decreased with age ($n = 57$; $\beta = 0.94$ and 0.94; *p-values* < 0.01 and 0.02, respectively; Fig. 2c), suggesting progressive age-related impairment of adaptive immunity, including humoral and cell-mediated responses (**S3 Table**). Monocytes and neutrophils also showed progressive declines with age, with a more

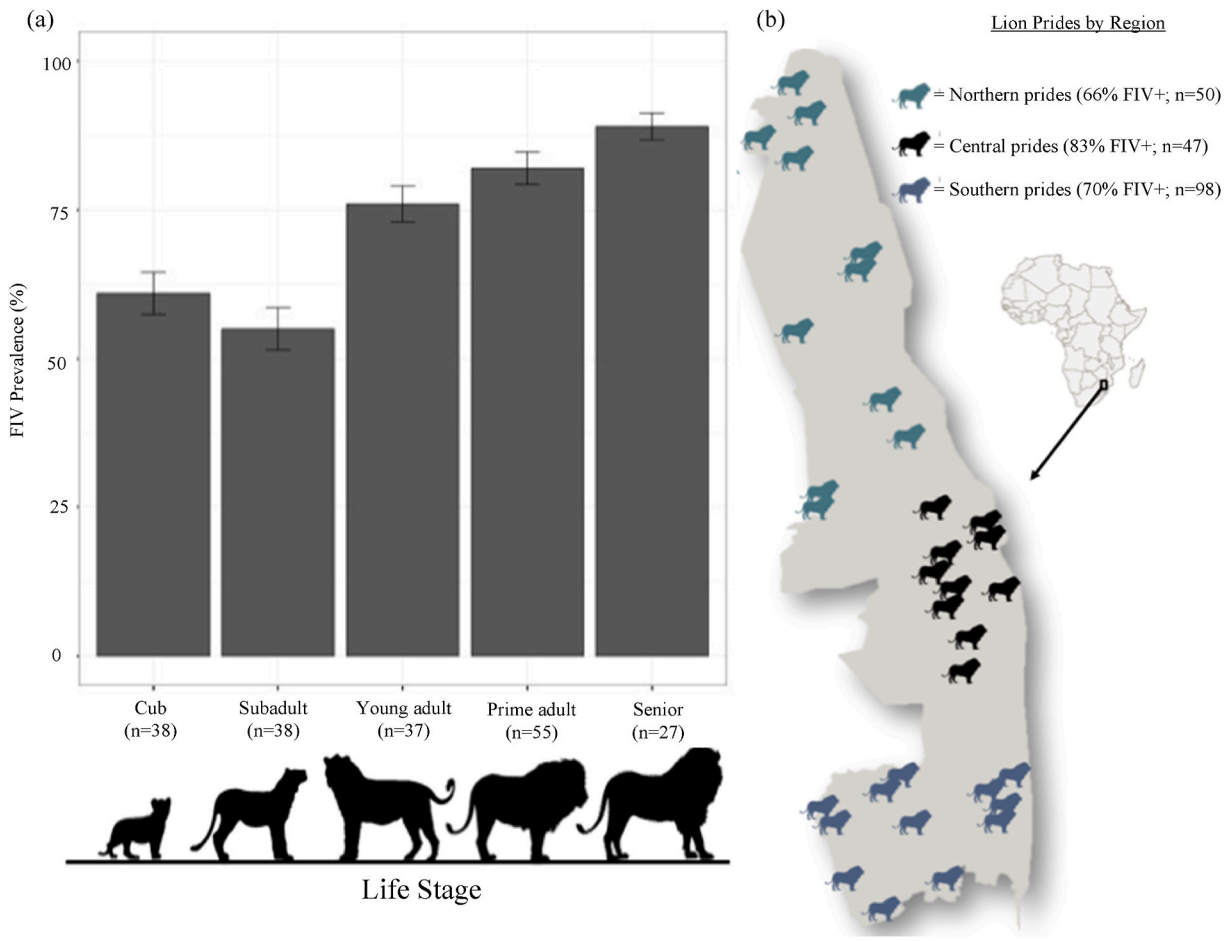

Fig. 1. FIV infection is common in lions of Kruger National Park. Within the sample population, 72.8% (142/195) of lions were infected with FIV. Prevalence of FIV infection was similar between males and females (75.6% versus 70.2%; $n = 74$ and 121, respectively), but increased with host age (a). For ease of visualization, age has been broken up by life stage into cubs (0–2yrs), subadults (2.1–4yrs), young adults (4.1–6yrs), prime adults (6.1–8yrs), and seniors (>8yrs) based on previous age classifications (Schaller, 1976). Regional prevalence of FIV was highest in the central region and lowest in the north (b). The map to the right shows locations where lion prides were sampled.

drastic decrease in FIV-infected lions, supporting a reduction in innate immunity ($n = 90$; $\beta = 0.85$ and 0.94; *p-values* < 0.01 and 0.03, respectively; Table 2 and **S2 Table**).

