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Defining Putative Koala Retrovirus-Associated Disease in Koalas

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Abstract. Koalas suffer from a wide range of diseases and illness, some of which are well understood, and others that are observed but have unclear aetiologies. A largely undescribed and poorly defined area in koala health is diseases presumed to be associated with koala retrovirus (KoRV) infection. Disease conditions putatively linked to KoRV infection are defined here as “putative KoRV-associated diseases” (PKAD). These include neoplasia, severe dermatological and oral conditions, life-threatening fungal and opportunistic infections, haematological disorders, chronic ill-thrift or poor body condition of undefined cause and other conditions suggestive of immune dysfunction. Multiple conditions are usually present at once and koalas invariably die despite treatment. The multifactorial nature of PKAD and the lack of clarity around KoRV’s role in many conditions means that developing a standard case definition encompassing all presentations is difficult. As such, presenting conditions have been defined as dysplastic/neoplastic versus those associated with immune dysfunction (putative immune dysfunction disorders—PIDDS).

Introduction
Koala retrovirus (KoRV) is present in almost all koalas (Phascolarctos cinereus) throughout Australia as both endogenous (integrated into the germ line and heritable) and exogenous (replication-competent, transmissible) virus. KoRV subtypes A-M exist, with only the subtype A showing evidence of endogenization and being ubiquitous in koalas from Queensland (QLD) and New South Wales (NSW) (Quigley & Timms, 2020; Blyton, Young, et al., 2022). Koalas in South Australia and Victoria do not appear to have endogenous forms of KoRV; however, there is evidence of recombinant variants of KoRV (recKoRVs) across the koala’s range which are thought to be largely defective, or non-replication competent (Löber et al., 2018; Tarlinton et al., 2022). Exogenous forms of KoRV have been detected across the koala’s range and there is mounting evidence to suggest that viral load may be an important factor to consider when investigating links to disease in koalas (Maher et al., 2019; Fabijan et al., 2020; Quigley & Timms, 2020; Blyton, Pyne, et al., 2022).

Koalas suffer from a wide range of diseases and illness, some of which are well understood and others that have unclear aetiologies. Diseases presumed to be associated with KoRV infection are a largely undescribed and poorly defined area in koala health. This knowledge gap is partly due to our poor understanding of how KoRV might act as an aetiological agent, but also the need to clearly define what constitutes KoRV-associated disease. Diseases putatively linked to KoRV infection are defined here as “putative KoRV-associated diseases” (PKAD).

Putative KoRV-associated diseases comprise a suite of conditions that present in koalas similarly to those caused by other pathogenic gammaretroviruses which affect other species (e.g., feline leukaemia virus, murine leukaemia virus, gibbon ape leukaemia virus) (Hanger & Loader, 2014). Examples of such conditions include leukaemia, lymphoma, aplastic anaemia, tumours, and immunodeficiency disorders (Beatty, 2014; MacLachlan & Dubovi, 2017). Despite compelling similarities between disease presentations in koalas and other species affected with gammaretroviruses, further research is required to substantiate a causal link.
between KoRV infection and these conditions.
To aid in defining PKAD, these conditions can be separated into two clearly defined groups (Fig 1): conditions clearly defined as bone marrow dysplasia and neoplasia, and conditions that reflect immune dysfunction, dysregulation, or disruption of normal cellular function, termed “putative immune dysfunction disorders” (PIDDS).

This manuscript aims to build on already published literature by Hanger & Loader (2014) outlining a suite of conditions that fall under the banner of PKAD.

Bone marrow dysplasia and Neoplasia group

Until recently, KoRV’s role in the development of neoplasia was speculative, at best. However, in 2021 McEwen et al., identified KoRV-A genomic integrations near oncogenes and established that there was dysregulation of genes in koalas affected by leukaemia and a variety of tumours (McEwen et al., 2021). This provides the most compelling evidence to date that KoRV integration (at least for subtype A) probably leads to neoplasia in koalas.

