

## Successful vaccination against *Onchocerca ochengi* infestation in cattle using live *Onchocerca volvulus* infective larvae

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### SUMMARY

Epidemiological evidence has led to the hypothesis that the concurrent and predominant transmission of *Onchocerca ochengi* by *Simulium damnosum* s.l. in sub-Saharan Africa could lead to the protection of humans against onchocerciasis caused by *Onchocerca volvulus* (zooprophylaxis). To gain support for this hypothesis, we investigated whether exposure to *O. volvulus* could protect cattle from *O. ochengi*. Gudali calves were vaccinated with live *O. volvulus*-infective larvae and subsequently challenged with *O. ochengi*-infective larvae whilst raised in a fly-proof house. Post-challenge adult parasite and microfilaria development, IgG1 and IgG2 subclass antibodies response to Ov10/Ov11 recombinant *Onchocerca* antigens, and peripheral blood lymphocyte proliferative responses to *O. ochengi* crude antigens were studied over a 1-year period. The vaccinated-challenged animals had 83–87% less adult *O. ochengi* parasites than non-vaccinated-challenged animals. IgG1 and IgG2 antibodies to Ov10/Ov11 recombinant *Onchocerca* antigens were invoked by non-vaccinated-challenged animals but not by most (80%) of the vaccinated-challenged animals. These findings support the idea of cross-protection (zooprophylaxis) due to inoculation of humans with *O. ochengi*-infective larvae under natural transmission conditions in endemic areas.

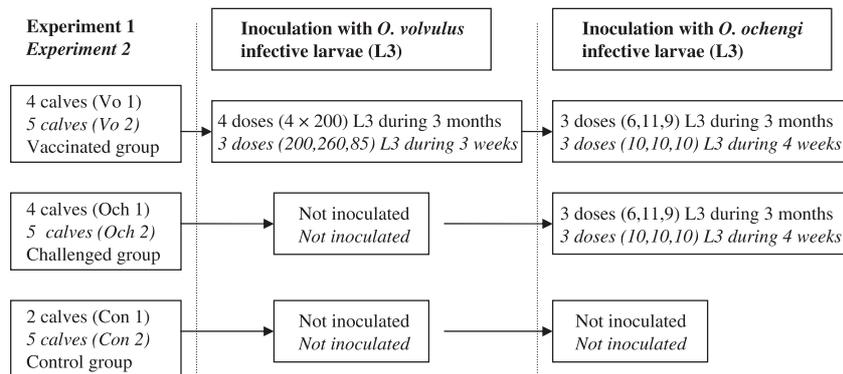
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Human onchocerciasis remains one of the diseases that significantly retards the socio-economic development of many parts of the African continent. *Onchocerca ochengi*, a bovine parasite, is phylogenetically the closest related species of the human filarial nematode *Onchocerca volvulus* (1). In sub-Saharan Africa, especially in West and Central Africa, *Simulium damnosum sensu lato* concurrently transmits both *O. volvulus* in humans and *O. ochengi* in cattle. About 70–90% of filarial larvae in *S. damnosum* s.l. females caught biting man in study sites in north Cameroon are *O. ochengi* (2,3). This high degree of zoophily implies that humans in the affected region are more frequently exposed to animal filaria-infective larvae than to those of *O. volvulus*. The specific conditions of the high Guinea savannah land of the Adamawa province in Cameroon are that a high population density of cattle is associated with a low human population and a very high rate of *O. ochengi* infestation in cattle (4).

The hypothesis of the ensuing longitudinal study was that the concurrent and predominant transmission of these cattle filarial parasites (*O. ochengi*) by *S. damnosum* s.l. leads to the protection of humans against human onchocerciasis (zooprophylaxis). An indirect means of seeking evidence for such protection was to use naïve calves vaccinated with live heterologous *O. volvulus*-infective larvae that were subsequently challenged with *O. ochengi*-infective larvae. It was expected that the *O. volvulus*-infective larvae were not likely to develop in cattle and would therefore most likely behave like other irradiation-attenuated filarial larvae reported in jirds (5). The experimental inoculation of a well-defined number of *O. ochengi*-infective larvae was also expected to provide information on the proportion of those infective larvae that successfully develop to the adult stage worms.

