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(71) **Demandeur/Applicant:**
VETERINARMEDIZINISCHE UNIVERSITAT WIEN, AT

(72) **Inventeurs/Inventors:**
HESS, MICHAEL, AT;
SCHACHNER, ANNA, AT;
MAREK, ANA, AT;
JASKULSKA, BARBARA, AT

(74) **Agent:** SMART & BIGGAR

(54) **Titre : VACCIN A ADENOVIRUS AVIAIRE**
(54) **Title: FOWL ADENOVIRUS VACCINE**

(57) **Abrégé/Abstract:**
Disclosed is a vaccine comprising fiber (2) protein of Fowl Adeno-virus C (FAdV-C) or an immunogenic fragment thereof for use in preventing hepatitis-hydropericardium Syndrome (HHS) in birds, preferably in poultry, especially in broilers.

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(71) **Applicant**: **VETERINÄRMEDIZINISCHE UNIVERSITÄT WIEN** [AT/AT]; Veterinärplatz 1, A-1210 Vienna (AT).

(72) **Inventors**: **HESS, Michael**; Josefsteig 71, A-3400 Klosterneuburg (AT). **SCHACHNER, Anna**; Veterinärplatz 1, A-1210 Vienna (AT). **MAREK, Ana**; Magdalenenstrasse 8/12, A-1060 Vienna (AT). **JASKULSKA, Barbara**; Veterinärplatz 1, A-1210 Vienna (AT).

(74) **Agent**: **SONN & PARTNER PATENTANWÄLTE**; Riemergasse 14, A-1010 Vienna (AT).

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(54) Title: FOWL ADENOVIRUS VACCINE

(57) Abstract: Disclosed is a vaccine comprising fiber (2) protein of Fowl Adeno-virus C (FAdV-C) or an immunogenic fragment thereof for use in preventing hepatitis-hydropericardium Syndrome (HHS) in birds, preferably in poultry, especially in broilers.

Fowl adenovirus vaccine

The invention relates to methods and compositions (formulations) for the prevention of hepatitis-hydropericardium syndrome (HHS).

HHS is an infectious disease of chickens, characterized by high mortality and severe economic losses, mainly in broiler flocks. After first reports of the disease in 1987 from Pakistan, outbreaks have been documented mainly in Asian countries, Central and South America. Initial assumptions pointed towards the involvement of an unknown agent in addition to an adenovirus which was later revised by reproducing the disease in specific pathogen-free birds following oral infection with virulent fowl adenovirus (FAdV) species C strains.

Fowl adenoviruses are members of the family *Adenoviridae* and genus *Aviadenovirus*. Five species (FAdV-A to FAdV-E) and 12 serotypes (FAdV-1 to 8a and 8b to 11), identified by cross-neutralization test, have so far been recognized.

Adenoviruses are non-enveloped particles with a double-stranded DNA genome and a diameter of 70 - 90 nm.

The major structural proteins of an adenovirus are hexons and pentons, constituting an icosahedral capsid of 252 subunits (capsomers), with hexons forming the facets and pentons capping the vertices of the icosahedron. The penton base anchors the antenna-like fiber protein, whose distal head domain, termed knob, harbors the receptor-binding site and is thus essential for initiating virus attachment to the host cell.

The FAdV capsid is characterized by a morphological peculiarity of two fiber proteins associated with each penton base, whereas mammalian adenoviruses feature only one fiber protein per vertex. Although the existence of dual fibers is common to all FAdVs, two fibers distinct in sequence and length, each encoded by a separate gene, are a specific feature of FAdV-1 (FAdV-A) (Hess et al., J. Mol. Biol. 252 (1995), 379-385). Based on the novel finding of two separate fiber-encoding genes in an FAdV-C isolate, it was recently demonstrated that this reflects, among all FAdV species with equally long fiber proteins, a feature exclusively attributed to members of FAdV-C (Marek et al., Vet. Microbiol. 156 (2012), 411-417).

Characterization of the knob as receptor-binding domain has established the fiber molecule as a critical factor associated with infection properties of adenoviruses, such as alterations in tissue tropism and virulence. However, many questions are still open in regard to the individual functionality of the dual fibers present in FAdVs, particularly in the context of interaction with host cell receptors.

As major surface-exposed capsid structures, fiber and hexon are key mediators of antigenicity in adenoviruses and carriers of a panoply of epitopes of subgroup- and type-specificity. It has also been shown that hexon- and fiber-specific antibodies account for most of the neutralizing activity in mammalian humoral response against adenovirus. Recently, *in vitro* trials demonstrated different degrees of neutralizing capacity of antibodies raised against recombinant hexon and fiber proteins of the egg-drop syndrome virus (EDSV (DAdV-A = DAdV-1)).

Owing to their antigenic properties, adenovirus capsid structures have been proposed as potential candidates for the design of epitope-based vaccines.

Strategies to combat HHS have concentrated on the prevention of infection and on the provision of attenuated fowl adenovirus vaccines (WO 03/039593) or inactivated vaccines from infected liver homogenates (Anjum et al. 1990) or grown up virus on primary cells (Alvarado et al. 2007). Due to the ubiquitous occurrence of FAdVs, however, applying such conventional vaccines and verification of effectiveness of the vaccination is of limited use due to the lack of discrimination between vaccination and infection.. A subunit vaccine against HHS based on the penton base (expressed in *E. coli*) was recently suggested (Shah et al., (Vaccine 30 (2012); 7153-7156)); however it is usually difficult to detect antibodies as an indicator of successful immunization because of the omnipresence of other fowl adenoviruses.

US 2011/165224 A1 discloses isolated FAdV strains of specific serotypes for inducing protective immunity. These compositions contain whole (live or killed) viruses, no subunit vaccines or isolated FAdV proteins. Griffin et al. (J. Gen. Virol. 92 (2011); 1260-1272) disclose coding potential and transcript analysis of FAdV-4. It is speculated that FAdV-4 fiber 2 (short fiber) which is "predicted to be protein-coding" (but not shown to be expressed) might bind a receptor and determine the tissue

tropism of FAdV-4, "perhaps leading to the unique clinical features associated with infection of virulent FadV-4". The authors correctly point out that both, avian FAdV-1 and the human enteric serotypes HAdV-40 and HAdV-41 (=HADV-F), contain two fiber genes. However, there are significant differences: Whereas in FAdV-1, as in all fowl AdVs, always two fibers per penton base are assembled together, there is only one fiber in the HAdV-Fs. Moreover, different quantities of both fibers are assembled into the HAdV-F virion although expression is the same on mRNA level (Song et al., Virology 432 (2012), 336-342). This shows that both fibers have different functions in the assembled virion (this has been verified in receptor studies). Moreover, Tan et al. (J. Gen. Virol. 82 (2001), 1465-1472) have shown that fiber 2 is involved in virus assembly and in the interaction with an unknown cellular receptor. Since FAdV-1 comprises - in contrast to all other FAdVs - two fibers of completely different lengths, such results cannot be transferred to other serotypes.

Marek et al. (Vet. Microbiol. 156 (2012); 411-417) discloses the fact that two fiber genes of nearly equal length are present in FAdV-C whereas other serotypes have only one fiber gene. Although it is mentioned that "fibers of FAdV play an important role in infectivity and pathogenicity of FAdV" (demonstrated in 1996!), this statement was identified by Marek et al. as "purely speculative" as far as FAdV-C is concerned. Furthermore, the likelihood that fiber proteins are involved in infectivity and pathogenicity does not automatically implicate the successful application of recombinant proteins as a vaccine.

Fingerut et al. (Vaccine 21 (2003); 2761-2766) disclose a subunit vaccine against the adenovirus egg-drop syndrome using part of its fiber protein.

It is an object of the present invention to provide a safe and specific vaccine for efficient prevention of HHS in birds, especially in poultry. The vaccine should be easy and cost-effective to produce and be suitable for administration on an industrial basis. Successful immunization with the vaccine should be easily detectable and confirmable.

Therefore, the present invention discloses a vaccine comprising a fiber 2 protein of Fowl Adenovirus C (FAdV-C) or an immunogenic fragment thereof for use in preventing hepatitis-hydropericardium syndrome (HHS) in birds, preferably in poultry,

especially in broilers.

The present invention provides the teaching that the fiber-2 protein of FAdV-C is an effective subunit vaccine that protects birds, especially chicken, completely from HHS. This finding was remarkable because fiber-1 protein of FAdV-C as well as hexon-derived subunit vaccines (hexon loop 1) did not show a protective effect. It is evident that the present vaccines with isolated subunits, i.e. isolated single proteins or protein fragments, essentially differ from vaccines that are based on live, attenuated or killed (whole) viruses. Accordingly, the present invention provides a completely novel and - in view of the teachings present in the present field for fiber and hexon-derived proteins in FAdVs - surprisingly effective strategy for vaccinating birds to manage prevention of HHS, IBH and GE.

For the present invention, any fiber-2 protein of FAdV-C can be used. In the examples of the present invention, fiber-2 protein from reference strain KR5 was used as reference (UniProt entry H8WQW9); however, also other fiber-2 protein sequences of FAdV-C can be used, e.g. from reference strains ON1 (GU188428 = NC_015323) or CFA20 (AF160185) or any other FAdV-C field isolates, e.g. isolates IV37, K99-97, K388-95, K88-95, K31, Peru53, Peru54, c344, K1013, AG234, C2B, 09-584, 09-8846, 09-2602, 922-1, Da60, K1013QT and INT4 (as disclosed by Marek et al., Vet. Microbiol. 156 (2012), 411-417); corresponding to UniProt entries H8WG65, H8WG69, H8WG72, H8WG77, H8WG70, H8WG73, H8WG66, H8WG76, H8WG60, H8WG61, H8WG62, H8WG75, H8WG67, H8WG78, H8WG63, H8WG68, H8WG64, H8WG74, H8WG71, H8WQZ7, H8WQZ2, H8WQW9, Q0GH78, O55281, and F2VJI5.

The term “fiber-2 protein” is accepted in the present field of technology. All fiber proteins, especially the sub-group of fiber-2 proteins are unified by a characteristic amino acid sequence resulting in a characteristic structure with specific and conserved amino acid motifs.