3.4. FIV may increase aggressive behaviors or delay tissue healing

FIV-positive lions exhibited a higher risk for severe wounds when compared to FIV-negative individuals ($n = 193$; $\beta = 3.76$; *p-value* $= 0.04$), suggesting increased fighting, more severe outcomes of fights, or reduced ability to heal wounds. Male FIV-infected lions had higher testosterone levels ($n = 73$; $\beta = 3.36$; *p-value* $= 0.03$) than their FIV-negative counterparts, again pointing towards a potential for increased aggression; however, FIV-positive females had lower testosterone levels than healthy females ($n = 119$; $\beta = 0.44$; *p-value* < 0.01; Fig. 2a; Table 2 and **S4 Table**).

3.5. FIV increases the richness and abundance of coinfecting parasites

This study measured exposure to 21 different parasite taxa known to infect lions. Overall, infections were common and diverse among KNP lions, with individuals typically hosting from one to twelve of the taxa we identified (*median = 7; IQR* = 3; Fig. 3a). Several of the taxa were detected only in FIV-infected lions: feline enteric coronavirus (4% prevalence), whipworms (*Trichuris* spp.; 9% prevalence), *Toxoplasma gondii* (12% prevalence), *Theileria annae (0.07%* prevalence*),* and *Theileria bicornis* (4% prevalence). Gastrointestinal parasite infections were strikingly common in FIV-positive lions (Fig. 3a). In addition to *T. gondii* and whipworms, infections with tapeworms (*Taenia* and *Echinococcus* spp.; $n = 114$; $\beta = 2.87$; *p-value* $= 0.03$), hookworms (*Ancylostoma* spp.; $n = 114$; $\beta = 8.51$; *p-value* $= 0.01$), and coccidia (*Isospora* spp.*;* $n = 114$; $\beta = 221.02$; *p-value* $= 0.02$) were increased among FIV-positive lions (**S5 Table**). Total gastrointestinal parasite burden reflected this trend and was increased in FIV-positive hosts ($n = 104$; $\beta = 4.73$; *p-value* $= 0.03$), largely driven by a marginal increase in the intestinal burden of hookworm species ($n = 48$; $\beta = 4.67$; *p-value* $= 0.09$; **S5 Table**). Finally, overall richness of gastrointestinal parasites was significantly higher in FIV-positive hosts ($n = 114$; $\beta = 1.61$; *p-value* $= 0.02$; Fig. 3b).

The prevalence of *Babesia microti,* a tick-transmitted hemoparasite, was also higher in FIV-positive lions ($n = 190$; $\beta = 2.19$; *p-value* $= 0.05$; Fig. 3a), as was hemoparasite richness ($n = 190$; $\beta = 1.35$; *p-value* $= 0.04$; Fig. 3c). Interestingly, despite these trends, overall parasite richness, including both gastrointestinal and hemoparasites, was not statistically different in FIV-positive and FIV-negative lions ($n = 111$; $\beta = 1.17$; *p-value* $= 0.12$; **S5 Table**), suggesting a trade-off in infection prevalence between groups.

3.6. FIV affects lion health directly and indirectly via immunity and coinfections

In our path analysis, we asked whether progressive wasting and organ damage associated with FIV are mediated directly due to FIV infection, or indirectly via increased coinfections. Our models reveal