To obtain *Onchocerca*-infective larvae, *O. volvulus*-infested humans in Kumba caught *S. damnosum* flies that came to feed on their legs. Kumba is a forest region of south-west Cameroon not prominent in cattle production. The flies were donated to the project and airlifted to the Wakwa

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**Figure 1** Experimental layouts for immunization and challenge of calves to test the zoonophylaxis of *Onchocerca volvulus* and *Onchocerca ochengi*. All actions or inoculations for experiment 2 are written in italics.

Regional Centre of the Institute of Agricultural Research for Development (IRAD) in North Cameroon, where they were maintained in the laboratory and dissected on the 8th to 9th day. The *O. ochengi*-infective larvae were produced in *Simulium* flies blood fed on *O. ochengi*-infested Zebu cattle. The *O. volvulus*- and *O. ochengi*-infective larvae recovered were transferred into RPMI 1640 medium supplemented with 2 mm glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin (Gibco, Ltd, Paisley, Scotland), and 1% (w/v) glucose (inoculation medium). In the two successive experiments (Figure 1) freshly recovered infective larvae from dissected *Simulium* flies were injected subcutaneously in Zebu Ngaoundere Gudali calves using inoculation medium within 2 h of recovery. The animals were raised together in a fly-proof facility at IRAD Wakwa. Counting, mapping of *O. ochengi* nodules, and estimation of microfilaria (mf) density, were undertaken as previously described (6). All nodules that had developed during the experiment were extirpated from the live animal at the end of the study, and dissected to isolate, examine, identify and count the worms therein. Female worms were also examined for embryogenesis and mf presence as previously described (7).

The percentage of infective larvae developing to adult worms was calculated using the formula: infective larvae development rate = [Number of adult worms recovered/Number of larvae inoculated] × 100. The Chi-square test was used to assess the variation in proportion of the adult worm parasites recovered from the vaccinated and non-vaccinated groups.

In both experiments, only *O. ochengi* parasites were recovered from the challenged and a few of the vaccinated calves. None of the control animals in the fly-proof facility was infested with any of the parasites. The *O. volvulus*-infective larvae that were inoculated in large doses in vaccinated animals did not develop to adult worms. A disproportionate ratio of male (17.9%) to female (82.1%) worms was recovered from both the non-vaccinated, challenged and vaccinated, challenged animals. Most female worms were not fertilized,

probably because the males were young or because most nodules contained no male worms. In bovine, as in human onchocerciasis, live male worms are found slightly less frequently than females during nodulectomy; presumably because the males are mobile and regularly leave the nodule (8). Nodulectomy was undertaken not long after the appearance of nodules when adult male worms could still be too young to mate. The average proportion of infective larvae that developed to adult worms for experiments I and II was 11% for the challenged animals as opposed to the 1.8% for the vaccinated group (Table 1). This difference was highly significant ( $P < 0.001$ ), with a Chi-square value of 18.0. In both experiments, the proportion of those infective larvae that successfully developed to the adult stage varied greatly amongst individuals. It ranged from zero to 38.5% and zero to 20% in individual animals in experiments I and II, respectively. The high proportion of protection (85.7%) recorded is higher than the 70% protection reported against *Dirofilaria immitis* infestation (9) following vaccination with radiation-attenuated infective larvae. It has been previously observed that cattle were highly exposed to natural transmission of *O. ochengi*-infective larvae in the area, with annual transmission potentials of up to 7732 infective larvae per animal and year (2). These findings suggest that a very high proportion of infective larvae inoculated into definitive hosts even under natural exposure conditions do not develop to adult worms, due to some form of density-dependent regulation and/or immune system control of such larvae.

