The Koala and its Retroviruses:
Implications for Sustainability and Survival

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Koala Retrovirus Related Diseases in European Zoo-based Koalas *(Phascolarctos cinereus)*

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Abstract. European zoos have housed koalas *(Phascolarctos cinereus)* for almost 25 years. From the time the first individual arrived on the old continent to the present population of 30 (15.15) animals, medical knowledge has improved significantly. During this time, 57 koala deaths have been recorded. With the discovery of the koala endogenous retrovirus (KoRV), the question remains whether it is involved in the various diseases found in captive population and specifically whether it was involved in the 57 deaths. This question is unfortunately difficult to answer as no real time tests were performed before and during the course of the diseases. A study of the detailed information of these records shows that almost half of them concern very young animals probably mainly because of joeys falling from the pouch and maternal neglect. A few deaths have no recorded information or are clearly not related to any infectious cause. 44% are due to neoplastic and opportunistic or non-opportunistic bacterial infectious process. While KoRV is thought to cause immunosuppression and tumour induction (mainly lymphomas), the link between disease and the virus has not been clearly established.

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The San Diego Zoo loaned the first koalas to European zoos in 1989 (Hamlin Andrus, 2011) to the London zoo. The first breeding pair arrived in 1991 at the Jardim Zoologico de Lisbon, Portugal. Twenty-five years later, seven institutions house 30 animals (15.15) (Hamlin Andrus, 2011).

Over the past 25 years, our knowledge in husbandry and care of this unique species has greatly improved. One of the major recent discoveries was the presence of an active endogenous retrovirus in the genome of a large portion of wild koalas and most (if not all) captive koalas (Canfield *et al.*, 1988; Hanger *et al.*, 2000; Tarlinton *et al.*, 2005). This retrovirus is thought to be responsible for an innate immunosuppression and neoplastic induction, especially, but not only, lymphoma (Tarlinton *et al.*, 2005). This detailed retrospective study of koala deaths that have occurred in the European captive population aims to look for a possible association with the presence of the retrovirus.

In 25 years, 57 deaths have been recorded in the European population (a list of every death with details can be found in Table 1). While every adult death has been examined in detail, some joey deaths, mainly during the first days of life, are missing, depending on the keepers observations.

Among these 57 deaths, 4% (2) have no record. 5% (3) are clearly not related to the retrovirus: one case of bladder stone with death occurring post surgery, one case of head trauma with hydrocephalus internus associated, and one case of ileum and colon torsion.

Deaths of joeys accounted for 47% (27) of all deaths. Most of these cases have no details, but we can assume most of them are related to joey falling from the pouch or maternal neglect (absence of pouch cleaning, joey rejected, etc.). A few cases have bacterial culture recorded and the results are listed among the other bacterial results (Table 2).

Table 1. European captive koala deaths. *institution,* where death recorded; *SB*, studbook number in the International studbook.

