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Short communication

Lack of antiviral antibody response in koalas infected with koala retroviruses (KoRV)

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ABSTRACT

Many wild koalas are infected with the koala retrovirus, KoRV, some of which suffer from lymphoma and chlamydial disease. Three subgroups, KoRV-A, KoRV-B and KoRV-J, have so far been described. It is well known that other closely related gammaretroviruses can induce tumours and severe immunodeficiencies in their respective hosts and a possible role for KoRV infection in lymphoma and chlamydial disease in koalas has been suggested. In many wild koalas, KoRV-A has become endogenised, *i.e.*, it is integrated in the germ-line and is passed on with normal cellular genes. In this study, sera from koalas in European zoos and from wild animals in Australia were screened for antibodies against KoRV-A. These naturally infected animals all carry endogenous KoRV-A and two zoo animals are also infected with KoRV-B. The antibody response is generally an important diagnostic tool for detecting retrovirus infections. However, when Western blot analyses were performed using purified virus or recombinant proteins corresponding to KoRV-A, none of the koalas tested positive for specific antibodies, suggesting a state of tolerance. These results have implications for koala vaccination, as they suggest that therapeutic immunisation of animals carrying and expressing endogenous KoRV-A will not be successful. However, it remains unclear whether these animals can be immunised against KoRV-B and immunisation of uninfected koalas could still be worthwhile.

Koala retroviruses (KoRV) have been isolated from wild and captive koalas in Australia as well as from koalas housed in zoos in other countries (Hanger et al., 2000; Fiebig et al., 2006; Xu et al., 2013; Miyazawa et al., 2011; Shojima et al., 2013). They are members of the genus gammaretrovirus and are most closely related to gibbon ape leukaemia virus (GaLV), feline leukaemia virus (FeLV), murine leukaemia virus (MuLV), and porcine endogenous retrovirus (PERV) (Hanger et al., 2000; Cui et al., 2012; Denner and Young, 2013). As well as leukaemia, GaLV, MuLV, and FeLV induce immunodeficiency in their respective hosts, leaving them susceptible to numerous opportunistic infections (Moloney, 1964; Rosenberg and Jolicoeur, 1997; Hardy, 1993; Gallo et al., 1978; Kannian and Green, 2010). As is the case with virus loads and AIDS in HIV-1 infected humans, there is a correlation between KoRV RNA levels in the plasma and neoplastic disease in koalas (Tarlinton et al., 2005; Mellors et al., 1997). KoRVs are likely the result of a relatively recent trans-species transmission from rodents or bats (see Denner and Young, 2013). At present, three KoRV subgroups have been described: KoRV-A, KoRV-B and KoRV-J (Hanger et al., 2000; Fiebig et al., 2006; Miyazawa et al., 2011; Xu et al., 2013). Interestingly, KoRV-A is present in the germline of some koalas, *e.g.*, the virus is endogenised (Tarlinton et al., 2006; Stoye, 2006), whereas KoRV-B and KoRV-J remain exogenous viruses.

Antibody responses are commonly observed in individuals infected with exogenous retroviruses and they are therefore of high diagnostic value (Daskalakis, 2011). To study the situation in koalas naturally infected with exogenous KoRV-B, and/or carrying endogenous KoRV-A sequences, sera from 16 koalas were tested for antibodies against the virus using viral lysates and recombinant viral proteins corresponding to KoRV-A. Surprisingly, none of the 16 koalas tested positive (Table 1), suggesting that the animals are tolerant against this virus. These data are in agreement with the lack of antibodies against PERV in normal pigs and in pigs immunised with the transmembrane envelope protein p15E of PERV (Keller et al., 2014), but differ from the situation in humans where antibodies against the human endogenous retrovirus HERV-K have been found in tumour patients and pregnant women (see below).

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Table 1
Animals analysed in this study.

Name	Origin	KoRV	Material analysed	Antibodies specific for	
				Virus	Recombinant proteins
Goonderra	Duisburg	A	Blood/serum	no	no
Coolongalook[a]	Antwerp	A + B	Blood/serum/tumour	no	no
Alora	Duisburg	A	Blood/serum	no	no
Birubi[a]	Duisburg	A	Blood/serum	no	no
Kambara[a]	Duisburg	A + B	Blood/serum/ascitis	n.t.	n.t.
Kangulandai[a]	Duisburg	A	Blood/serum	no	no
Irwin	Duisburg	A	Blood/serum	no	no
Posh spice	Australia	+	Serum	no	n.t.
Neil	Australia	+	Serum	no	n.t.
Minkey	Australia	+	Serum	no	no
Bubbles	Australia	+	Serum	no	no
Matt	Australia	+	Serum	no	no
Karen	Australia	+	Serum	no	no
Setch	Australia	+	Serum	no	no
Emma	Australia	+	Serum	no	no
Clay	Australia	+	Serum	no	no

[a] Died; n.t., not tested due to lack of material; +, the animals are from a region in Australia where all are infected with KoRV-A at least.