The *O. volvulus* recombinant antigens, Ov10/Ov11 that are cross-reactive amongst *Onchocerca* species (6,10) were employed to examine anti-*Onchocerca* antibody reactivity in five of the vaccinated and five of the non-vaccinated animals for 350 days post-inoculation with *O. ochengi*. Whereas four out of five non-vaccinated, challenged cattle showed an increased IgG response to the antigens, this was only the case with one out of five in the vaccinated group (and this particular animal developed two *O. ochengi* nodules) (results not shown). An exponential rise in IgG1 antibody

**Table 1** Proportion of *Onchocerca ochengi*-infective larvae that successfully develop to adult worms in vaccinated and non-vaccinated challenged calves

Group	No. of animals	No of <i>O. ochengi</i> infective larvae inoculated	Adult <i>O. ochengi</i> worms recovered by nodulectomy			Microfilaria		Infective larvae percentage development	Protection rate (%)
			Female	Male	Total	Intra-uterine	Dermal		
Vaccinated (Vo)	9 <sup>a</sup>	224 <sup>a</sup>	4	0	4	0		1.8	85.7
Non-vaccinated challenged (Och)	9	254	23	5	28	Yes in one animal	Yes in one animal	11.0	
Control	7	0	0	0	0	0	0	0	

Data from experiments I and II combined i.e. not considering vaccine dose (number of *O. volvulus*-infective larvae inoculated). Group Vo, calves were vaccinated with live infective larvae of *O. volvulus* and then challenged by inoculation of each calf with infective larvae of *O. ochengi*. Group Och, calves not vaccinated but each calf inoculated with live infective larvae of *O. ochengi*. The control group received none of these larvae and these animals were used to monitor the efficiency of the fly-proof house. Percent protection is based on the formula: [mean adult worm recovery from vaccinated but challenged calves subtracted from adult worm recovery from non-vaccinated challenged calves] divided by mean recovery from non-vaccinated challenged calves. <sup>a</sup>One calf died 4 months from start of experiment, so data for this animal were deleted from all analysis.

response was associated with the development of dermal mf in the lone non-vaccinated, challenged animal that developed skin mf. A similar increase in antibody levels has been reported in a study involving another bovine *Onchocerca* sp. (*O. lienalis*) in its natural host (11). One of the nodules found in the lone animal with dermal mf in the non-vaccinated, challenged group, suffered from spontaneous suppuration and a severe dermal inflammatory reaction similar to that observed in one of the two nodules of the vaccinated animal described earlier. Both cases resulted in the formation of an open sore from which debris of female worms, too disintegrated to be identified as either *O. ochengi* or *O. volvulus*, were recovered. This massive inflammatory reaction around the two nodules is consistent with eosinophil infiltration observed during increased killing of a challenge infestation (12). It is the first time this has been observed in the *O. ochengi* cattle system. It has been reported in man in Liberia (13). Unlike the non-vaccinated, challenged group the vaccinated animals were also observed to have no peripheral blood mononuclear cell *in vitro* proliferative response to *O. ochengi* whole worm PBS extract (result not shown). A similar lack of PBMC response has been reported in a *Mandrillus sphinx* (14), which showed the greatest resistance to experimental infestation with *Loa loa*. Collectively, these data were considered as consistent with successful vaccination.

Little has become known regarding zooprophylaxis as a natural strategy for the control of certain diseases since this theory was postulated (15). The partial protection of cattle to challenge with *O. ochengi*-infective larvae induced by prior exposure to *O. volvulus* larvae and the degree of immunity observed in the present study is most likely related to the antigenic homology (16) and the phylogenetic closeness

of the human parasite *O. volvulus* and its analogue in cattle, *O. ochengi* (1). The present results portray a great potential for the development of a vaccine against *O. volvulus* as they support the earlier suggestions (3,17,18) that *O. ochengi* infestations under the specific conditions in the Adamawa plateau have a zooprophylactic effect against human onchocerciasis.

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#### REFERENCES

- 1 Bain O. Le genre *Onchocerca*: Hypothese sur son évolution et clé dichotomique des espèces. *Ann Parasitol (Paris)* 1981; **56**: 503–526.
- 2 Achukwi MD, Harnett W & Renz A. *Onchocerca ochengi* transmission dynamics and the correlation of *O. ochengi* microfilaria density in cattle with the transmission potential. *Vet Res* 2000; **31**: 611–621.