institution	SB	sex	birth date	death date	death information
LISBON	116	female	07/06/82	20/07/00	Presumably due to pelvic infection but not possible to determine the origin. Septic metritis. Bladder suppurative inflammation. Arteriosclerosis. Splenic atrophy.
LISBON	117	female	14/07/82	09/11/92	Lymphoid neoplasia—lymphoblastic lymphosarcoma in the abdominal cavity.
DUISBURG	120	male	18/05/83	08/07/04	Unknown cause of death.
LONDON	124		13/06/84	07/11/91	Disseminated lymphoma.
LISBON	148	male	04/06/89	26/12/07	Large Intestine adenocarcinoma—glandular proliferation of the mucosa and submucosa.
DUISBURG	173	male	20/07/92	31/08/05	Moderate alveolar lung emphysema, gastritis, lymphadenitis of mesenterial lymph nodes, volvulus, ulcerative, haemorrhagic and necrotising colitis, focal necrosis of gall bladder, myocardial degeneration.
LISBON	176	female	26/09/92	08/02/93	Malnutrition during her last month on the pouch.
LISBON	184	female	09/06/93	13/12/93	Unknown cause of death (joey).
DUISBURG	185	female	01/07/93	08/10/07	Lungs: focal acute purulent pneumonia *(Enterobacter cloacae, E.coli)*, partly subacute to chronic granulomatous parabronchial lesions with storage of crystalline structures.
DUISBURG	194	male	16/05/94	22/09/04	Unknown cause of death (joey).
LISBON	195	unknown	15/06/94	25/06/94	Rejection by the mother.
LISBON	200	female	14/10/94	15/01/02	Renal carcinoma—tumoral mass (9×5 cm) in the left perirenal area.
DUISBURG	201	male	26/10/94	18/05/06	Alveolary lung emphysema, focal purulent-necrotizing esophagitis, haemorrhagic gastroenteritis, fatty liver, interstitial fibrosis of pancreas.
BEAUVAL	204	male	24/05/95	11/01/07	Polymorphous lymphosarcoma (mesenteric lymph nodes, lungs, liver, bone marrow). Typhlocolitis.
DUISBURG	206	unknown	07/06/95	07/06/95	Unknown cause of death (joey).
PLANCKNDL	212	female	21/10/95	11/04/04	Fatty degeneration of the liver with necrosis, pneumonitis and atrophy of lymphoid tissues.
DUISBURG	224	unknown	01/01/97	18/02/97	Unknown cause of death (joey).
PLANCKNDL	225	male	29/03/97	13/08/01	Leukaemia.
DUISBURG	239	unknown	29/03/98	29/03/98	Unknown cause of death (joey).
LISBON	242	unknown	06/06/98	09/06/98	Foetus found on the floor, replaced. Disappeared next day.
PLANCKNDL	250	male	09/03/99	27/06/07	Chronic proliferative lymphoid inflammatory process. Liver, pancreas, abdominal fluid: *Enterobacter* sp., *Citrobacter* sp.
PLANCKNDL	253	unknown	17/05/99	23/05/99	Unknown cause of death (joey).
LISBON	256	female	27/06/99	28/12/99	Lung: oedema, mild to moderate. Liver: congestion, moderate, with possible individual cell necrosis.
DUISBURG	260	unknown	06/11/99	09/11/99	Unknown cause of death (joey).
PLANCKNDL	262	unknown	18/11/99	15/03/00	Unknown cause of death (joey).
DUISBURG	270	female	03/04/00	06/07/01	Cervical tumours, aspiration pneumonia, infarctions in left heart chamber, liver necrosis centrolobullary.
LISBON	272	female	06/05/00	03/06/03	Serosal tumour—generalized sarcoma (nodules in the stomach, intestines, peritoneum) from fusiform cells originally from peritoneal serosa.
BEAUVAL	269	female	01/06/00	22/03/08	Septicaemia and suspected mesenteric lymphoma.
VIENNA	297	male	24/02/01	26/04/08	Colon leiomyosarcoma, colon stenosis, osteolysis carpus.
MADRID Z	298	male	10/03/01	08/11/09	Multicentric lymphoid leukaemia.
DUISBURG	299	male	17/03/01	17/11/07	Hydrocephalus internus, degenerative changes in the cortex and massive subacute degenerative/necrotising changes of the skeletal musculature most probably caused by trauma.
PLANCKNDL	309	female	02/02/02	20/09/12	Ulcerative membranous necrotic enteritis-cecitis. Lymphoid hyperplasia.
PLANCKNDL	338	female	10/07/02	11/10/03	Diffuse necrotizing hepatitis, strong suspicion of ulcus, glomerulonephrosis, exhaustion of the immune system. *Pseudomonas aeruginosa* (liver, lung, brain, intestinal ulcer).
DUISBURG	343	unknown	13/11/02	13/11/02	Unknown cause of death (joey).
LISBON	344	unknown	27/12/02	03/06/03	Death during hand rearing process.
DUISBURG	349	unknown	26/06/03	28/03/04	*Pneumocystis carinii* in lungs and brain.

Table 1 *(continued)*.