Instead of using the whole fiber-2 protein of FAdV-C, only immunogenic fragments of fiber-2 protein can be used as vaccines according to the present invention. Immunogenic fragments can be any polypeptide from a fiber-2 protein of a naturally occurring FAdV-C isolate with a minimum length of 7 amino acid residues, preferably with a minimum length of 8 amino acid residues, especially with a minimum length of 9 amino acid residues. These

minimum lengths provide sufficient MHC binding. Suitable motifs can be verified experimentally or via computer prediction (see e.g. Wallny et al., PNAS 103(2006), 1434-1439 ; Huo et al., PLoS ONE 7 (2012): e39344. doi:10.1371). Preferred lengths of the immunogenic fragments are therefore 7 to 100 amino acids, preferably 8 to 50 amino acids, more preferred 8 to 20 amino acids, especially 8 to 16 amino acids. For example, the immunogenic fragments according to the present invention may contain octapeptides or nonapeptides based on the peptide-binding motifs of chicken MHC class I molecules belonging to the B4, B12, B15, and B19 haplotypes (Wallny et al., 2006; Huo et al., 2012). The motifs were as follows: B4: x-(D or E)-x-x-(D or E)-x-x-E; B12: x-x-x-x-(V or I)-x-x-V and x-x-x-x-(V or I)-x-x-x(V); B15: (K or R)-R-x-x-x-x-x-Y and (K or)-R-x-x-x-xx-x-Y; B19: x-R-x-x-x-x-x-(Y, P, L, F) and x-R-x-x-x-x-x-x-(Y, P, L, F).

The fiber-2 protein has a tail domain (amino acid 1 to 65), a shaft domain (amino acid 66 to 276) and a head domain (amino acid 277 to 479; all amino acid sequence numbers in this general specification are based on the fiber-2 protein of the KR5 reference strain (UniProt H8WQW9; Marek et al., 2012)). Preferred immunogenic fragments of the present invention contain the following motifs (based on amino acid numbering according to fiber-2 of KR5): 400 to 450, preferably 410 to 440, more preferred 420-440; 70 to 95, preferably 75 to 93, especially 75 to 90; 20 to 70, preferably 25 to 65, especially 45 to 65 and 25 to 47; 200 to 225, 265 to 290, 350 to 385, 460 to 480, 165 to 190, 320 to 350 and 290 to 320.

Examples of immunogenic fragments are fragments comprising one or more of the following amino acid sequences of fiber-2 protein (again according to the amino acid sequence of fiber-2 of KR5 and corresponding to the alignment in Fig. 5):

Most preferred:			424	to	438	253	to	267	26	to	40
23	to	37	421	to	435	425	to	439	403	to	417
24	to	38	422	to	436	254	to	268	404	to	418
22	to	36	423	to	437	78	to	92	405	to	419
25	to	39	424	to	438	424	to	438	406	to	420
21	to	35	425	to	439	424	to	438	407	to	421
424	to	438	426	to	440	79	to	93	408	to	422
254	to	268	427	to	441	77	to	91	409	to	423
423	to	437	423	to	437	423	to	437	69	to	83

255	to	269
20	to	34
325	to	339
355	to	369
425	to	439
423	to	437
45	to	59
46	to	60
47	to	61
48	to	62
49	to	63
50	to	64
51	to	65
326	to	340
255	to	269
326	to	340

Highly	pre-		207	to	221	426	to	440	308	to	322
ferred:			208	to	222	324	to	338	283	to	297
424	to	438	209	to	223	167	to	181	71	to	85
26	to	40	210	to	224	168	to	182	425	to	439
27	to	41	267	to	281	169	to	183	426	to	440
28	to	42	268	to	282	170	to	184	77	to	91
29	to	43	269	to	283	171	to	185	422	to	436
30	to	44	270	to	284	172	to	186	68	to	82
31	to	45	271	to	285	173	to	187	426	to	440
32	to	46	272	to	286	356	to	370	282	to	296
75	to	89	273	to	287	70	to	84	426	to	440
76	to	90	353	to	367	353	to	367	356	to	370
77	to	91	354	to	368	192	to	206	281	to	295
78	to	92	355	to	369	54	to	68	284	to	298
79	to	93	356	to	370	55	to	69	78	to	92
80	to	94	357	to	371	322	to	336	310	to	324
81	to	95	358	to	372	323	to	337	311	to	325
354	to	368	359	to	373	324	to	338	53	to	67
283	to	297	69	to	83	325	to	339	183	to	197
425	to	439	76	to	90	326	to	340	313	to	327
422	to	436	425	to	439	422	to	436	314	to	328
325	to	339	327	to	341	422	to	436	315	to	329
282	to	296	68	to	82	327	to	341	316	to	330
422	to	436	252	to	266	56	to	70	317	to	331
253	to	267	309	to	323	294	to	308	318	to	332
423	to	437	442	to	456	295	to	309	319	to	333
322	to	336	256	to	270	296	to	310	52	to	66
323	to	337	426	to	440	297	to	311	252	to	266
324	to	338	68	to	82	298	to	312	183	to	197
325	to	339	69	to	83	299	to	313	297	to	311
326	to	340	70	to	84	300	to	314	422	to	436
327	to	341	71	to	85	355	to	369	328	to	342
328	to	342	72	to	86	325	to	339	59	to	73
70	to	84	73	to	87	191	to	205	60	to	74
425	to	439	74	to	88	355	to	369	61	to	75
423	to	437	464	to	478	71	to	85	62	to	76
424	to	438	465	to	479	441	to	455	63	to	77
204	to	218	310	to	324	421	to	435	64	to	78
205	to	219	80	to	94	256	to	270	65	to	79
206	to	220	443	to	457	79	to	93	463	to	477

184	to	198	188	to	202
254	to	268	76	to	90
309	to	323	194	to	208
207	to	221	77	to	91
43	to	57	326	to	340
324	to	338	193	to	207
52	to	66	79	to	93
53	to	67	282	to	296
54	to	68	69	to	83
55	to	69	184	to	198
56	to	70	298	to	312
57	to	71	23	to	37
58	to	72	70	to	84
185	to	199	379	to	393
323	to	337	283	to	297
444	to	458	296	to	310
324	to	338	283	to	297
356	to	370			
78	to	92			
206	to	220			
364	to	378			
376	to	390			
377	to	391			
378	to	392			
379	to	393			
380	to	394			
381	to	395			
382	to	396			
71	to	85			
192	to	206			
378	to	392			
421	to	435			
192	to	206			
297	to	311			
182	to	196			
183	to	197			
184	to	198			
185	to	199			
186	to	200			
187	to	201			

Preferred:			283	to	297	127	to	141	355	to	369
424	to	438	284	to	298	186	to	200	425	to	439
354	to	368	285	to	299	465	to	479	421	to	435
27	to	41	286	to	300	310	to	324	206	to	220
426	to	440	284	to	298	257	to	271	283	to	297
255	to	269	440	to	454	421	to	435	80	to	94
282	to	296	261	to	275	323	to	337	308	to	322
357	to	371	310	to	324	208	to	222	312	to	326
193	to	207	251	to	265	378	to	392	91	to	105
261	to	275	363	to	377	72	to	86	92	to	106
307	to	321	207	to	221	93	to	107	93	to	107
352	to	366	24	to	38	207	to	221	94	to	108
354	to	368	319	to	333	320	to	334	95	to	109
75	to	89	282	to	296	86	to	100	96	to	110
261	to	275	182	to	196	458	to	472	97	to	111
295	to	309	260	to	274	459	to	473	69	to	83
44	to	58	22	to	36	460	to	474	424	to	438
207	to	221	293	to	307	461	to	475	169	to	183
169	to	183	428	to	442	462	to	476	165	to	179
253	to	267	429	to	443	281	to	295	166	to	180
311	to	325	296	to	310	280	to	294	101	to	115
292	to	306	168	to	182	281	to	295	102	to	116
185	to	199	260	to	274	77	to	91	103	to	117
464	to	478	318	to	332	205	to	219	104	to	118
465	to	479	78	to	92	308	to	322	105	to	119
283	to	297	309	to	323	193	to	207	106	to	120
423	to	437	79	to	93	204	to	218	107	to	121
206	to	220	191	to	205	261	to	275	191	to	205
42	to	56	192	to	206	260	to	274	298	to	312
287	to	301	193	to	207	170	to	184	259	to	273
288	to	302	194	to	208	377	to	391	423	to	437
289	to	303	195	to	209	282	to	296	309	to	323
290	to	304	196	to	210	167	to	181	205	to	219
291	to	305	197	to	211	190	to	204	261	to	275
292	to	306	282	to	296	310	to	324	357	to	371
293	to	307	28	to	42	189	to	203	256	to	270
357	to	371	317	to	331	190	to	204	291	to	305
280	to	294	245	to	259	309	to	323	295	to	309
281	to	295	206	to	220	284	to	298	327	to	341
282	to	296	67	to	81	76	to	90	294	to	308

170	to	184	258	to	272	443	to	457	161	to	175
283	to	297	328	to	342	307	to	321	162	to	176
347	to	361	203	to	217	81	to	95	163	to	177
421	to	435	322	to	336	29	to	43	355	to	369
208	to	222	128	to	142	94	to	108	204	to	218
281	to	295	262	to	276	311	to	325	421	to	435
191	to	205	85	to	99	284	to	298	406	to	420
244	to	258	55	to	69	421	to	435	118	to	132
464	to	478	452	to	466	80	to	94	71	to	85
57	to	71	75	to	89	72	to	86	294	to	308
58	to	72	422	to	436	202	to	216	346	to	360
425	to	439	77	to	91	79	to	93	426	to	440
348	to	362	353	to	367	323	to	337	280	to	294
349	to	363	321	to	335	281	to	295	45	to	59
350	to	364	28	to	42	312	to	326	290	to	304
351	to	365	78	to	92	87	to	101	297	to	311
352	to	366	262	to	276	28	to	42	320	to	334
284	to	298	453	to	467	281	to	295	119	to	133
379	to	393	123	to	137	285	to	299	20	to	34
311	to	325	124	to	138	465	to	479	21	to	35
327	to	341	125	to	139	356	to	370	22	to	36
260	to	274	126	to	140	194	to	208	23	to	37
182	to	196	127	to	141	309	to	323	24	to	38
445	to	459	128	to	142	306	to	320	25	to	39
262	to	276	129	to	143	252	to	266	404	to	418
422	to	436	265	to	279	306	to	320	442	to	456
55	to	69	266	to	280	443	to	457	289	to	303
450	to	464	320	to	334	405	to	419	281	to	295
451	to	465	321	to	335	54	to	68	170	to	184
452	to	466	366	to	380	41	to	55	258	to	272
453	to	467	367	to	381	204	to	218	259	to	273
454	to	468	368	to	382	24	to	38	260	to	274
455	to	469	369	to	383	380	to	394	261	to	275
456	to	470	370	to	384	251	to	265	262	to	276
310	to	324	371	to	385	208	to	222	263	to	277
70	to	84	372	to	386	348	to	362	264	to	278
208	to	222	284	to	298	157	to	171	442	to	456
353	to	367	322	to	336	158	to	172	443	to	457
205	to	219	246	to	260	159	to	173	444	to	458
328	to	342	260	to	274	160	to	174	445	to	459