Virtually all neoplastic presentations in koalas are fatal, either via expansion and compression of surrounding structures impeding function or through carcinogenesis. Hanger & Loader (2014) provide a comprehensive review of neoplastic conditions observed in koalas, describing in detail leukaemia, lymphoma, osteochondroma, fibrosarcoma and mesothelioma. Many other types of neoplasia have been reported in koalas (Gillett, 2014; Tong, 2019) but their relationship with KoRV infection still requires investigation.

Bone marrow dysplasia of humans is referred to as “myelodysplastic syndrome” or “myelodysplastic neoplasia” and is characterized by ineffective haematopoiesis or the failure of normal bone marrow stem cells to mature into normal functioning blood cells (Hasserjian, 2019; Sekeres & Taylor, 2022). Bone marrow dysplasia in koalas presents as a similar spectrum of haematopoietic disorders to those described in humans, with diagnoses in koalas usually assigned as aplastic anaemia, leukaemia or myelodysplasia.
Definitive causes of bone marrow dysplasia in koalas are unknown, though genetic inheritance, KoRV-induced germline variants in haematopoietic stem cells and exogenous viral infection remain plausible aetiologies. Antimicrobials such as chloramphenicol may induce short-term aplastic anaemia in some koalas but is extremely rare. A bone marrow assessment guide for koalas is available to assist with diagnoses of bone marrow dysplasia (Gillet & Hanger, 2019).

**The putative immune dysfunction disorders (PIDDS) group**

Koalas in this group often present with chronic ill thrift and poor body condition and are suffering from a variety of disorders consistent with immune incompetence, suppression, dysregulation or dysfunction. It is possible that gene dysregulation, immune dysfunction from KoRV integration, or a direct immunosuppressive effect of the KoRV transmembrane envelope protein pL5E is responsible for manifestations of PIDDS, but further investigation is required. Individuals affected by PIDDS often die prematurely, though it is not uncommon for some koalas to suffer repeated episodes or combinations of disorders for months to years before their demise. Koalas colloquially referred to as “poor-doers” or suffering from “wasting” syndrome might also fall into this group.

A diagnosis of PIDDS may be complicated by coinfecting pathogens and, in some cases, these may exacerbate disease severity. It is not always clear if coinfections are primary or secondary in nature. For example, Phascolarctid gammaherpesvirus 1 & 2 (PhaHV 1&2) has been identified more commonly in koalas affected with chlamydial disease (Vaz et al., 2019), and trypanosome infection in koalas may cause severe anaemia (regenerative), dullness, lethargy, anorexia, peritoneal effusions, and nervous signs (tremors and seizures). As such, when faced with conditions included in this group, clinicians should be cautious of arriving at a diagnosis of PIDDS without first carrying out comprehensive clinical and diagnostic assessments to investigate other feasible causes of disease.

A presumptive classification of PIDDS should be considered in koalas concurrently afflicted by two or more conditions described in the PIDDS group. For example, concurrent planar/plantar hyperkeratosis, oral ulceration and severe candidiasis; or severe ulcerative tongue lesions, stomatitis, oral candidiasis, marginal anaemia and hypomelanosis of planar and plantar surfaces.

Conditions within the PIDDS group have been separated into 7 subgroups. These include:

1. **Severe or atypical dermatological or keratinous conditions.**

   Severe or atypical dermatological or keratinous conditions include cutaneous growth disorders and proliferations, giant cell dermatosis (Fig. 2A), hyperkeratosis of the planar and plantar surfaces (Fig. 2B), hypomelanosis of the planar and plantar surfaces (Fig. 2C), autoimmune mediated dermatitis such as discoid lupus erythematosus, allergic dermatitis and severe generalized dermatitis which may or may not be associated with primary or secondary fungal or parasitic infections (Fig. 2D).