Box 2a: Independent variables for health evaluated in this study

CATEGORY	VARIABLE	CHANGE WITH FIV
Health	Alkaline Phosphatase (U/L) - ALT	↑
	Alanine Aminotransferase (U/L) - ALP	↑
	Blood urea nitrogen (mg/dl) -BUN	↓
	Creatinine (mg/dl) - CRE	Ø
	Glucose (mg/dl) - GLU	Ø
	Total protein (g/dl) -TP	Ø
	Gingivitis (% prevalence)	Ø
	Oral papillomas (% prevalence)	↓
	Ocular lesions (% prevalence)	↑
	Lymph node enlargement (% prevalence)	Ø
	Hygromas (% prevalence)	Ø
Nutrition	Dehydration (% prevalence)	↑
	Leptin (ng/ml) - GHR	↓
	Ghrelin (pg/ml) - Lept	↑
Immunity	Packed cell volume (%)	Ø
	Total solids (g/dl)	↑
	Leukocytes (cells/ul)	Ø
	Neutrophils (cells/ul)	Ø
	Lymphocytes (cells/ul)	↓
	Monocytes (cells/ul)	Ø
	Eosinophils (cells/ul)	Ø
	Basophils (cells/ul)	Ø
	CD4+ T lymphocytes (cells/10000)	Ø
	CD8+ cytotoxic T lymphocytes (cells/10000)	Ø
	CD21+ B lymphocytes (cells/10000)	Ø
	Erythrocyte sedimentation rate (units 120 min) -ESR120	↑
	Bactericidal activity whole blood (BKA) - BKaWB	Ø
	Bactericidal activity plasma (BKA) - BKaPlasma	Ø
Aggression	Testosterone in males (ng/ml)	↑
	Testosterone in females (ng/ml)	↓
	Severe wounds (% prevalence)	↑

↑Increase ↓Decrease Ø No change

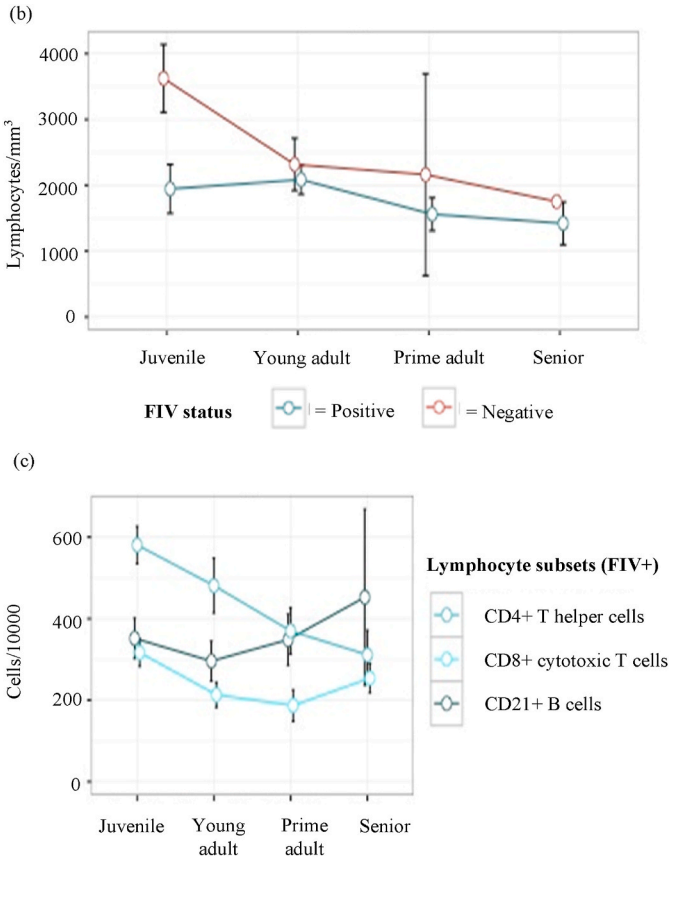

Fig. 2. FIV has broad effects on lion health and is associated with progressive immune impairment. Box (2a) above provides a complete list of the health metrics measured for the purposes of this study. Arrows to the right of each variable summarize the directionality of statistically significant changes with FIV infection. Descriptive statistics and reference values can be found in Table 2. Complete model output can be found in supplementary tables S1–S4. For ease of visualization, each parameter has been broken into categories of clinical relevance. To the right, age-related changes in lymphocyte profiles are shown for total lymphocyte counts in FIV-positive versus FIV-negative lions (b); as well as specific lymphocyte subsets in FIV-positive lions (c). Due to small sample size for lymphocyte subsets, subadults, and cubs have been included together under the 'juvenile' category.

three major findings. First, FIV, hemoparasites, and gastrointestinal parasites have direct negative effects of similar magnitude on lion health (Fig. 4 and Tables 3a and 3b), but they explain different aspects of morbidity. All three contribute to emaciation based on body condition score, but FIV and hemoparasites are associated with liver pathology (ALT) while GI parasites are correlated with the most variation in leptin and ghrelin levels (Fig. 4, Table 3a). Second, FIV-related immune impairment itself contributes strongly to ill health in lions in addition to the infections that we measured (Fig. 4). In fact, the effects of immunity on health parameters are equal to, or stronger than, direct effects of FIV and coinfections (Tables 3a and 3b). Negative health trends are associated primarily with low B cell populations and inflammation (ESR; Tables 3a and 3b). Finally, FIV shows strong indirect associations with hemoparasitic and gastrointestinal coinfections through its effects on host immunity (Fig. 4 and Tables 3a and 3b). GI parasite coinfections show strong associations with low B cell populations and increased inflammation (ESR); whereas hemoparasites were associated with low general populations of lymphocytes, as well as poor bactericidal activity of whole blood (Tables 3a and 3b).