446	to	460	262	to	276	308	to	322	117	to	131
447	to	461	376	to	390	444	to	458	70	to	84
448	to	462	21	to	35	254	to	268	346	to	360
347	to	361	311	to	325	195	to	209	310	to	324
186	to	200	170	to	184	126	to	140	25	to	39
68	to	82	294	to	308	189	to	203	29	to	43.
403	to	417	54	to	68	93	to	107	465	to	479
213	to	227	29	to	43	295	to	309	292	to	306
25	to	39	298	to	312	192	to	206	345	to	359
27	to	41	364	to	378	194	to	208	164	to	178
259	to	273	290	to	304	227	to	241	137	to	151
27	to	41	259	to	273	71	to	85	263	to	277
169	to	183	205	to	219	70	to	84	209	to	223
407	to	421	92	to	106	23	to	37	305	to	319
321	to	335	280	to	294	316	to	330	171	to	185
365	to	379	28	to	42	1	to	15	136	to	150
442	to	456	33	to	47	206	to	220	280	to	294
299	to	313	34	to	48	293	to	307	66	to	80
227	to	241	35	to	49	56	to	70	67	to	81
308	to	322	36	to	50	1	to	15	181	to	195
309	to	323	37	to	51	243	to	257	358	to	372
310	to	324	38	to	52	369	to	383	284	to	298
311	to	325	205	to	219	325	to	339	295	to	309
312	to	326	80	to	94	225	to	239	325	to	339
377	to	391	39	to	53	226	to	240	129	to	143
72	to	86	40	to	54	227	to	241	94	to	108
29	to	43	245	to	259	228	to	242	307	to	321
348	to	362	262	to	276	229	to	243	291	to	305
307	to	321	426	to	440	230	to	244	251	to	265
345	to	359	69	to	83	231	to	245	252	to	266
346	to	360	280	to	294	76	to	90	253	to	267
347	to	361	312	to	326	349	to	363	254	to	268
361	to	375	464	to	478	378	to	392	255	to	269
362	to	376	319	to	333	451	to	465	256	to	270
363	to	377	354	to	368	296	to	310	257	to	271
364	to	378	187	to	201	69	to	83	288	to	302
365	to	379	322	to	336	356	to	370	285	to	299
169	to	183	325	to	339	228	to	242	254	to	268
311	to	325	308	to	322	76	to	90	228	to	242
168	to	182	67	to	81	71	to	85	257	to	271

319 to 333
440 to 454

The vaccine according to the present invention preferably contains a fiber-2 protein of FAdV-C, selected from the sequences UniProt entries H8WG65, H8WG69, H8WG72, H8WG77, H8WG70, H8WG73, H8WG66, H8WG76, H8WG60, H8WG61, H8WG62, H8WG75, H8WG67, H8WG78, H8WG63, H8WG68, H8WG64, H8WG74, H8WG71, H8WQZ7, H8WQZ2, H8WQW9, Q0GH78, O55281, and F2VJI5, as well as the protein sequences provided in Fig. 5 and Table 3, especially H8WQW9, or immunogenic fragments thereof; or immunogenic sequences with at least 80, preferably at least 90, especially at least 95% amino acid identity, or immunogenic fragments thereof (based on alignment with the Clustal Omega program; identity is calculated by the ratio of identical amino acids divided by the total number of amino acids (of the shorter sequence, if sequences are not of the same length), times 100 (for %)). For example, amino acid residues on position (based on the KR5 sequence H8WQW9) 29, 31, 36, 91, 93, 114, 115, 213, 219, 232, 235, 279, 291, 294, 295, 299, 300, 302 to 307, 319, 324, 329, 343, 338, 343 to 346, 372, 378, 380, 391, 393, 400, 403, 405, 406, 411, 413, 421, 427, 433, 435, 439, 453, 459, 476, or 478 can be changed (as evidenced by the isolates of UniProt sequences H8WG65, H8WG69, H8WG72, H8WG77, H8WG70, H8WG73, H8WG66, H8WG76, H8WG60, H8WG61, H8WG62, H8WG75, H8WG67, H8WG78, H8WG63, H8WG68, H8WG64, H8WG74, H8WG71, H8WQZ7, H8WQZ2, H8WQW9, Q0GH78, O55281, and F2VJI5); or deletion of sequences, such as at the N-terminus (e.g. up to position 21), 123 to 139, 250 to 272, 364, or at the C-terminus, e.g. positions 464 to 479 (as also evidenced by the above UniProt sequences; alignments made by the UniProt alignment software (Clustal Omega program)). Further naturally occurring amino acid variations, deletions and insertions are exemplified in Fig. 5 and derivable from the sequences in Table 3.

Preferably, the vaccine according to the present invention further comprises an adjuvant, preferably selected from the group consisting of Freund's complete adjuvant, Freund's incomplete adjuvant, aluminum hydroxide, Bordetella pertussis, saponin, muramyl dipeptide, ethylene vinyl acetate copolymer, oil, a vegetable oil or a mineral oil, in particular peanut oil or silicone oil, and combinations thereof.

Adjuvants are substances that enhance the immune response to immunogens. Adjuvants, can include aluminum hydroxide and aluminum phosphate, saponins e.g., Quil A, water-in-oil emulsion, oil-in-water emulsion, water-in-oil-in-water emulsion. The emulsion can be based in particular on light liquid paraffin oil (European Pharmacopea type); isoprenoid oil such as squalane or squalene; oil resulting from the oligomerization of alkenes, in particular of isobutene or decene; esters of acids or of alcohols containing a linear alkyl group, more particularly plant oils, ethyl oleate, propylene glycol di(caprylate/caprate), glyceryl tri(caprylate/caprate) or propylene glycol dioleate; esters of branched fatty acids or alcohols, in particular isostearic acid esters. The oil is used in combination with emulsifiers to form the emulsion. The emulsifiers are preferably nonionic surfactants, in particular esters of sorbitan, of mannide (e.g. anhydromannitol oleate), of glycerol, of polyglycerol, of propylene glycol and of oleic, isostearic, ricinoleic or hydroxystearic acid, which are optionally ethoxylated, and polyoxypropylene-polyoxyethylene copolymer blocks, in particular the Pluronic(R) products, especially L121. For example the adjuvant-containing vaccine is prepared in the following way: 50 to 90 v/v of aqueous phase comprising the immunogen are emulsified in 1 to 10% w/v of anhydromannitol oleate, 1 to 10% w/v of oleic acid ethoxylated with 11 EO (ethylene oxide) and 5 to 40% v/v of light liquid paraffin oil (European Pharmacopea type) with the aid of an emulsifying turbomixer. An alternative method for preparing the emulsion consists in emulsifying, by passages through a high-pressure homogenizer, a mixture of 1 to 10% w/v squalane, 1 to 10% w/v Pluronic(R) L121, 0.05 to 1% w/v of an ester of oleic acid and of anhydrosorbitol ethoxylated with 20 EO, 50 to 95% v/v of the aqueous phase comprising the immunogen. It is also possible to formulate with synthetic polymers (e.g., homo- and copolymers of lactic and glycolic acid, which have been used to produce microspheres that encapsulate immunogens, e.g., biodegradable microspheres). A further instance of an adjuvant is a compound chosen from the polymers of acrylic or methacrylic acid and the copolymers of maleic anhydride and alkenyl derivative. Advantageous adjuvant compounds are the polymers of acrylic or methacrylic acid which are cross-linked, especially with polyalkenyl ethers of sugars or polyalcohols. These compounds are

known by the term carbomer, e.g. acrylic polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups, preferably not more than 8, the hydrogen atoms of at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing from 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under the name Carbopol(R) (BF Goodrich, Ohio, USA) are particularly appropriate. They are cross-linked with an allyl sucrose or with allyl pentaerythritol. Among then, there may be mentioned Carbopol(R) 974P, 934P and 971P. Among the copolymers of maleic anhydride and alkenyl derivative, the copolymers EMA(R) (Monsanto) which are copolymers of maleic anhydride and ethylene, linear or cross-linked, for example cross-linked with divinyl ether, are preferred. The dissolution of these polymers in water leads to an acid solution that will be neutralized, preferably to physiological pH, in order to give the adjuvant solution into which the immunogenic, immunological or vaccine composition itself will be incorporated. The carboxyl groups of the polymer are then partly in COO^- form.

Preferably, a solution of adjuvant according to the invention, is prepared in distilled water, preferably in the presence of sodium chloride, the solution obtained being at acidic pH. This stock solution is diluted by adding it to the desired quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with NaCl, preferably physiological saline (NaCl 9 g/l) all at once in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4), preferably with NaOH. This solution at physiological pH will be used as it is for mixing with the vaccine, which may be especially stored in freeze-dried, liquid or frozen form. From this disclosure and the knowledge in the art, the skilled artisan can select a suitable adjuvant, if desired, and the amount thereof to employ in an immunological, immunogenic or vaccine composition according to the invention, without undue experimentation.