   Histopathological findings may include parakeratotic hyperkeratosis, epidermal hyperplasia and dysplasia, lymphoplasmacytic, lymphohistiocytic and neutrophilic infiltrates and interstitial fibrosis.

2. **Oral disorders in the form of severe gingivitis, periodontal disease and ulceration of the tongue, buccal surfaces and lips.**

   Ulcerative lesions may appear as deep dry lesions along or at the base of the tongue (Fig. 3A) or buccal surface. Ulcers may be obscured by a layer of firmly adhered masticated leaf. Cracking and deep fissures around the commissures of the mouth are often observed (Fig. 3B). Advanced periodontal disease includes extensive gingival recession, particularly around the incisors, inflamed gingiva and purulent exudate from affected sites (Fig. 3C). Oral candidiasis is usually present and may extend into the oesophagus and around the oesophageal sphincter. Affected koalas often display ptalism, leaf drop, and pain associated with eating.

3. **Non-antibiotic related gastrointestinal cacocholic dysbiosis/typhlocolitis syndrome (CDTS).**

   CDTS results in altered caeco-colic homeostasis, disrupted motility, chemical and epithelial function, altered water content, and inflammatory changes in the caecum and proximal colon (Gillet & Hanger, 2019). The aetiology behind CDTS is unclear and likely multifactorial but is commonly associated with antimicrobial use (Gillet & Hanger, 2019).

   However, a subset of captive or wild koalas will develop this syndrome in the absence any predefined risk factors.

   Affected koalas develop diarrhea, seemingly spontaneously, and suffer rapid weight loss, dehydration and have normal or reduced appetites. Affected koalas may be found suffering from these symptoms in the wild, often sitting low or at the base of a tree, or they may develop this condition in captivity and often die despite attempts at treatment. There is no prior history of antibiotic or antifungal use in these animals.

   At gross necropsy the caecum presents with varying degrees of pallor and content consistency. In some cases, the caecal content may be liquid and malodorous but the mucobacterial lining remains intact (Fig. 4A). Other cases may show complete separation of the caecal mucobacterial layer (resulting in a translucent caecal wall) and either soft, firm or dry caecal content (Fig. 4B). Histopathology usually reveals lymphoplasmacytic infiltrates in the lamina propria and submucosal layers of the gastrointestinal tract.

4. **Severe life-threatening or widely disseminated opportunistic or fungal infections, including multifocal cryptococcal and candidal infections.**

   Multifocal cryptococcal lesions may be observed in the long bones, pelvis, mandible, maxilla, soft tissue structures, and dermis. Severe systemic candida infections with infiltration of the oesophageal and gastrointestinal mucosal layers and myocardial micro-abcssesation have been observed (Hanger & Loader, 2014).

5. **Chronic ill-thrift or poor body condition of undefined cause.**

   Both adult and joey koalas may present with ill-thrift and poor body condition, with some joeys also failing to thrive during their development to
The processes behind why a joey might fail to thrive are currently unknown and are likely to be multifactorial, though investigations in this area are currently underway (D. Higgins, pers. comm.). Intense plasma cell and other mononuclear cell infiltrates within the gastrointestinal lining have been identified in some koalas with ill-thrift (Hanger & Loader, 2014) and suggest an underlying aetiology.

6 Haematopoietic disorders including marginal anaemias, lymphopaenias, and severe anaemia associated with trypanosome infection.

Anaemia and lymphopaenia of undefined cause have been observed in koalas. Affected individuals may be in moderate to poor body condition and may or may not be suffering from other disease manifestations suggestive of immune suppression. Bone marrow cytology appears normal in these cases.

Several koalas have been observed with clinical signs consistent with trypanosome infection in other species including severe regenerative anaemia, dullness, lethargy, anorexia, peritoneal effusions, nervous signs (tremors and seizures), and irregular parasitaemia. Affected animals include joeys and adults. Koalas affected by neurological symptoms invariably die within days, whilst those showing regenerative anaemias only may recover with blood transfusions and supportive care.