4. Discussion

As indicated by our results, FIVple-infection in lions with viral subtypes A, D, or E (as identified via PCR in a partner study; Kerr et al., 2018) is characterized by immune impairment and dysfunction associated with nutrient imbalance and progressive wasting, as well as clinical and biochemical changes commonly associated with feline AIDS (FAIDS) (Roelke et al., 2009; Hartmann, 2011, 2012). Importantly, findings suggest that these morbidities may be driven in similar parts by FIV itself, and by gastrointestinal and hemoparasitic coinfections, which appear to be facilitated by FIV-related immune dysfunction. Overall, these findings suggest that coinfections may play a strong role in shaping FIV-mediated disease progression in lion hosts.

Using quantitative metrics, alterations in immune parameters were common in FIV-positive hosts. Total lymphocyte counts were lower; $CD4^+$ and $CD8^+$ T cell populations decreased with age in infected animals, as did counts of innate effector cells including monocytes and neutrophils; and inflammation, as measured by erythrocyte sedimentation rate (ESR) and total solids, was elevated. Observed changes to immune parameters were consistent with trends previously identified in domestic cats infected with FIV-fca, which includes broader cell tropism than HIV and SIV, a difference that leads to infection and destruction of B lymphocytes, T lymphocytes, regulatory T cells, and macrophages (Ackley et al., 1990; Brown et al., 1991; Yamamoto et al., 2007, 2010; Reggeti et al., 2008; Elder et al., 2008; Murphy et al., 2014). Immune dysfunction may be further supported in FIV-positive lions by elevations in ESR and total solids. In humans and domestic cats, these elevations have been shown to correlate well with a shift towards general immune overstimulation – a compensatory response to immune impairment due to $CD4^+$ T-helper cell depletion (Bendinelli et al., 1995; Ndakotsu et al., 2008; Roelke et al., 2009; Hartmann, 2012).

Coinfections were overwhelmingly common in the lion population.