Accordingly, the vaccine according to the present invention preferably comprises a pharmaceutically acceptable diluent and/or carrier, preferably selected from the group consisting of

water-for-injection, physiological saline, tissue culture medium, propylene glycol, polyethylene glycol, vegetable oils, especially olive oil, and injectable organic esters such as ethyl oleate.

The Fiber 2 protein of FAdV-C can be produced by any suitable expression system. Preferably, production is effected in a eukaryotic expression system. Specifically preferred expression systems are a baculovirus expression system, an *E. coli* expression system, or a *Pichia pastoris* expression system. However, virtually any suitable expression system or vector can be used in the production of the vaccine provided by this invention. By way of illustration, said suitable expression or vector systems can be selected, according to the conditions and needs of each specific case, from plasmids, bacmids, yeast artificial chromosomes (YACs), bacteria artificial chromosomes (BACs), bacteriophage P1-based artificial chromosomes (PACs), cosmids, or viruses, which can further have a heterologous replication origin, for example, bacterial or of yeast, so that it may be amplified in bacteria or yeasts, as well as a marker usable for selecting the transfected cells different from the gene or genes of interest. These expression systems or vectors can be obtained by conventional methods known by persons skilled in the art.

The vaccines according to the present invention can be produced in industrial amounts; the individual vaccine dose given to the animals can be in the ranges also applied for other vaccines. Preferably, the fiber-2 protein of FAdV-C or an immunogenic fragment thereof is contained in the vaccine in an amount of 0.1 µg/ml to 10 mg/ml, preferably of 1 µg/ml to 1 mg/ml, especially of 10 to 100 µg/ml.

In a preferred form, the vaccine according to the present invention consists of

- fiber-2 protein of FAdV-C or an immunogenic fragment thereof, preferably in an amount of 0.1 µg to 10 mg, preferably of 1 µg to 1 mg, especially of 10 to 100 µg; and
- a pharmaceutically acceptable carrier and/or diluent and/or adjuvant.

The vaccine according to the present invention preferably comprises a pharmaceutically acceptable vehicle, especially if provided as commercially sold vaccine product. The suitable vehicles may be both aqueous and non-aqueous. Examples of non-

aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate.

With the present invention, an efficient method for preventing HHS in birds is provided. Accordingly, the present invention relates to another aspect to a method for preventing HHS in birds, preferably in poultry, especially in parent flocks, comprising administering to poultry, especially to the parent flock, a vaccine containing fiber-2 protein of FAdV-C or an immunogenic fragment thereof. The vaccine is administered to the birds in an effective amount at a suitable point in time. Typical ways of administration are intravenous, subcutaneous, intramuscular, oral, *in ovo* or intracloacal administration. Preferably, vaccination in chicken is effected in week 17 to 19, especially in week 18 of life.

A specific advantage of the present invention is that vaccination of the parent flock provides sufficient protection for the progeny, especially to broilers, to safeguard sufficient protection e.g. up to at least 30, preferably at least 40, especially at least 60 days, to the progeny of vaccinated animals. It is therefore advantageous that the present invention provides sufficient protection of the broilers by vaccination of the parent animals. Accordingly, protection of broilers is effected by immunization of the parental animal in poultry, especially in chicken.

According to another aspect, the present invention also provides a kit comprising a fiber-2 protein of FAdV-C or an immunogenic fragment thereof immobilised on a solid surface. Preferably, the kit is a serological kit for detection of anti-fiber-2 antibodies (within the meaning of the present invention) in samples, especially blood samples of animals. This kit is specifically suitable for the present invention to detect the successful vaccination by determining specific anti-fiber-2 antibodies in the vaccinated animals. In the course of establishing the present invention it was found that specific detection of anti-fiber-2 antibodies in the vaccinated animals is difficult or even impossible by commercially available FAdV-test kits, especially FAdV-ELISAs, or by usual serum neutralization tests (SNTs). It was observed that only detection with fiber-2-specific tests (e.g. Fib-2 ELISAs and the like) was possible.

This was due to type specificity and the non-neutralizing capacity of the antibodies elicited by the vaccination according to the present invention. Nevertheless ((and even more remarkable)), sufficient protection is provided with the vaccine according to the present invention.

This shows that there was also a need to provide a specific test and test system to establish whether protection is given (by the determining the presence of specific antibodies against fibre-2 protein of FAdV-C). This could be provided by the kit according to the present invention that - in contrast to the commercially available FAdV-ELISAs and SNTs (that might produce false negative results) - successfully and reliably confirm successful vaccination. The kit of the present invention also provides a means for detecting infection with FAdV viruses, because fiber-2 protein of FAdV-C is very specific for the individual viruses. Moreover, the kit according to the present invention is also suitable for determining whether antibody protection is still present in progeny of vaccinated animals or whether an active immunization of the progeny is indicated.

Preferably, the kit according to the present invention further comprises means for detection of the binding of an antibody to the immobilised fiber-2 protein of FAdV-C or the immobilised immunogenic fragment thereof, preferably an antibody being specific for bird antibodies, especially an anti-chicken IgG antibody or an anti-turkey IgG antibody. Of course, any suitable detection (capturing) means for the binding event between fiber-2 protein protein and an antibody from the vaccinated bird is suitable for the present kit; however, (secondary) antibodies or suitable (secondary) antibody fragments that are able to bind to the anti-fiber-2 antibodies antibodies to be detected in a (blood) sample of the vaccinated bird are specifically preferred.

It is specifically preferable to provide a solid phase test kit with a labelled agent that detects the binding event to the immobilised fiber-2 protein. Accordingly, detection agent for the binding event, especially the anti-chicken IgG antibody or the anti-turkey IgG antibody, is a labelled agent, especially a labelled antibody. For example, the agent (antibody/antibody fragment) is labelled with a colourigenic, fluorescent, luminescent or radioactive label.

Suitable labels are therefore e.g. fluorescent compounds, isotopic compounds, chemiluminescent compounds, quantum dot labels, biotin, enzymes, electron-dense reagents, and haptens or proteins for which antisera or monoclonal antibodies are available. The various means of detection include but are not limited to spectroscopic, photochemical, radiochemical, biochemical, immunochemical, or chemical means.

The label may be of a chemical, peptide or nucleic acid molecule nature although it is not so limited. Other detectable labels include radioactive isotopes such as ^{32}P, luminescent markers such as fluorochromes, optical or electron density markers, etc., or epitope tags such as the FLAG epitope or the HA epitope, biotin, avidin, and enzyme tags such as horseradish peroxidase, β-galactosidase, etc. The label may be bound to a peptide during or following its synthesis. There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels that can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for the agents (antibodies/antibody fragments) described herein, or will be able to ascertain such, using routine experimentation. Furthermore, the coupling or conjugation of these labels to the peptides of the invention can be performed using standard techniques common to those of ordinary skill in the art.

The invention is further illustrated by the following examples and figures, yet without being restricted thereto.

Fig. 1. Survival rates of birds of group I (Fib-1 vaccinated), group II (Fib-2 vaccinated) and group III (Hex L1 vaccinated), together with groups IV (positive control) and V (negative control), after infection with virulent FAdV strain AG234.

Fig. 2. Pathologic lesions as manifested by focal necroses in the liver and pericardial sac filled with straw-coloured fluid in a bird from the positive control group IV that died 3 days post challenge (d.p.c.).

Fig. 3. Results of antibody investigation as detected by (a) commercial FAdV Group-1 ELISA (results indicated as Sample to Positive (S/P) ratio of the mean OD value of maximum ten tested sera from each group, starting measurements on day 21 (before

challenge), (b) Serum neutralization test (SNT) (results indicated as log_2 transformed mean titers of maximum ten tested sera from each group, starting measurements on day 21; titres ≤ 3 were considered negative), and (c) custom-made ELISA using recombinant Fib-2 protein (results indicated as mean OD values measured from sera of all Fib-2 vaccinated birds as well as positive and negative control birds, starting on day 7).

Fig. 4. Immunoblots of purified recombinant KR5 proteins incubated with chicken sera collected on 21st day of life (preabsorbed with insect cell powder, diluted 1:2000). A, lanes 1, 2 and 3 purified Fib-1 incubated with sera from Fib-1 vaccinated birds. B, lanes 1, 2 and 3 purified Fib-2 incubated with sera from Fib-2 vaccinated birds. C, lanes 1, 2 and 3 purified Hex L1 incubated with sera from Hex L1 vaccinated birds. D, lane 1 purified Hex L1, lane 2 purified Fib-1, lane 3 purified Fib-2, incubated with serum from a bird of the positive control group (vaccinated with purified, non-infected insect cell material). E, lane 1 purified Hex L1, lane 2 purified Fib-1, lane 3 purified Fib-2, incubated with serum from a bird of the negative control group (non-vaccinated). Proteins are detected by serum antibodies as bands migrated to estimated molecular weight sizes of 51 kDa (Fib-1), 56 kDa (Fib-2) and 36 kDa (Hex L1).

Fig. 5. Alignment of fiber proteins according to the present invention (Fib-2 of FAdV-C).

Examples

In the examples of the present invention, fiber-1, fiber-2 and the loop-1 region of hexon of an FAdV-C reference strain (KR5), were recombinantly expressed in the baculovirus system. In a vaccination trial, the efficacy of these capsid components to induce protective immunity in chickens was assessed by challenging birds with virulent FAdV. Hence, this is the first study of its kind to employ both fiber proteins individually in an *in vivo* experiment with the aim to further elucidate the functional significance of the investigated FAdV capsid proteins in the infection process and to address their potential use as candidate subunit vaccines for the control of HHS.

1. Materials and methods

1.1. Virus propagation and DNA extraction

FAdV-C (= FAdV-4) reference strain KR5 and the challenge virus AG234 were propagated on primary chicken-embryo liver (CEL) cells according to a protocol described by Schat and Sellers, A Laboratory Manual for the Isolation and Identification of Avian Pathogens,(2008), 195-203). Viral titer was determined according to the method of Reed and Muench (Am. J. Hyg. 27 (1938), 493-497) by endpoint titration. DNA extraction from cell culture supernatant was carried out with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany).