Histopathology of neurological trypanosome infected cases revealed an intense lymphocytic/plasmocytic choroiditis, extensive lymphocytic infiltration of the meninges, marked perivascular lymphocytic infiltration and macrophage reaction within the choroid plexus which extended into the surrounding meninges. Adjacent cerebral and cerebellar vessels were congested, and free trypanosomes were observed in blood vessels.

Figure 2. Severe and atypical dermatological presentations in koalas. (A) Cutaneous proliferations caused by giant cell dermatosis. (B) Hyperkeratosis of the plantar surface. (C) Hypomelanosis of the plantar surface. (D) Severe crusting and ulceration of the face and limbs with secondary fungal infection.
Figure 3. Oral disorders that may be found in koalas with a presumptive diagnosis of PIDDS. (A) Deep ulceration of the tongue, which may be obscured by or impacted with masticated leaf. (B) Cracking and deep fissures around the commissures of the mouth. (C) Severe stomatitis and advanced periodontal disease. Candida may also be present in the mouth and oesophagus.

Figure 4. Pathological findings in koalas affected by caeco-colic dysbiosis/typhlocollitis syndrome (CDTS). (A) Liquid and malodorous caecal content with the mucobacterial lining grossly intact and an opaque caecal wall. (B) Extensive breakdown of the mucobacterial lining in a koala with chronic illness where the caecal content has fallen away from the mucosa entirely leaving a transparent caecal wall.
7 Severe chronic or treatment-refractive chlamydial disease.

Severe chlamydial disease is more common in northern koala populations with a positive association suggested between KoRV infection and chlamydial disease severity (Quigley & Timms, 2020). Other factors could contribute to chlamydial severity such as chlamydial virulence plasmids, environmental conditions affecting nutrition, and co-infection with other bacterial or viral pathogens.

Clinical and diagnostic evaluation

When aiming to establish a disease diagnosis in koalas, thorough physical examinations should be followed by comprehensive diagnostic tests (Gillet & Hanger, 2019). Diagnostic testing of any ill or compromised koala should include (at minimum) haematocrit, total plasma protein, blood film examination, bone marrow aspirate and cytological examination, abdominocentesis with cytological examination, urinalysis, abdominal ultrasonography, and full body radiographs. Additional tests could include a full haematology and biochemistry panel, blood gas analysis, and computed tomography or magnetic resonance imaging.

Most diagnostic techniques applied in koala medicine are similar to those in domestic species and can be easily performed by a skilled clinician. Details of techniques used in koalas have been described (Gillet & Hanger, 2019).

Discussion

Although the role of KoRV infection in the aforementioned conditions is still putative, there is growing evidence to suggest that it is highly likely that KoRV influences the immune system of koalas (Mathew et al., 2014; Higgins, 2019; Maher et al., 2019) and that viraemic load may be an important factor to consider.

Conditions within the PIDDS group may have multiple potential aetiologies, and at times affected koalas may appear to respond temporarily to targeted treatment. However, where multiple conditions are present in combination the likelihood of clinical resolution is extremely low, and koalas invariably die despite treatment. As access to molecular tests such as PCR for identifying KoRV subtypes become more widely available, there is the potential that clinicians may be tempted to infer a diagnosis or assign a prognosis based on KoRV test results. However, such testing should always be viewed in light of the koala’s clinical presentation, particularly until reliable evidence is found to link KoRV subtype or viral load to particular disease syndromes.

Thorough clinical and diagnostic evaluations are critical in disease diagnosis and where veterinarians are not intimately familiar with koala medicine and health, advice should be sought from those with experience in this area. It is recommended that fresh postmortem tissues (disease affected and unaffected) and whole blood in EDTA be collected and stored frozen (-80°C where possible) for future research into KoRV’s relationship with clinical disease.

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