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E fi ... DNA ... *Cryptosporidium baileyi* ...

... C ..., A ... D *

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... *Cryptosporidium baileyi* ... *Cryptosporidium* ... I ... EGF ... OM ... A ... *C. baileyi* ... (*P* < 0.05), ... B ... (*P* < 0.05) ... 100 μ ... EGF ... OM, ... B ... A ... *C. baileyi* ... 2, ... 100 μ ... EGF ... OM ... 71.3%. ... fi ... (*P* > 0.05), ... fi ... (*P* < 0.05). ...

— 2016 E... B. ... A...

1. Introduction

Cryptosporidium, ... A ... C ... *Cryptosporidium*, ... I ... (N ... ., 1999).

Cryptosporidium ... 30 ... (N ... ., 2006). A ... *Cryptosporidium baileyi*, *Cryptosporidium meleagridis* ... *Cryptosporidium galli* (C ... ., 1986; ... 2001; ... 1994, 1999; ... ., 2003). A ... , *C. baileyi* ... *Cryptosporidium* ... (C ... ., 1986), ... *C. meleagridis*

I [illegible] *Plasmodium* (B [illegible] ., 2006), *Toxoplasma gondii* (B [illegible] ., 2005), *Eimeria tenella* (L [illegible] ., 2006), *Cryptosporidium parvum* [illegible] *Cryptosporidium andersoni* ([illegible] ., 2009). [illegible] ([illegible] ., 2012), [illegible] ([illegible] ., 2004). A [illegible] *T. gondii* (B [illegible] ., 2005). D [illegible] (B [illegible] ., 2005; D [illegible] , 2005). [illegible] *Cryptosporidium*, [illegible] *C. parvum* [illegible] (C [illegible] OM) [illegible] (2007) [illegible]

A [illegible] *C. baileyi rhomboid* [illegible] EGF [illegible] OM [illegible] *C. baileyi* [illegible]

2. Materials and methods

2.1. Parasites

C. baileyi J [illegible] H [illegible] , C [illegible] 3 [illegible] 2.5% [illegible] 4 °C [illegible] 2 [illegible]

B (B 0.05% 20), 5% B 37 °C 1 1:100 5% 37 °C 2 A H I G (1:10,000) 37 °C 1 4 O (O D) ( ) 30% H_2O_2 10 0.1 M (H 5.0) 100 μ 15 50 μ 2 M H_2O_4 (OD) 492 . A

2.9. Peripheral blood lymphocyte proliferation assay

5 10 . 1.0 × 10^7 / ML 1640 (G) 10% (FB), 100 8 / 100 / . 96 fl (50 μ /) 37° C 5% CO_2 50 μ A L (20 μ /) 48 . M 5 / ( ) (10 μ) 4 100 μ (DM O) 570 (OD570). E

2.10. Evaluation of protective efficacy of DNA vaccine against C.

DNA (B G). I 27 *C. baileyi* (O G) . B 3, 33 60 B G (B G 1 = ; B G 2 =) . F 30 .

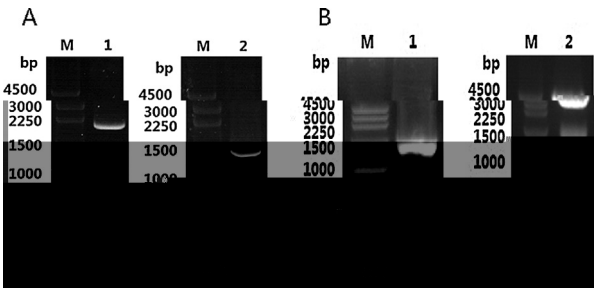

Fig. 1. A fi DNA *C. baileyi* fi EGF OM. (A) A fi DNA *C. baileyi* N . M: 250 DNA L ; 1: ; 2: . (B) I fi EGF OM C . M: 250 DNA L ; 1: EGF OM; 2: EGF OM, K I/ I.