1.2. Cloning and initial protein expression

Primers were designed on the basis of the complete genomic KR5 sequence (GenBank accession number HE608152) and contained 5´-terminal restriction sites for cloning into the pFastBac transfer vector (Invitrogen, Vienna, Austria) (Table 1). The entire encoding regions for fiber-1 and fiber-2 (nucleotides 30438 to 31739 and 31723 to 33162, respectively) and the hexon loop-1 region (nucleotides 20481 to 21366) were amplified from the FAdV-C reference strain KR5 using a proofreading DNA polymerase (Invitrogen, Vienna, Austria). Following intermediate cloning into the pCR4Blunt-TOPO vector (Invitrogen) and digestion with *BamHI/StuI* (Fib-1), *StuI/XbaI* (Fib-2) and *NcoI/XhoI* (Hex L1) fragments were ligatedinto the cleaved pFastBac vector at the respective restriction sites. After determining the correct insertion of each product into pFastBac by sequencing, the construct was transformed into competent *E. coli* DH10Bac cells (Invitrogen, Vienna, Austria). Recombinant baculovirus DNA was isolated from transformed colonies using the S.N.A.P. Miniprep Kit (Invitrogen, Vienna, Austria). The genes of interest were expressed in *Spodoptera frugiperda* Sf9 cells (Invitrogen, Vienna, Austria) as His-tag fusion proteins according to the manufacturer´s protocol.

1.3. Identification of recombinant proteins

To verify expression of the recombinant proteins and to optimize the expression conditions, SDS-PAGE was performed on the soluble and membrane-bound fractions of the cell lysate, collected from infected Sf9 monolayer cultures at different time

intervals (24, 48, 72, 96 h) post-infection. Recombinant proteins were identified by immunoblot using anti polyhistidine antibody (Sigma-Aldrich, Vienna, Austria). Non-infected Sf9 cells were processed in the same way to serve as negative control.

1.4. Expression and purification of recombinant proteins

For expression, Sf9 suspension cultures (50 ml) were infected with amplified recombinant baculovirus at an MOI of 3. Cultures collected after 72 h inoculation in a shaking incubator were concentrated by centrifugation for 5 min at 3500 rpm. The resulting cell pellet was disrupted by resuspension in lysis buffer (containing 20 mM sodium phosphate, 0,5 M NaCl, 20-40 mM imidazole, 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM PMSF and proteinase inhibitors) and sonication, with subsequent incubation on ice for 1 h. Clarified supernatants obtained by centrifugation of the crude cell lysates at 14000 rpm for 20 min at 4°C were used for purification on affinity chromatography columns (His GraviTrap, GE Healthcare, Freiburg, Germany). Hexon L1 protein presented as insoluble material in the pellet fraction was solubilised with phosphate buffer containing 8 M urea. The 0.45 µm-filtered sample was loaded on columns equilibrated with phosphate buffer containing 8 M urea, and the protein was eluted after step-washing the columns with decreasing concentrations of urea. Samples from each purification fraction were subsequently analyzed for presence of the proteins of interest by SDS-PAGE and immunoblotting.

Prior to *in vivo* administration, the recombinant proteins were transferred into sterile PBS (Gibco/Invitrogen, Vienna, Austria) by buffer exchange in Slide-A-Lyzer 7K Dialysis Cassettes (Thermo Scientific, Vienna, Austria). Protein Hex L1 was additionally processed through Amicon Ultra-15 size exclusion spin columns (Millipore, Vienna, Austria) to remove eluted insect cell proteins and to concentrate the target protein.
Protein concentrations were determined by Bradford assay (Thermo Scientific, Vienna, Austria).

1.5. Animal experiment

A total of 112 SPF (specific pathogen-free) chickens (VALO, Lohmann Tierzucht GmbH, Cuxhaven, Germany) were divided into five groups that were housed separately in isolator units (Mon-

tair Andersen bv, HM 1500, Sevenum, Netherlands). At first day of life, a 500 µl injection was administered intramuscularly to each animal, containing 50 µg of the recombinant protein, with group I (*n* = 26) receiving fiber-1 (Fib-1), group II (*n* = 28) receiving fiber-2 (Fib-2) and group III (*n* = 26) receiving hexon loop-1 (Hex L1), mixed 1:1 with GERBU Adjuvant LQ #3000 (GERBU Biotechnik GmbH, Heidelberg, Germany; a sterile aqueous suspension of lipid particles with excipients and emulsifiers).

Equally, birds of group IV (*n* = 23) were injected with purified and dialysed material from non-infected insect cells to serve as a positive control. Birds of group V (*n* = 9) were treated as a negative control and received an injection of 500 µl sterile PBS.

At day 21 of life, animals of groups I to IV were intramuscularly challenged with 200 µl of 10^7 50% tissue culture infective dose ($TCID_{50}$)/ml of the virulent FAdV-C virus AG234. Birds of the negative control group were administered the same amount of sterile PBS intramuscularly.

Upon challenge, the birds were monitored daily for clinical signs. Necropsy was performed on all animals that died or had to be euthanized in the course of the study. Samples taken at regular intervals included blood (collected on days 7, 11, 14, 21, 28, 35 and 42) for detection of antibodies and cloacal swabs (collected on days 21, 28 and 35) or tissue from the large intestine (taken on day 42) for detection of virus excretion at regular intervals.

All remaining birds were killed at the termination of the experiment on day 42 of life.

The trial and all of the included procedures on experimental birds were discussed and approved by the institutional ethics committee and licensed by the Austrian government (license number BMWF-68.205/0196-II/3b/2012).

1.6. Antibody response

Commercial FAdV enzyme-linked immunosorbent assay (ELISA)

Commercially available FAdV Group 1 Antibody Test Kit was obtained from BioChek (Reeuwijk, Holland) to test antibody levels in sera of each group before (day 21) and after challenge (days 28, 35 and 42).

Serum neutralization test (SNT)

Test sera were inactivated at 56°C for 30 min. CEL cells were prepared from 14-day-old chicken embryos and plated in 96-well plates (Sarstedt, Wiener Neudorf, Austria) with a density of 1 x 10^6 cells/ml. The assay was performed according to a constant virus diluted serum method using 100 $TCID_{50}$/100 µl KR5. The plates were inoculated at 37°C in 5% CO_2 and investigated for CPE after 5 days.

Fib-2 ELISA

After predetermining optimal virus- and serum-dilutions by checker-board titrations, 96-well ELISA plates (Nunc Medisorb, Roskilde, Denmark) were coated with 100 µl recombinant affinity-purified Fib-2 protein per well, diluted in coating buffer (0.015 M Na_2CO_3, 0.035 M $NaHCO_3$, pH 8.4) to a final concentration of 0.05 µg/ml. After 24 h, plates were washed and 100 µl of the test sera, diluted 1:100 in blocking buffer (Starting Block T20 PBS, Thermo Scientific), were added to each well for 1 h. Following a washing step, 100 µl Goat-Anti-Chicken-IgG-HRP (Southern Biotechnology, Birmingham, USA) diluted 1:5000 in PBS-0.05 % v/v Tween 20 (Calbiochem, Darmstadt, Germany) were added to each well and incubated for 1 h. After another washing step, 100 µl TMB (tetramethylbenzidine) substrate (Calbiochem, Darmstadt, Germany) were added to each well and the plates were incubated for 15 min in the dark. The reaction was stopped with 100 µl 0.5 M sulphuric acid/well and the optical density (OD) of each well was measured with an ELISA reader (Sunrise-Basic, Tecan, Grödig, Austria) at a wavelength of 450 nm.

On each plate, a positive and a negative control were included. All sera were tested in duplicate and the OD is indicated as the mean value of the duplicates. A tentative cut-off value was established as the arithmetic mean of all OD values plus three times the standard deviation determined from serum samples from the negative control group.

1.7. Western blot analysis

Purified recombinant Fib-1, Fib-2 and Hex L1 proteins were boiled for 5 min in sample buffer containing 4% SDS and 10% mercaptoethanol, separated by 12% SDS-PAGE and electrotransferred onto BioTrace PVDF Transfer Membrane (Pall, Vienna, Austria).

After 3 h of blocking with 3% (w/v) skim milk, the membrane was cut into strips which were incubated separately in the test sera (preabsorbed with 1% Sf9 cell powder, diluted 1:2000) for 1 h. After several washes with PBS-0.05% Tween 20, the membrane strips were incubated for 1 h with rabbit anti-chicken IgG-HRP conjugate (Sigma-Aldrich, Vienna, Austria) diluted 1:2500, followed by several washes and incubation with Clarity Western ECL substrate (Bio-Rad Laboratories GmbH, Vienna, Austria). Visualization was performed on x-ray film (Super RX, Fuji, Japan) after exposure for 12 sec.

1.8. Real-time (rt) PCR from cloacal swabs and intestine

Excretion of challenge virus was investigated from cloacal swabs taken on days 7 and 14 post challenge (p.c.) and tissue samples taken from the large intestine at termination of the study (day 21 p.c.) from five birds of each group, using an rt PCR assay based on the 52K gene, following DNA extraction with a commercial system (Qiagen, Hilden, Germany) (Günes et al., J. Virol. Meth. 183 (2012), 147-153).

2. Results

2.1. Expression of proteins

Characteristic morphologic changes were exhibited by Sf9 cell cultures within 48 - 96 h after inoculation with recombinant baculovirus. Recombinant proteins were detected by SDS-PAGE and Western blot as bands migrated to estimated molecular weight sizes of 51 kDa (Fib-1), 56 kDa (Fib-2) and 35 kDa (Hex L1) with peak expression around 72 h after inoculation. Furthermore, expression analysis showed that large fractions of Fib-1 and Fib-2 were expressed as soluble proteins in the supernatant, whereas Hex L1 protein was preferentially found in the pellet.

2.2. Protection of recombinant proteins against virulent FAdV

Following challenge, clear-cut differences in severity of clinical signs and mortality rates were noticed between individual groups (Fig. 1). The difference in mortality between the groups was found to be highly significant by chi-square analysis (χ^2=46; p<0,01) and significant differences were also indicated in the pairwise comparison of mortality between the Fib-2 vaccinated group and all other challenged groups (Bonferroni cor-

rected chi-square test).