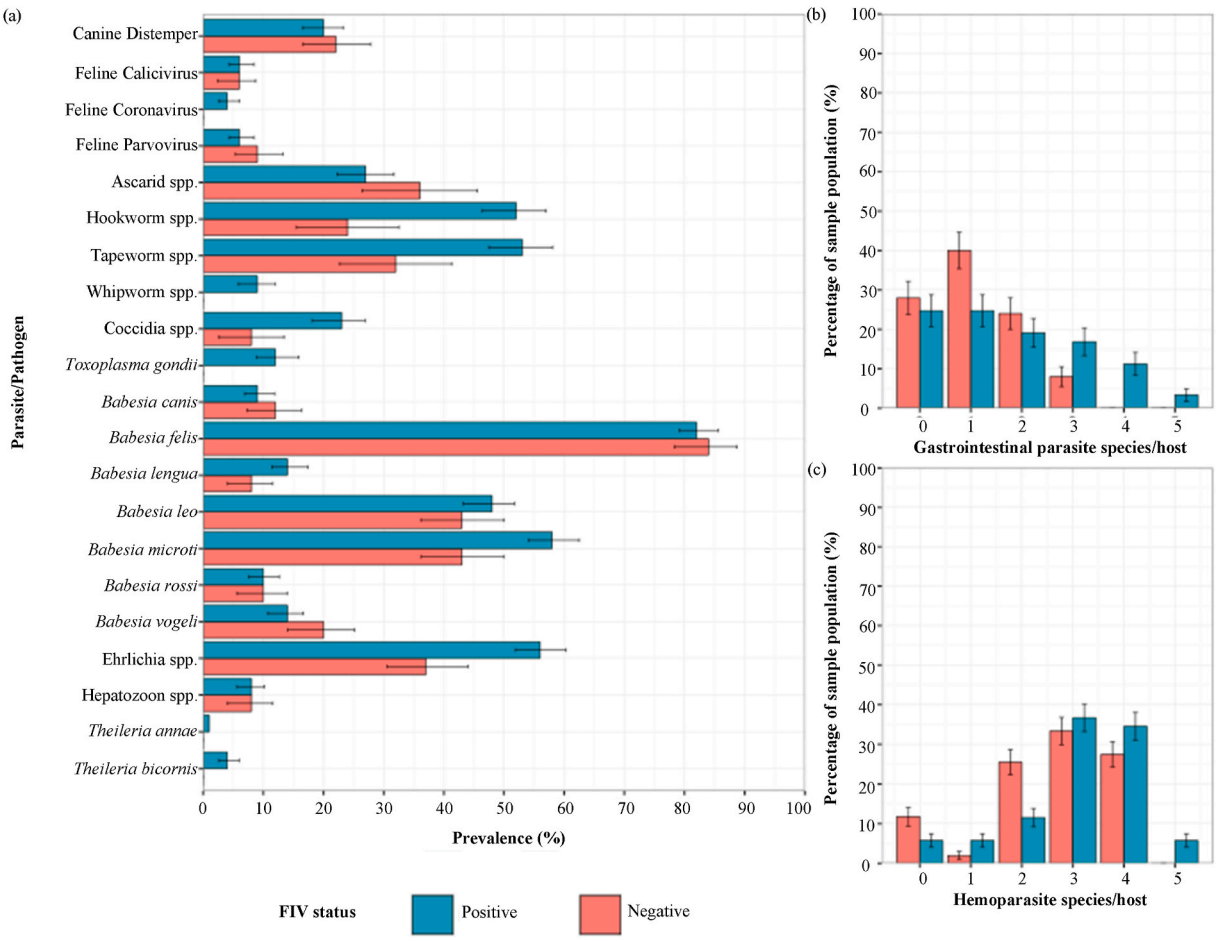

Fig. 3. FIV significantly increases the prevalence of select gastrointestinal and hemoparasitic coinfections, as well as overall parasite richness for both groups. Graph (a) shows the prevalence of coinfecting parasites isolated in lions from this study. Sample size for the parasite groups included is as follows: n = 114 for gastrointestinal parasites; n = 190 for hemoparasites; and n = 195 for viral parasites. Coinfections are broken down by FIV status (positive versus negative). The two additional graphs illustrate the relationship between FIV status and gastrointestinal parasite richness (b) and hemoparasite richness (c).

FIV-positive lions regularly exhibited increases in the richness and abundance of co-infecting parasites, which in-turn may contribute to FIV disease progression. Positive associations with FIV were particularly striking for the gastrointestinal parasites we investigated, with prevalence of hookworms, tapeworms, and coccidia increased, and whipworms and *Toxoplasma gondii* exclusively found in FIV-positive lions. Overall intestinal parasite burdens were increased with FIV infection and were driven largely by increased egg shedding by hookworm species based on parasite eggs/gram detected in feces. While mechanisms explainging these findings have not been fully identified, observed changes may be due to impaired gastrointestinal immunity, as similar lentiviruses (HIV and FIV-fca) have a strong affinity for $CD4^+$ T cells exhibiting either CCR5 (HIV) or CXCR4 (FIV-fca) chemokine coreceptors commonly found in gut associated lymphoid tissue (GALT) (Reggeti et al., 2008; Hartmann, 2011). As such, the correlations between FIV-infection and gastrointestinal parasite prevalence may reflect a breakdown in gut immunity as observed with HIV in humans and FIV in domestic cats (Willett et al., 2006; Walson et al., 2009; McSorley and Maizels, 2012; Murphy et al., 2014; Costiniuk and Angel, 2012).

Alongside gastrointestinal parasites, the richness of hemoparasite infections was increased in FIV-positive lions. This alteration was most apparent in the prevalence of *Babesia microti,* which was significantly higher in FIV-infected hosts. *B. microti* infection occurred against a backdrop of other *Babesia*, *Ehrlichia*, *Theileria*, and *Hepatozoon* species that were also prevalent in combination, including one unknown *Hepatozoon* species that was visually identified in most of the blood smear samples despite a lack of affinity for the PCR markers. Of the species detected via PCR, hemoparasite coinfections increased as immune cell populations and bactericidal capacity of blood decreased, likely reflecting the role of adaptive and innate immune defenses in limiting the invasion, growth, and persistence of these intracellular parasites (Olivier et al., 2003; Levinson, 2012). Hemoparasites may thus benefit directly from a decrease in humoral and cell-mediated immunity brought on by clinical AIDS (Sher et al., 1992; Dean et al., 1998; Olivier et al., 2003).