2.11. Statistical analyses

ANO A G 5 . $P < 0.05$ fi .

3. Results

3.1. Nested-PCR amplification and construction of recombinant plasmid pEGFP-CbROM

OM O F fi N C DNA 1422 (F . 1A) EGF C2, EGF OM. fi C *Sac I*/*Kpn I*. (F . 1B). OM B L . OM *C. parvum* (**XM_628467.1**), *C. hominis* (**XM_661677.1**) *C. muris* (**002139608.1**) G B 63.6%, 63.9% 36.5% . A *C. parvum* (**XP_628469.1**), *C. hominis* (**XP_666769.1**) *C. muris* (**XP_002139644.1**) G B 60.3%, 66.8% 51.1% .

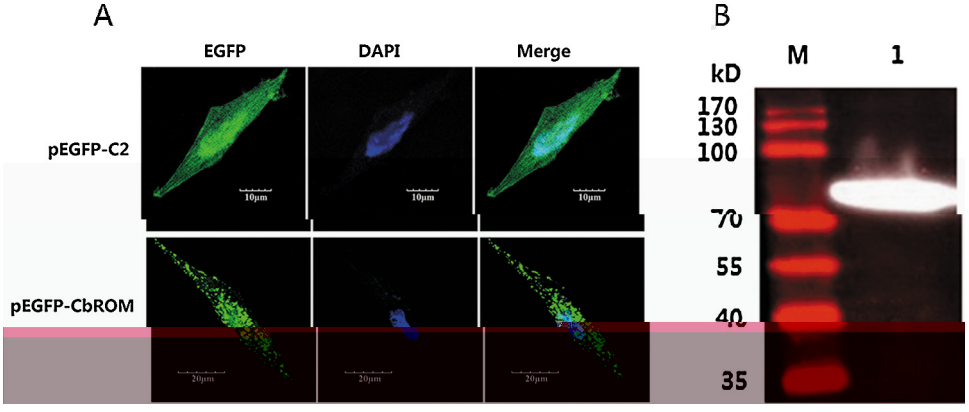

Fig. 2. D EGF OM CEF . (A) D OM CEF CEF CEF EGF OM CEF EGF . A 48 , CEF EGF fl fl CEF EGF OM . (B) B EGF OM. GF M: M ; 1: EGF OM CEF 48 . (FC fi .)

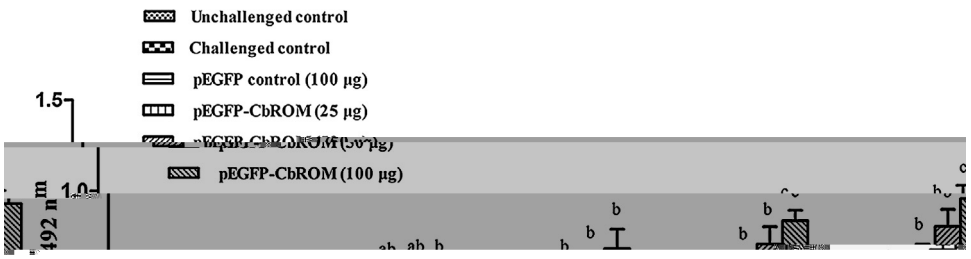

Fig. 3. H C b EGF . G OM DNA . C EGF . G OM, EGF C, B C 5 C fi *C. baileyi* C b ELI A. A492 C 1:100 C . E b OD (± .E., =5). B C ( ) C fi (*P*<0.05).