Onset of mortality was recorded on day 3 p.c., in coincidence with the overall peak of mortality. Dead birds were observed until day 5 p.c., and after that no more animals died. After infection with the virulent virus, birds of group IV (positive control) showed severe clinical depression as manifested by huddling together with ruffled feathers, and 18 out of 23 animals (78 %) died. In contrast, birds in group II (Fib-2 vaccinated) displayed no apparent clinical symptoms and only one dead animal out of 28 on day 3 p.c. after the challenge was recorded. Birds of group I (Fib-1 vaccinated) partially showed clinical symptoms and 10 out of 26 animals died resulting in an overall mortality of 38%. In group III (Hex L1 vaccinated), severity of clinical affection was comparable to the positive control group, and 19 out of 26 animals (73%) died. Necropsy revealed severe lesions in heart and liver of all animals found dead or those which had to be euthanized during the experiment. Characteristic findings included straw-colored fluid in the pericardial sac and focal necrosis in the livers (Fig. 2).

Surviving animals of clinically affected groups experienced full recovery by 26 days of life. No more lesions were recorded in any of the surviving animals at termination of the experiment on day 42 of life. In group V (negative control), no clinical signs were observed at any time of the experiment and no pathological lesions were noticed at termination of the study.

2.3. Detection of antibodies

Commercial FAdV ELISA and SNT

No antibodies were detected with the commercial ELISA and the SNT prior to challenge at day 21 in any of the groups (Fig. 3a and 3b). Following challenge, birds of groups I - IV developed an increase in antibody levels detectable by both commercial ELISA and SNT. In the vaccinated groups, antibodies measured by commercial ELISA increased until 7 d.p.c. and after that gradually declined, whereas antibody levels in the positive control group display a continuous increase until termination of the experiment. Development of neutralizing antibodies p.c. continuously increased in groups I - IV with highest titres obtained in non-vaccinated birds

No antibodies were detected in negative control animals at any of the tested time points during the experiment.

Fib-2 ELISA

To investigate a specific antibody response against Fib-2 prior to and after challenge a custom-made ELISA using recombinant purified protein was developed. Starting measurements in Fib-2 vaccinated birds on day 7, the ELISA first detected an increase in mean OD value above the determined cut-off on day 11 and peaked at 7 d.p.c. (Fig. 3b). Until termination of the experiment, mean Fib-2 antibody levels declined only slightly. Of note, the antibody response of the bird that did not survive challenge was only 0.21 and differed significantly from all other birds.

Birds of the positive control group were tested negative for Fib-2 antibodies on day 21. Survivors, however, developed a strong anti-Fib-2 response p.c., reaching the level of vaccinated birds by the end of the experiment.

Sera obtained from the negative control group before and after challenge were tested negative in the Fib-2 ELISA (Fig. 3c), similarly to sera from Fib-1 and Hex L1 vaccinated groups.

2.4. Western Blot

Immunoblots with sera from three birds of each group I - III obtained on day 21 after administration of recombinant proteins confirmed the presence of antibodies against Fib-1, Fib-2 and Hex L1, respectively (Fig. 4). No antibodies were detected in sera from one bird of the positive and negative control group when tested against each of the purified recombinant proteins in the immunoblot.

2.5. Virus excretion

No virus excretion was detected in any of the samples taken from negative control animals (Table 2). Following challenge, viral excretion was noticed in all tested birds of groups I-IV, at 7 d.p.c with no evident difference in viral load between protein-vaccinated and positive control birds. Shedding was verified until termination of the experiment and the majority of birds were recorded positive for virus excretion in the faeces. The large intestine of half of the infected birds was positive

at termination of the study, with positive birds in each of the groups I-IV.

3. Discussion

While human adenoviruses are well studied on a molecular basis for their use as vaccine and gene therapy vectors, current understanding of FAdV-host interaction and molecules involved is still limited. Interaction between capsomer and host cell has been established as the critical factor in formation of host immunity, rendering adenovirus capsid proteins interesting candidates for subunit vaccine development. In regard to the prevention of HHS, *E. coli* expressed penton base was recently proposed as a potential subunit antigen. In the present study, the efficacy of fiber subunit immunization derived from FAdV-C was investigated by utilizing for the first time the novel finding of two distinct fiber-encoding genes in FAdV-C. In addition, hexon loop-1, a surface-exposed structure with immunogenic potential, was investigated.

The choice of the baculovirus expression system was based on evidence for possible post-translational modifications of such adenovirus proteins.

Upon challenge with the virulent strain AG234, different degrees of protection were observed in chickens vaccinated with recombinant FAdV capsid proteins. Although Hex L1-specific antibodies were detected prior to challenge, this protein could not be proven as an effective subunit antigen in our study. In comparison, an immune response directed against Fib-2 is highly efficacious as it prevents any clinical signs of disease. This could indicate a key role of the Fib-2 protein in the initial steps of infection, possibly by mediating attachment to host cell receptors. Cellular attachment via binding of fiber to the ubiquitously present coxsackievirus-adenovirus receptor (CAR) is a well-known mechanism in human adenoviruses. However, knowledge about CAR-fiber interaction is primarily derived from *in vitro* studies and the role of CAR as primary receptor for adenovirus entry into the host cell is increasingly questioned. In this context, binding to primary receptors specific for avian - but not mammalian, -cells was suggested to be mediated by the short fiber of CELO. Previous phylogenetic data show a higher degree of relatedness of FAdV-C Fib-2 with the short fiber gene of CELO

and the single fiber gene found in other FAdV species, as compared to Fib-1. Based on these informations, together with the actual finding of highly efficacious immune response directed against FAdV-C Fib-2, Fib-2 could serve as the primary ligand for induction of a host-cell dependent infection pathway.

Antibodies raised against Fib-2 following vaccination were detected with the exception of one bird, indicating a correlation with protection, in contrast to the commercial ELISA which failed to detect antibodies before challenge. Obviously, the type specificity of the fiber antigen results in a binding incompatibility of the induced antibodies within the commercial ELISA test system. The results obtained from SNT indicate that antibodies directed against Fib-2 do not possess neutralizing capacity, which is in agreement with previously reported observations of weak or lacking serum neutralization activity elicited by fiber if administered as an isolated virus component.

The challenge virus was detected in cloacal swabs of groups I-IV alike, demonstrating that vaccination does not prevent virus excretion and shedding, even in birds protected from clinical disease. This finding is supported by a previous study that reports excretion of challenge virus even in birds clinically fully protected by a live attenuated FAdV vaccine (Schonewille et al., Avian Dis. 54 (2010), 905-910).

In summary, identification of virulent strains of FAdV-C as causative agents of HHS together with the limitations faced by currently employed inactivated vaccines argue for the development of next-generation immunization strategies. The findings presented in the present invention shows high efficacy of recombinant Fib-2 protein for the development of an effective and safe subunit vaccine.

Tables

Table 1: Primers used.

Primer name	Sequence (5´-3´)	Position	Purpose
KR5-b Fib-1 f	5´-GGATCCATGTCGGCCCTAATCG-3´	30438 - 30453 [a]	Amplification of the fiber-1 gene of strain KR5 and cloning into the pFastBac vector
KR5-b Fib-1 r	5´-AGGCCTTTAGGGGCTCGGAGC-3´	31725 - 31739 [a]	Amplification of the fiber-1 gene of strain KR5 and cloning into the

			pFastBac vector
KR5-b Fib-2 f	5´-AGGCCTATGCTCCGAGCCCCTA-3´	31723 - 31738 [a]	Amplification of the fiber-2 gene of strain KR5 and cloning into the pFastBac vector
KR5-b Fib-2 r	5´-TCTAGATTACGGGACGGAGGCTG-3´	33146 - 33162 [a]	Amplification of the fiber-2 gene of strain KR5 and cloning into the pFastBac vector
FAV f	5´-AATTCCATGGACAAGTTCAGGCAGAC-GGTCGT-3´	20481 - 20502 [a]	Amplification of the hexon loop-1 gene region of strain KR5 and cloning into the pFastBac vector
FAV r	5´-TAACTCGAGCTAGTGATGCCGG-GACATCAT-3´	21347 - 21366 [a]	Amplification of the hexon loop-1 gene region of strain KR5 and cloning into the pFastBac vector
52K-fw	5´-ATGGCKCAGATGGCYAAGG-3´	13075 - 13093 [b]	Amplification of the 52k gene in rt-PCR
52K-rv	5´-AGCGCCTGGGTCAAACCGA-3´	13250 - 13232 [b]	Amplification of the 52k gene in rt-PCR

a Position is indicated for the complete genomic KR5 sequence (HE608152).

b Position is indicated for the complete genomic CELO sequence (U46933).