Another hallmark of AIDs in humans and FAIDS in cats is progressive wasting and malnourishment. Here, these findings were evident through high levels of ghrelin, a gastric hormone released in response to nutrient deprivation and stomach shrinkage; and low levels of leptin, which is produced by adipose cells in respone to high nutrient balance (Appleton et al., 2000; Shibata et al., 2003; Ida et al., 2007; Klok et al., 2007; Martin et al., 2010; Depauw et al., 2012; Jensen et al., 2015; Witzel et al., 2015). Further support for malnourishment was provided by consistently low levels of blood urea nitrogen (BUN) in FIV-positive hosts, even in the face of dehydration. While BUN is more frequently used to evaluate renal health, low values of BUN may reflect decreased protein balance and subsequent muscle wasting due to insufficient alimentary intake or increased losses through the kidneys and intestines (Thrall, 2004; Stockham and Scott, 2008). Of the parasites diagnosed, GI parasites contributed most profoundly to these changes and were more tightly associated with low levels of leptin and high levels of ghrelin than FIV itself or hemoparasites. Given that reduced immunity strongly

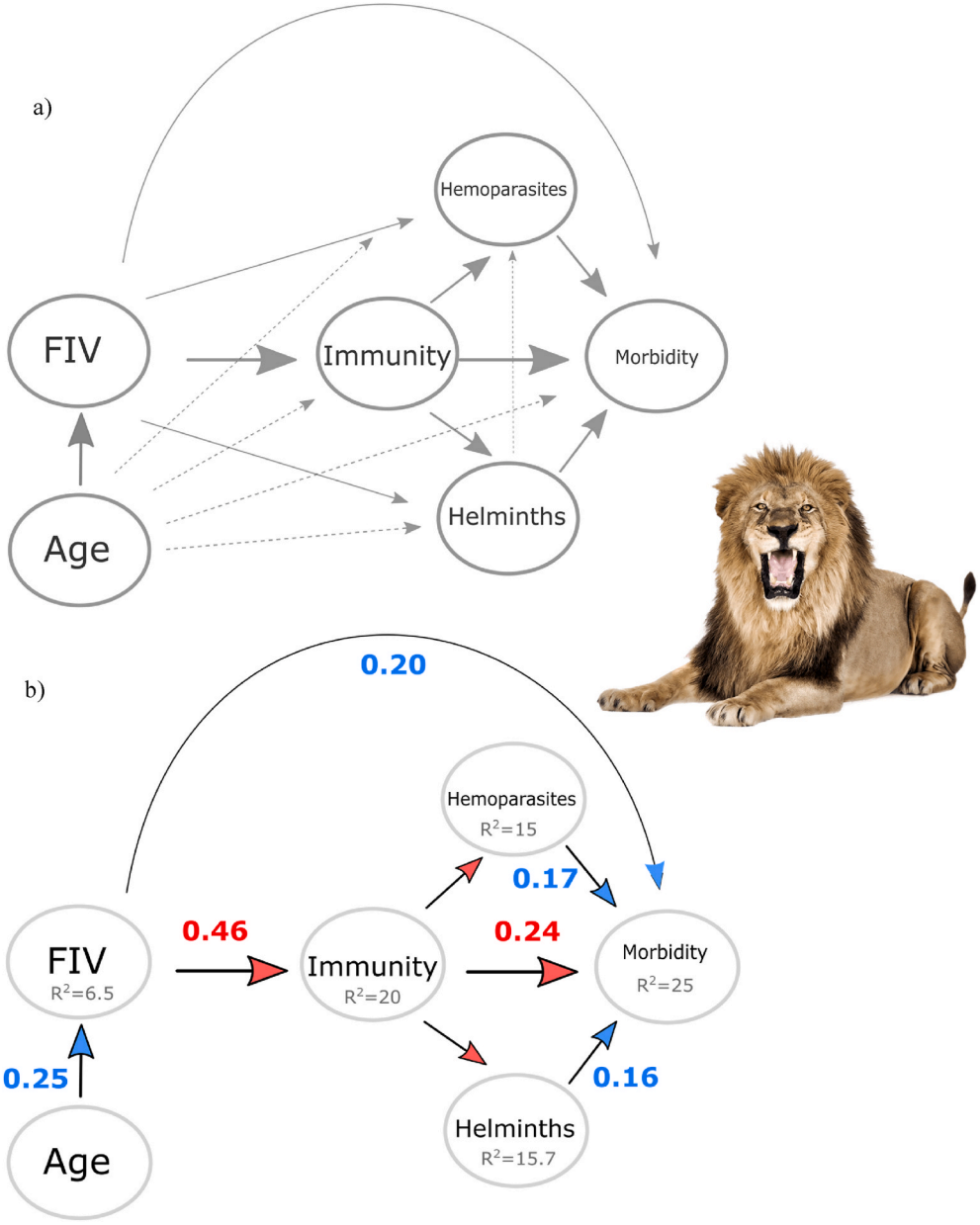

Fig. 4. FIV has strong direct and indirect effects on overall health. The final path model (a) shows only statistically significant relationships between manifest variables (rectangles) and latent variables (circles) for FIV infection, immune response (IMM), co-infections with hemoparasites (Hemoparasites), co-infections with gastrointestinal parasites (Helminths), and morbidity (Morbidity). Note that the parameter $\beta_{x,z}$ between each variable of interest represents the path coefficient obtained from least squares regression examining the relationship between one latent variable and the next (for example, FIV to IMM). Blue arrows represent positive relationships, whereas red arrows denote negative relationships. Final sample size was 106 lions.

and positively affects the prevalence, richness, and abundance of GI parasites, these findings suggest that lions with FAIDS may maintain condition while they are able to mount compensatory immune responses, but that metabolic homeostasis may decline as B and T cells wane.

In AIDS patients, systemic inflammation and antigen deposition along filtration barriers due to primary and secondary infections often leads to extensive immunopathology in the form of glomerulonephropathies and enteropathies (Yamamoto et al., 1989; Freeman et al., 2004; Roelke et al., 2009). While markers for these pathologies were included in our biochemistry panels, BUN levels in FIV-positive lions typically remained within the normal range (Broughton et al., 2017), as did creatinine, suggesting that they do not suffer from overt renal pathology. Liver enzymes were increased with FIV-infection relative to uninfected lions, but again were within the normal