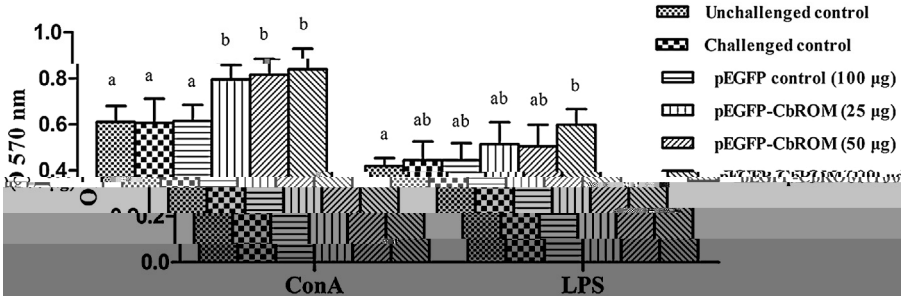

Fig. 4. A C C C C b C C EGF . G OM. OD570 C M. (=5). 570 ELI A. B C (b) C fi (*P*<0.05).

3.2. CbROM protein expression in CEF cells

A 48 C fl C b CEF EGF . G OM, fl C EGF . C2 (F . 2A). C b G OM C CEF .

3.3. Western-blot analysis of CbROM systhesized in vitro

b C C CEF C EGF . G OM, C 79 D (F . 2B), GF C C C b C H b C C I G.

3.4. Evaluation of specific antibody responses

C ELI A C C G C b C C C F . 3. C b C EGF . G OM C C C EGF C B fi (*P*<0.05), b C fi G C C EGF . G OM 10 20 I (*P*>0.05). I EGF . G OM C OD C fi I G C 30 I, C EGF . C2 B C C C b C C G C b fi C C EGF . G OM (100 μ) C C C C (*P*<0.05). C EGF . G OM C fi b C C .

3.5. Evaluation of lymphocytes proliferation

C b M C C F . 4. A 30 I, b C C C C C C fi C C C b EGF . G OM C C C C C (<0.05), C b C B C C C fi C C C EGF . G OM (100 μ) C C C C (*P*<0.05). C C b *Cryptosporidium* C C DNA C fi C C C C C *Cryptosporidium* C .

3.6. Protective efficacy of DNA vaccination against C b

C DNA b C C C *C. baileyi* C C C C 27 C 10^6 C b C C C C . I C C C C C C C 11 13 C C C (F . 5). C EGF .

Table 1
E [illegible] EGF [illegible] OM [illegible].

G[illegible]	3 ( )	33 ( )	60 ( )	A[illegible] B[illegible] G 1 ( )	A[illegible] B[illegible] G 2 ( )
[illegible]	31.26 ± 1.500	255.0 ± 22.92	490.9 ± 42.23	223.8 ± 23.08	253.9 ± 43.02
C[illegible]	31.27 ± 1.836	252.6 ± 17.12	421.7 ± 25.05	221.3 ± 16.51	169.2 ± 30.30
EGF [illegible] (100 μ)	31.24 ± 1.642	258.9 ± 22.11	420.7 ± 41.67	227.7 ± 21.74	161.7 ± 35.28
EGF [illegible] OM (25 μ)	29.73 ± 1.951	245.0 ± 18.38	455.5 ± 35.15	215.3 ± 18.15	210.5 ± 28.08
EGF [illegible] OM (50 μ)	30.43 ± 2.001	240.5 ± 24.58	464.4 ± 33.76	210.1 ± 24.76	

(100 μ) A, EGF OM (*P* < 0.05), B L (100 μ) (*P* < 0.05). *Cryptosporidium* *Cryptosporidium* (H 1984).

EGF OM *C. bailey* 10^6 *C. bailey* 10. 2. 100 μ EGF OM, 71.3%. (*P* > 0.05) (*P* < 0.05). (*P* > 0.05) B G2 EGF OM *C. baileyi* A 100 μ EGF OM 50 μ DNA . A EGF OM B G1 OM *Cryptosporidium* *E. tenella* *C. parvum and C. hominis* (2012). A DNA (100 μ) OM DNA *Cryptosporidium* *Cryptosporidium*.

Conflict of interest

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K M (NC. 2012C12009. 2)

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