Table 2: Detection of viral excretion in cloacal swab samples (taken on days 21, 28 and 35) and tissue from the large intestine (taken on day 42) by real-time PCR from five birds of each group. Results are shown as number of positive samples/number of samples tested.

d [a]	group I (Fib-1)	group II (Fib-2)	group III (Hex L1)	group IV (positve control)	group V (negative control)
21	-	-	-	-	-
28	5/5	5/5	5/5	5/5	0/5
35	3/5	4/5	4/5	4/5	0/5
42	1/5	3/5	5/5	1/5	0/5

[a] Day of life

Table 3: List of examples of fiber proteins useable according to the present invention:

Fowl adenovirus 4 isolate Punjab 1 fiber gene, complete cds
1,386 bp linear DNA
DQ864436.1 GI:112735223
Fowl adenovirus 4 isolate Punjab 2 fiber gene, complete cds
1,386 bp linear DNA
DQ864434.1 GI:112735219
Fowl adenovirus 4 short fiber gene, complete cds
1,482 bp linear DNA
AY340863.1 GI:33359662
Fowl adenovirus 10 short fiber protein gene, complete cds
1,496 bp linear DNA
AF007579.1 GI:2674070
Fowl adenovirus 4 isolate Kr-Yeoju short fiber gene, complete cds
1,425 bp linear DNA
HQ709232.1 GI:318040046
Fowl adenovirus 4 isolate Kr-Gunwi short fiber gene, complete cds
1,425 bp linear DNA
HQ709231.1 GI:318040044
Fowl adenovirus 4 isolate Kr-Andong short fiber gene, complete cds
1,425 bp linear DNA
HQ709230.1 GI:318040042
Fowl adenovirus 4 isolate Kr-Changnyeong short fiber gene, complete cds
1,425 bp linear DNA
HQ709229.1 GI:318040040
Fowl adenovirus partial sf gene for short fiber protein, isolate OTE
1,197 bp linear DNA
FN557186.1 GI:315455213
Fowl adenovirus partial sf gene for short fiber protein, isolate 08-5769
1,197 bp linear DNA
FN557185.1 GI:315455211
Fowl adenovirus partial sf gene for short fiber protein, isolate 08-3622
1,197 bp linear DNA
FN557184.1 GI:315455209
Fowl adenovirus 4 isolate Bareilly fiber protein gene, complete cds
1,437 bp linear DNA
FJ949088.1 GI:238683632
Fowl adenovirus C pVIII gene, U-exon gene, fiber-1 gene, fiber-2 gene and
ORF22, isolate C2B
4,345 bp linear DNA
HE608154.1 GI:381214073
Fowl adenovirus C pVIII gene, U-exon gene, fiber-1 gene, fiber-2 gene and
ORF22, isolate AG234
4,321 bp linear DNA
HE608153.1 GI:381214067
Fowl adenovirus C complete genome, isolate KR5
45,810 bp linear DNA
HE608152.1 GI:381214017
Fowl adenovirus C partial fiber-2 gene, isolate K388-95
1,395 bp linear DNA
FR872927.1 GI:381214013
Fowl adenovirus C partial fiber-2 gene, isolate 09/8846
1,440 bp linear DNA
FR872926.1 GI:381214011
Fowl adenovirus C partial fiber-2 gene, isolate 09/584
1,440 bp linear DNA
FR872925.1 GI:381214009
Fowl adenovirus C partial fiber-2 gene, isolate 09/2602
1,329 bp linear DNA
FR872924.1 GI:381213952
Fowl adenovirus C partial fiber-2 gene, isolate K99-97
1,340 bp linear DNA
FR872923.1 GI:381213950
Fowl adenovirus C partial fiber-2 gene, isolate Peru54
1,421 bp linear DNA
FR872922.1 GI:381213948
Fowl adenovirus C partial fiber-2 gene, isolate Peru53
1,416 bp linear DNA
FR872921.1 GI:381213946
Fowl adenovirus C partial fiber-1 gene, isolate K1013
1,184 bp linear DNA
FR872898.1 GI:381213900
Fowl adenovirus C partial fiber-1 gene, isolate 922/1
1,311 bp linear DNA
FR872897.1 GI:381213898
Fowl adenovirus C partial fiber-1 gene, isolate C2B
1,302 bp linear DNA
FR872896.1 GI:381213896

Fowl adenovirus C partial fiber-1 gene, isolate Da60
1,302 bp linear DNA
FR872895.1 GI:381213894
Fowl adenovirus C partial fiber-1 gene, isolate KR5
1,302 bp linear DNA
FR872894.1 GI:381213892
Fowl adenovirus C partial fiber-1 gene, isolate INT4 (QT-cell passaged AG234)
1,188 bp linear DNA
FR872893.1 GI:381213890
Fowl adenovirus C partial fiber-1 gene, isolate AG234
1,302 bp linear DNA
FR872892.1 GI:381213888
Fowl adenovirus C partial fiber-1 gene, isolate K31
1,181 bp linear DNA
FR872891.1 GI:381213886
Fowl adenovirus 4 isolate Kr-Yeoju short fiber gene, complete cds
1,425 bp linear DNA
HQ709232.1 GI:318040046
Fowl adenovirus 4 isolate Kr-Gunwi short fiber gene, complete cds
1,425 bp linear DNA
HQ709231.1 GI:318040044
Fowl adenovirus 4 isolate Kr-Andong short fiber gene, complete cds
1,425 bp linear DNA
HQ709230.1 GI:318040042
Fowl adenovirus 4 isolate Kr-Changnyeong short fiber gene, complete cds
1,425 bp linear DNA
HQ709229.1 GI:318040040
Fowl adenovirus partial sf gene for short fiber protein, isolate OTE
1,197 bp linear DNA
FN557186.1 GI:315455213
Fowl adenovirus partial sf gene for short fiber protein, isolate 08-5769
1,197 bp linear DNA
FN557185.1 GI:315455211
Fowl adenovirus partial sf gene for short fiber protein, isolate 08-3622
1,197 bp linear DNA
FN557184.1 GI:315455209
Fowl adenovirus 4 isolate Bareilly fiber protein gene, complete cds
1,437 bp linear DNA
FJ949088.1 GI:238683632
Fowl adenovirus 4 short fiber gene, complete cds
1,482 bp linear DNA
AY340863.1 GI:33359662
Fowl adenovirus 4 isolate Punjab 1 fiber gene, complete cds
1,386 bp linear DNA
DQ864436.1 GI:112735223
Fowl adenovirus 4 isolate Punjab 2 fiber gene, complete cds
1,386 bp linear DNA
DQ864434.1 GI:112735219
Fowl adenovirus 10 short fiber protein gene, complete cds
1,496 bp linear DNA
AF007579.1 GI:2674070

The nature of the sequence, the FAdV species/serotypes, the-length of the sequence, the GenBank accession number and the version is indicated for each of the sequences.

Table 4: List of species in the genus Aviadenovirus:

Falcon adenovirus A	
Falcon adenovirus 1 [AY683541]	(FaAdV-1)
Fowl adenovirus A	
Fowl adenovirus 1 (CELO) [U46933 =_ AC_000014]	(FAdV-1)
Fowl adenovirus B	
Fowl adenovirus 5 (340) [AF508952]	(FAdV-5)
Fowl adenovirus C	
Fowl adenovirus 4 (ON1) [GU188428 = NC_015323]	(FAdV-4)
Fowl adenovirus 10 (CFA20) [AF160185]	(FAdV-10)

Fowl adenovirus D
Fowl adenovirus 2 (P7-A) [AF339915] (FAdV-2)
Fowl adenovirus 3 (75) [AF508949] (FAdV-3)
Fowl adenovirus 9 (A2-A) [AF083975 = AC_000013] (FAdV-9)
Fowl adenovirus 11 (380) [AF339925] (FAdV-11)
Fowl adenovirus E
Fowl adenovirus 6 (CR119) [AF508954] (FAdV-6)
Fowl adenovirus 7 (YR36) [AF508955] (FAdV-7)
Fowl adenovirus 8a (CFA40) [AF155911] (FAdV-8a)
Fowl adenovirus 8b (764) [AF508958] (FAdV-8b)
Goose adenovirus
Goose adenovirus 1 (GoAdV-1)

Species names are in italic script; names of types and isolates ( ) are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

New Claims:

1. Vaccine comprising fiber 2 protein of Fowl Adenovirus C (FAdV-C)for use in preventing hepatitis-hydropericardium syndrome (HHS) in birds, preferably in poultry, especially in broilers;
wherein the vaccine is a subunit vaccine.

2. Vaccine for use according to claim 1 further comprising an adjuvant, preferably selected from the group consisting of Freund's complete adjuvant, Freund's incomplete adjuvant, aluminum hydroxide, Bordetella pertussis, saponin, muramyl dipeptide, ethylene vinyl acetate copolymer, oil, a vegetable oil or a mineral oil, in particular peanut oil or silicone oil, and combinations thereof.

3. Vaccine for use according to claim 1 or 2 wherein the fiber-2 protein of FAdV-C is selected from the protein sequences provided in Fig. 5 .

4. Vaccine for use according to claim 3 wherein the fiber-2 protein of FAdV-C is the protein sequence FIBER-2_KR5 provided in Fig. 5.

5. Vaccine for use according to any one of claims 1 to 4 further comprising a pharmaceutically acceptable diluent and/or carrier, preferably selected from the group consisting of water-for-injection, physiological saline, tissue culture medium, propylene glycol, polyethylene glycol, vegetable oils, especially olive oil, and injectable organic esters, especially ethyl oleate.

6. Vaccine for use according to any one of claims 1 to 5 wherein the fiber-2 protein of FAdV-C has been recombinantly produced, preferably in a baculovirus expression system, in an *E. coli* expression system, or in a *Pichia pastoris* expression system.

7. Vaccine for use according to any one of claims 1 to 6 wherein the fiber-2 protein of FAdV-C is contained in an amount

of 0.1 µg/ml to 10 mg/ml, preferably of 1 µg/ml to 1 mg/ml, especially of 10 to 100 µg/ml.

8. Vaccine for use according to any one of claims 1 to 7 consisting of

- fiber-2 protein of FAdV-C, preferably in an amount of 0.1 µg to 10 mg, preferably of 1 µg to 1 mg, especially of 10 to 100 µg; and
- a pharmaceutically acceptable carrier and/or diluent and/or adjuvant.

9. Method for preventing HHS in birds, preferably in poultry, especially in broilers, comprising administering to poultry, especially parent flock, a vaccine comprising fiber-2 protein of Fowl Adenovirus Serotype C (FAdV-C) thereof;
wherein the vaccine is a subunit vaccine.

10. Kit comprising a fiber-2 protein of FAdV-C or an immunogenic fragment thereof immobilised on a solid surface.

11. Kit according to claim 9 further comprising means for detection of the binding of an antibody to the immobilised fiber-2 protein of FAdV-C or the immobilised immunogenic fragment thereof, preferably an antibody being specific for bird antibodies, especially an anti-chicken IgG antibody or an anti-turkey IgG antibody.

12. Kit according to claim 10 wherein the anti-chicken IgG antibody or the anti-turkey IgG antibody is a labelled antibody, especially an antibody labelled with a colourigenic, fluorescent, luminescent or radioactive antibody.