- 3 Wahl G, Enyong P, Ngosso A *et al.* *Onchocerca ochengi*: epidemiological evidence of crossprotection against *Onchocerca volvulus* in man. *Parasitology* 1998; **116**: 349–362.
- 4 Wahl G, Achukwi MD, Mbah D, Dawa O & Renz A. Bovine onchocerciasis in North Cameroon. *Vet Parasitol* 1994; **52**: 297–311.
- 5 Lucius R, Textor G, Kern A & Kirsten C. *Acanthocheilonema viteae*: vaccination of jirds with irradiation-attenuated stage-3 larvae and with exported larval antigen. *Exp Parasitol* 1991; **73**: 184–196.
- 6 Achukwi MD, Harnett W, Bradley J & Renz A. *Onchocerca ochengi* acquisition in zebu Gudali cattle exposed to natural transmission: parasite population dynamics and IgG antibody subclass responses to Ov10/Ov11 recombinant antigens. *Vet Parasitol* 2004; **122**: 35–49.
- 7 Renz A, Tress AJ, Achukwi D, Edwards G & Wahl G. Evaluation of suramin, ivermectin and CGP 20376 in a new macroparasitocidal drug screen, *Onchocerca ochengi*. *Trop Med Parasitol* 1995; **46**: 31–37.
- 8 Schulz-Key H & Karam M. Periodic reproduction of *Onchocerca volvulus*. *Parasitol Today* 1986; **2**: 284–286.
- 9 Mejia SJ & Carlow CKS. An analysis of the humoral immune response of dogs following vaccination with irradiated infective larvae of *Dirofilaria immitis*. *Parasite Immunol* 1994; **16**: 157–164.
- 10 Kuo YM & Bianco AE. Stage-specific and species cross-reactive antibody responses in experimental *Onchocerca* infections of cattle. *Am J Trop Med Hyg* 1995; **53**: 624–632.
- 11 Kuo YM & Bianco AE. Temporal changes in the humoral immune response of cattle during experimental infections with *Onchocerca lienalis*. *Parasite Immunol* 1995; **17**: 393–404.
- 12 Lange AM, Yutanawiboonchai W, Scott P & Abraham D. IL-4- and IL-5-dependent protective immunity to *Onchocerca volvulus* infective larvae in BALB/cBYJ mice. *J Immunol* 1994; **153**: 205–211.
- 13 Albiez EJ, Buttner DW & Duke BOL. Diagnosis and extirpation of nodules in human onchocerciasis. *Trop Med Parasit* 1988; **39**: 331–346.
- 14 Leroy E, Baize S, Wahl G, Egwang TG & Georges AJ. Experimental infection of a nonhuman primate with *Loa loa* induces transient strong immune activation followed by peripheral unresponsiveness of Helper I cells. *Infect Immun* 1997; **65**: 1876–1882.
- 15 Nelson G. Parasitic Zoonoses. In England PT & Sher A (eds): *The Biology of Parasitism: A Molecular and Immunological Approach*. New York, Alan R. Liss, MBL lectures in Biology; 1988; **9**: 13–41.
- 16 Hoch B, Wahl G, Enyong P, Lüder CGK, Harnett W & Renz A. Serological recognition of specific and crossreactive antigens of *Onchocerca ochengi* and *O. volvulus* by infected cattle and humans. *Trop Med Parasitol* 1992; **43**: 206–207.
- 17 Renz A, Enyong P & Wahl G. Cattle, worms and zooprophylaxis. *Parasite* 1994; **1**(Suppl. 1): 4–6.
- 18 Seidenfaden R, Fischer A, Bonow I, Ekale D, Tanya V & Renz A. Combined benefits of annual mass treatment with ivermectin and cattle zooprophylaxis on the severity of human onchocerciasis in northern Cameroon. *Trop Med Internat Health* 2001; **6**: 715–725.