range previously established for this species (Broughton et al., 2017). Recent studies on the pathological mechanisms of FIV in lions of Botswana found similar trends in liver enzymes, which were attributed to parasite visceral larval migrans as diagnosed via gross assessment and histopathology (Roelke et al., 2009). We worked with live animals only, and so were not in a position to evaluate liver histology; however, the tight association of FIV and GI parasite infections in our study is consistent with this explanation. Contrastingly, increased richness and prevalence of hemoparasites contributed much more strongly to elevations in ALT alongside clinical wasting despite normal nutrient balance (as measured through leptin and ghrelin), suggesting a dominant role for hemoparasites in driving liver pathology in the form of hepatocellular injury (Reyers et al., 1998; Solano-Gallego et al., 2016). In addition, while some changes in biochemistry and endocrine markers were subclinical, in that they did not fall outside reference intervals established for healthy wild lions, clinical signs of FIV infection including gingivitis and ocular lesions were present in FIV-positive lions, especially as they aged. Within the context of environmental variation to which wild animals are exposed, particularly variation in food availability and parasitic exposures, the observed subtle, subclinical changes in organ function and minor clinical manifestations may thus be relevant to animal health (Munson et al., 2008).

While this was one of the most comprehensive predator disease

Table 3

3a and 3b. Correlations and % contributions between latent and manifest variables describing the final partial least squares path model examining the relationships between FIV infection, immune status, coinfection with helminths or hemoparasites, and morbidity in 195 lions from Kruger National Park, South Africa.

Box 3a: Final cross correlations between significant manifest variables (MVs) and their respective latent variables (LVs)

Variables	FIV	Immunity (IMM)	Hemoparasites	Helminths	Health Impairment (HealthIMP)
Age	0.25	−0.28	0.34	−0.11	0.12
Immunity (IMM)	−0.46	1	−0.38	−0.39	−0.43
$CD4^+$	−0.38	0.62	−0.48	−0.05	−0.11
$CD21^+$	−0.29	0.88	−0.25	−0.46	−0.45
BKAWB	−0.39	0.57	−0.39	0.01	−0.16
ESR120	0.28	−0.56	0.03	0.41	0.23
Hemoparasites	0.22	−0.39	1	−0.01	0.29
Helminths	0.2	−0.4	−0.01	1	0.28
Burden	0.19	−0.29	0.03	0.86	0.29
Richness	0.19	−0.41	0.02	0.95	0.24
Health Impairment	0.36	−0.43	0.29	0.28	1
Emaciation	0.15	−0.15	0.18	0.11	0.44
ALT	0.34	−0.36	0.29	0.15	0.86
Ghrelin (GHR)	0.08	−0.19	−0.01	0.28	0.4
Leptin (Lep)	−0.04	0.14	0.01	−0.15	−0.23

Box 3b: Contribution (%) of each latent variable (LV) to global observed variability in health impairment

Latent Variables	**β**	**Correlation**	**Contribution to global R2 (%)**
FIV	0.2	0.36	**27**
Immunity	−0.24	−0.424	**38**
Helminths	0.16	0.28	**17**
Hemoparasites	0.17	0.29	**18**