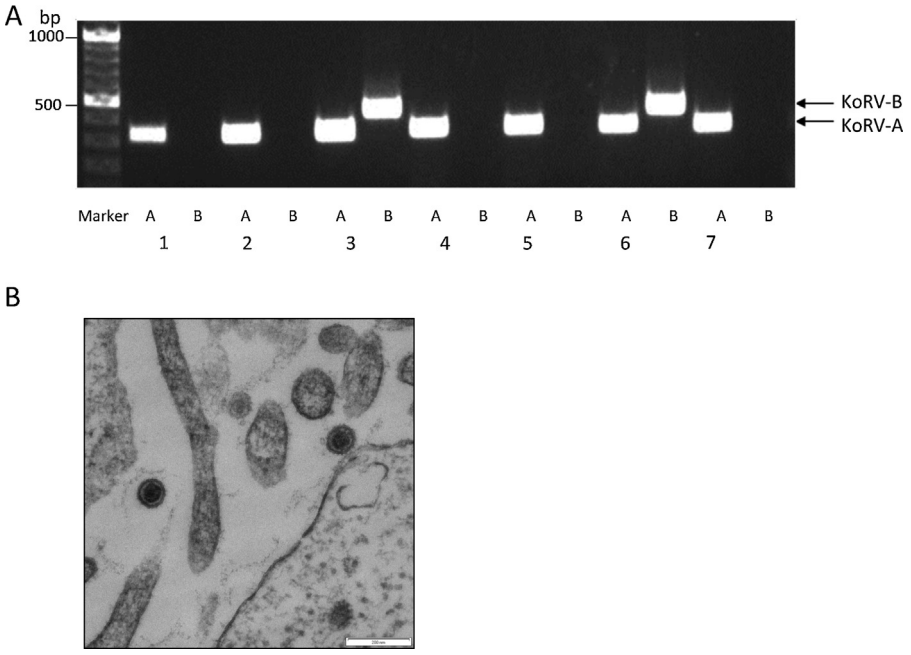

Fig. 1. (A) Results of the PCR analysis using primers specific for KoRV-A and KoRV-B. "A" indicates the amplicon using primers for KoRV-A, "B" for KoRV-B. DNA from the following koalas was tested: 1-Irwin, 2-Goonderah, 3-Coolongalook, 4-Alora, 5-Birubi, 6-Kambara, 7-Kangulandai. (B) Electron microscopy of human 293 cells producing KoRV particles. Infectivity was demonstrated in parallel infection assays.

Sera taken from koalas housed at the Duisburg Zoo in Germany, the Antwerp Zoo in Belgium and from a wild population just south of Brisbane, Australia (Table 1) were analysed by Western blot. Provirus integration in DNA isolated from serum, peripheral blood mononuclear cells (PBMCs), ascites or tumour tissues was tested by PCR using primers specific for KoRV-A and KoRV-B (Table 2). Although all animals carried KoRV-A, two animals, one from the zoo in Duisburg (Kambara) and one from Antwerp (Coolongalook), both originally from the San Diego Zoo, were also found to be infected with KoRV-B (Fig. 1A). Interestingly, a recent study failed to find additional KoRV-B infected animals at the San Diego Zoo, whereas a number were identified at the Los Angeles Zoo (Xu et al., 2013). This suggests that the animals were either infected in Europe or that KoRV-B is the result of a recombination between KoRV-A and other endogenous sequences. The wild animals came from a region of Australia in which all are at least infected with KoRV-A, although the prevalence of KoRV-B remains unknown. As

Table 2
Primers used for detection of the corresponding KoRV.

Virus	Sequence	References
KoRV-A		
Forward	CTAATAAAAGGGCCCATAGA	Hanger et al., 2000
Reverse	GTTGAACCATCCCTCGTACC	
KoRV-B		
Forward	CGGTGAAGGTTGACGGTATT	Xu et al., 2013
Reverse	ACCCCAAGGTTCCATAGCTC	
KoRV-A real-time		
Forward	CTAATAAAAGGGCCCATAGA	
Reverse	GTTGAACCATCCCTCGTACC	
Probe	Fam-CCATGGATACAGACCTTAGGGCCC-BHQ1	