institution	SB	sex	birth date	death date	death information
DUISBURG	353	male	12/08/03	25/10/10	Severe purulent and emphysematous prostatitis, high amount of neutrophil granulocytes. *E.coli haemolytica* (+++) and *Klebsiella pneumoniae* (+).
PLANCKNDL	354	unknown	13/08/03	15/08/03	Unknown cause of death (joey).
PLANCKNDL	358	unknown	24/11/03	03/02/04	Unknown cause of death (joey).
LISBON	362	male	28/05/04	22/04/11	Malignant round cells tumour—right nasal cavity with infiltration of retro-orbital area, right frontal lobe, mandibular lymph node, adrenal gland and prostate.
PLANCKNDL	364	unknown	22/08/04	06/10/04	Unknown cause of death (joey).
DUISBURG	372	unknown	20/06/05	21/06/05	Unknown cause of death (joey).
DUISBURG	375	female	03/10/05	06/10/09	Bladder stone. Died post surgery. Sepsis and circulatory failure due to torsion. *Citrobacter freundii*. Tubuli calcification.
PLANCKNDL	397	male	16/05/06	28/10/08	Toxoplasmosis.
MADRID Z	398	unknown	06/06/06	01/12/06	Consolidated area on the lungs (aspiration pneumonia or septicaemia) and brain oedema (septicaemia). *Klebsiella pneumoniae* from all samples (pouch incl.).
MADRID Z	409	female	12/05/07	04/02/08	Found dead on the floor. *Enterobacter intermedius*.
PLANCKNDL	408	female	12/05/07	29/10/08	Toxoplasmosis.
MADRID Z	417	female	30/08/08	04/03/09	Joey fell several times from pouch. Found dead. Spleen, liver and kidney: *Pseudomonas fluorenscen*; lungs: *Moraxella* sp.
BEAUVAL	418	female	18/11/08	07/12/10	Multicenter lymphoma and leukaemia. Severe lipidosis and haemosiderosis.
MADRID Z	426	unknown	21/04/09	29/08/09	Unknown cause of death (joey).
MADRID Z	434	male	10/10/09	13/09/10	Ileum and colon torsion. Cecum and proximal colon vascularization had congestive mucosa with haemorrhagic areas. Emphysema in the distal area of the lungs. *E. coli* in liver and intestine.
DUISBURG	439	unknown	06/06/10	06/09/10	Unknown cause of death (joey).
DUISBURG	446	unknown	25/10/10	25/10/10	Unknown cause of death (joey).
PLANCKNDL	?	unknown	28/06/11	29/06/11	Unknown cause of death (joey).
PLANCKNDL	?	unknown	22/08/11	29/08/11	Unknown cause of death (joey).
MADRID Z	?	female	22/09/11	02/04/12	Neutrophilic margination in the brain suggests terminal endotoxemia/bacteraemia; isolation of *Klebsiella* from the spleen and *E. coli/Staphylococcus* from kidney.
PLANCKNDL	?	unknown	10/08/12	01/09/12	Unknown cause of death (joey).

Deaths associated with neoplasms accounted for 23% (13) of all deaths. Among those, six cases are lymphoma and one case a leukaemia. These cases present a high suspicion of association with KoRV. The remaining neoplastic processes are leiomyosarcoma, malignant round cell tumour, adenocarcinoma, generalized sarcoma, renal carcinoma, and an unidentified cervical tumour. Whereas these tumours have never been described in association with KoRV, we cannot exclude the relationship. Retroviruses have been described in all mammals including humans being directly associated with different type of carcinoma, sarcoma, and lymphoma. Human immunodeficiency virus (HIV) is indirectly associated with carcinoma and sarcoma of various organs and leiomyosarcoma (Gessain, 2013).

5% (3) of deaths are described as being caused by an opportunistic infection. Two cases of toxoplasmosis with tachyzoites of *Toxoplasma gondii* found on histology and one case of lung and brain infection by *Pneumocystis jirovecii (carinii)*. Toxoplasmosis is opportunistic in many mammal species, but marsupials are known to be specifically sensitive (Bultel *et al.*, 2013). *Pneumocystis jirovecci* is described to be opportunistic in any species (Dugdale *et al.*, 2013). The relationship of these opportunistic diseases with the immunosuppression caused by KoRV can be suspected in these cases.

Finally 16% (9) of deaths were associated with bacterial infections, with pneumonia most predominant. A list of all bacteria cultured can be found in Table 2. Some of these bacteria are often described as opportunistic and the others are generally found as part of the normal intestinal flora (Euzéby, 2013). Again immunosuppression caused by KoRV is suspected.

In conclusion, we can say that 44% (25) of the 57 deaths recorded during 25 years of captive management of koala in Europe could potentially have been related to KoRV. However, as no tests have been performed on the population, and no viral load kinetics on animals before and during the course of disease exist, it is not possible to definitively associate any of these deaths with the virus. It is also difficult to make the distinction between naturally occurring disease and induced disease as the population and number of cases are too small to statistically correlate this with what can be found in other species. Although not definitive, based on the current knowledge of KoRV in koalas there is a high index of suspicion that the seven cases of death due to lymphoma/leukaemia in this retrospective study may have been associated with the virus.

Table 2. List of bacteria cultured at necropsy.

Bacteria name	Gram staining	Family	Opportunistic status
Pseudomonas sp. incl. *aeruginosa* & *fluorenscen*	Gram –		opportunistic
Enterobacter sp. incl. *intermedius* & *cloacae*	Gram –	Enterobacteriaceae	opportunistic
Citrobacter sp. incl. *freundii*	Gram –	Enterobacteriaceae	
Escherichia coli incl. *haemolytica*	Gram –	Enterobacteriaceae	
Klebsiella pneumoniae	Gram –	Enterobacteriaceae	
Staphylococcus sp.	Gram +		opportunistic
Moraxella sp.	Gram –		opportunistic

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