*Relationships between latent variables are denoted by bold face font.

studies to date, capture logistics within a free-ranging predator population did impose several limitations. First, we were unable to investigate the role of directly transmitted viruses in FIV disease progression because our diagnostics for viruses were limited to detection of antibodies at a single time point. As such, we cannot be sure whether viral exposures were current or occurred at some point in the past and whether they occurred prior to, or after FIV infection. Directly transmitted viruses would be of particular interest here due to the potential feedback between increased aggressive behaviors in FIV positive hosts and enhanced transmission – both of FIV itself, and of other directly transmitted infections. Indeed, we found evidence of increased aggression or delayed wound healing particularly in FIV-infected male lions, which showed a higher prevalence of combative wounds and elevations in circulating levels of testosterone. These results are consistent with findings in domestic cats with FIV and humans with HIV, which show upregulation of sex steroid levels and an increased propensity to engage in "risky" behaviors (increased fighting in cats and sexual encounters in humans; Fromont et al., 1997; Podell et al., 1999; Lloyd-smith et al., 2005). Based on lion behavioral ecology, similar findings in this species would be likely to increase host contact rates, with the possibility of increasing transmission of FIV and other directly transmitted infections (Fromont et al., 1997, 2003). In addition, this study's finding of strong direct effects of FIV-driven immunosuppression on host health (independent of coinfections) could also be related to the diagnostic limitations of this study, which failed to detect relevant parasites. Future studies would benefit from the use of NextGen Sequencing and longitudinal data collection in order to assign causal pathways with more certainty as they pertain to coinfections.

Finally, while we attempted to avoid sampling bias by catching entire prides and conducting captures across all areas of KNP, our sampling methods were not truly random. Lions that were too weak to respond, or to compete with other lions at the carcass, were unlikely to be sampled. We may thus have missed the most emaciated individuals, as well nomadic sub adults, as these animals often do not associate with a pride (Schaller, 1976). On the other hand, lions that had recently eaten might not respond to a bait carcass, and as such we might also have missed especially well-fed individuals with higher fitness.

5. Conclusions

With a sample population of 195 wild lions out of 1700, which equates to 11.5% of the total population living in Kruger National Park, as well as diagnostic and health data on 22 different parasites, ours is one of the largest and most comprehensive infectious disease studies of wild predators. Findings from this study significantly extend current knowledge of FIV's immune effects in lions, as well as its potential role for structuring parasite communities via these immune alterations. Both FIV's direct mechanisms, and indirect pathology mediated by the higher richness and abundance of other parasites in FIV-infected individuals, may in turn contribute to this pathogen serving as a key mediator of host pathology through organ injury, malnutrition, and further immune dysfunction. Most importantly, this is the first study to show strong supportive evidence that secondary infections may play an essential part of FIV disease progression in lions, rather than just a symptom, a finding that may also have clinical relevancy for other lentiviral impacted animal and human populations with high exposure to neglected parasites.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Lion captures and sample collection for this study would not have been possible without the supporting infrastructure of a larger study on lion behavior, demographics, and disease dynamics (PIs Dr. Paul Funston and Dr. Sam Ferreira), as well as the continued efforts and expertise of staff and researchers from SANParks Veterinary Wildlife and Scientific Services. Specifically, we would like to thank Drs. Sam Ferreira, Peter Buss, Michelle Miller, and Markus Hofmeyr, as well as technician Jennifer Joubert-Hofmeyr, for their continued diligence and help initiating and orchestrating this project. In addition, collection of information regarding pride dynamics and demographics would not have been possible without the dedicated efforts of the lion behavioral monitoring team comprised of Darius Scholtz, Nkabeng Maruping, Trevor Valoyi,

Travis Smit, and Katlego Makutulela. Coupled with this information, laboratory efforts for this study were widely supported by the participation of several graduate and undergraduate students including Tanya Kerr, Zaya McSky, Emily Magnuson, Kadie Anderson, Elena Alonso, and Ignatius Viljoen, as well as Dr. Patrick Chappell at Oregon State University. This work was supported by Oregon State University, SANPARKs Scientific Services, Ajubatus, and Morris Animal Foundation [Grant #D13ZO-420], as well as the Spanish Ministerio de Economia y Competitividad (MINECO) through a Ramon y Cajal agreement [RYC-2016-21120] and private donors through Morris Animal Foundation's research sponsor program. Sponsors of this research did not participate in the design, data collection, analysis, interpretation, writing, or decision for submission of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.07.003.

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