13. Kit according to any one of claims 10 to 12, comprising a fiber-2 protein of FadV-C.

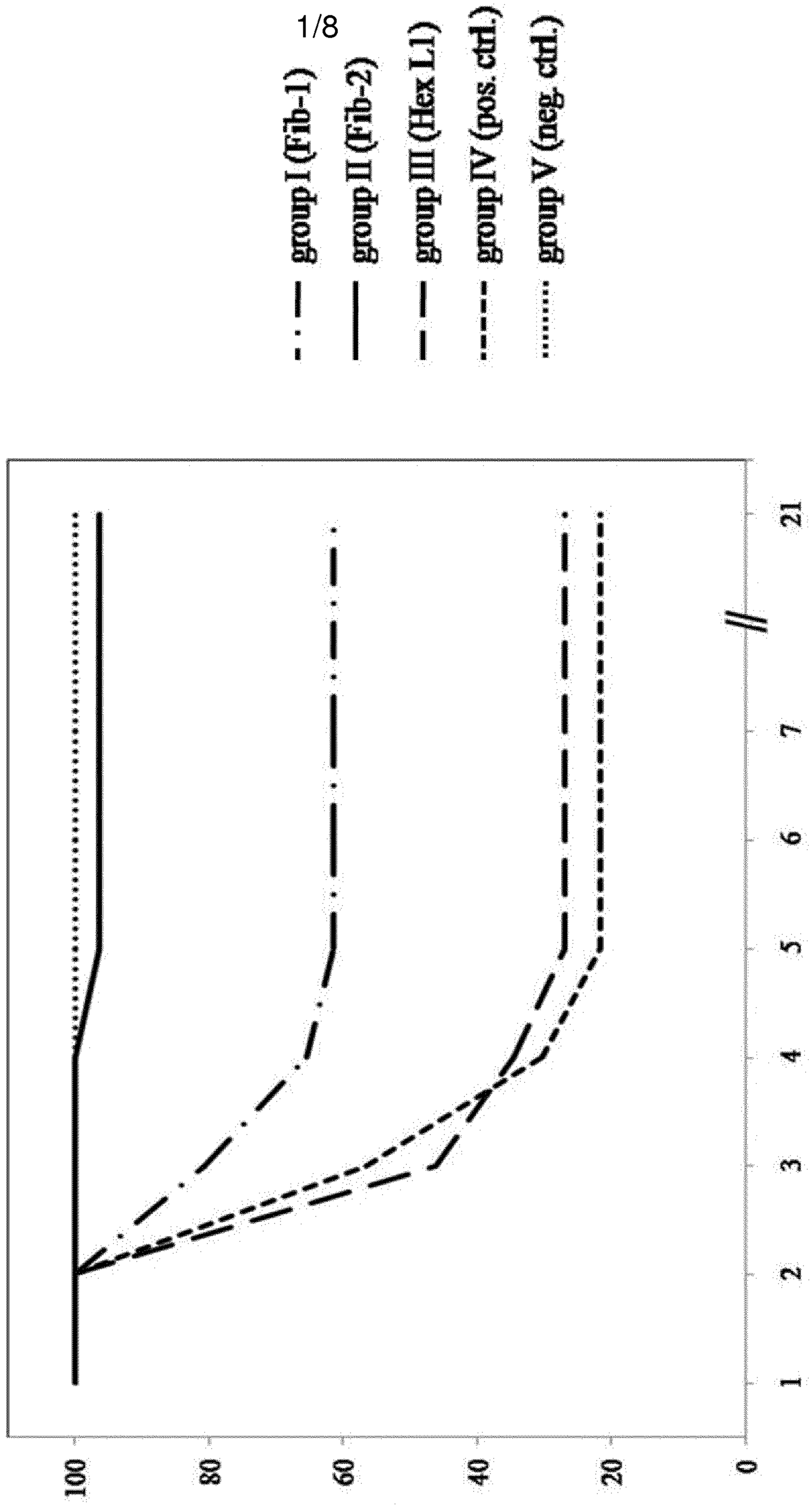

group I (Fib-1)
group II (Fib-2)
group III (Hex L1)
group IV (pos. ctrl.)
group V (neg. ctrl.)
Surviving birds (%)
100
80
60
40
20
0
1
2
3
4
5
6
7
21
Day post challenge

Fig. 1

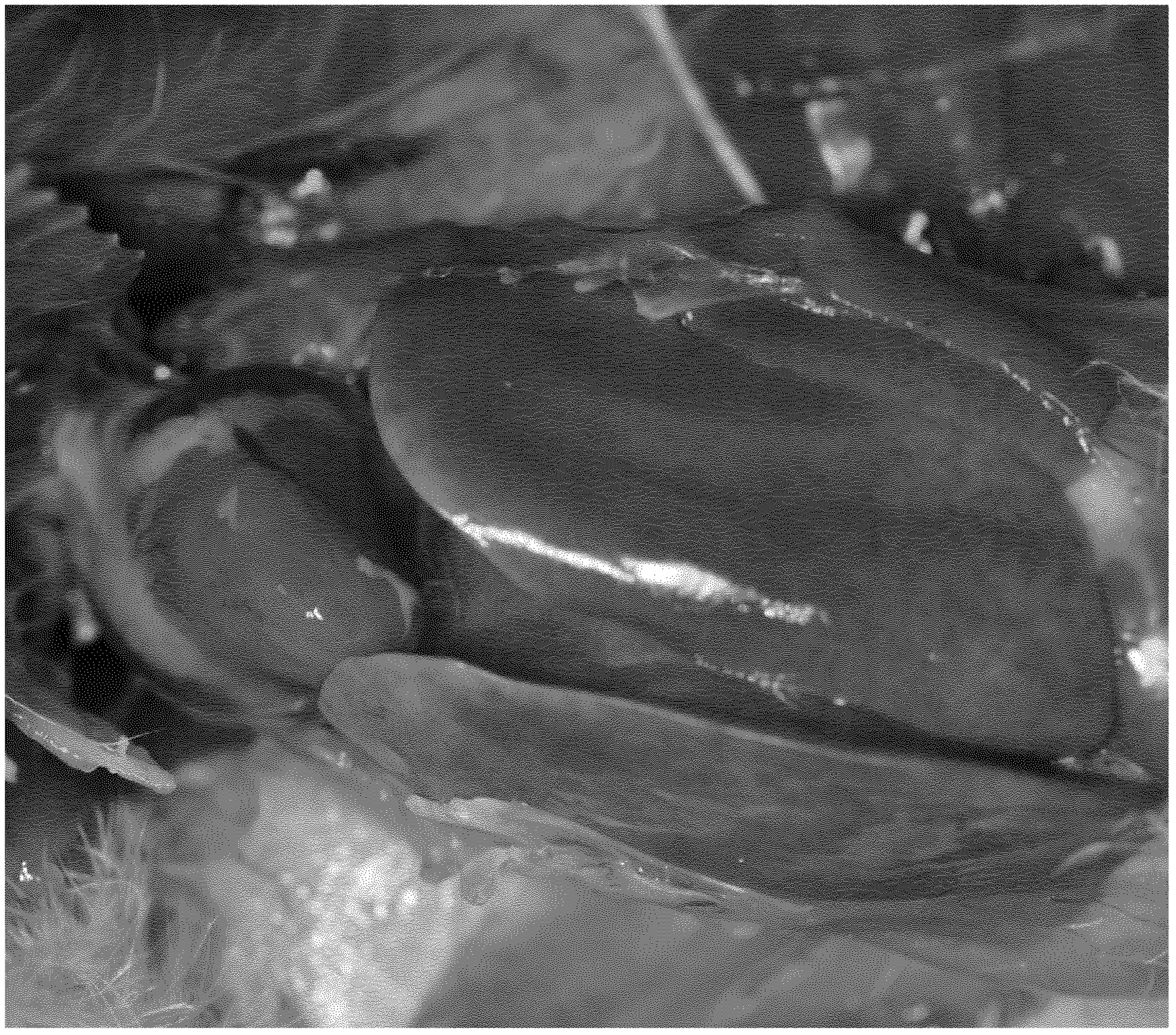


Fig. 2

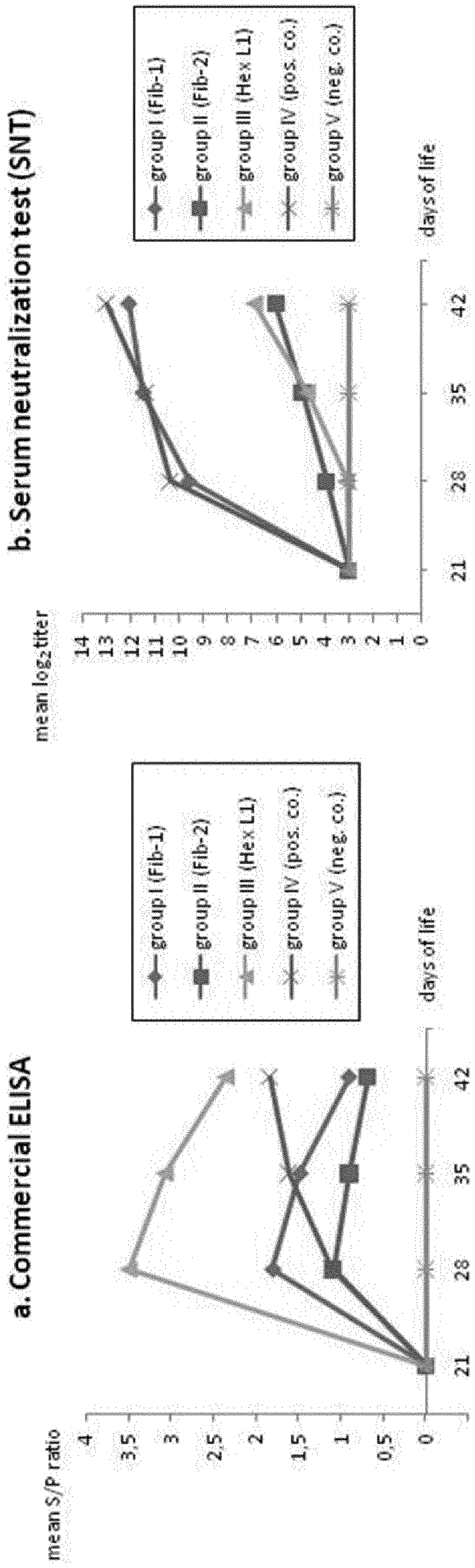

a. Commercial ELISA
mean S/P ratio
4
3,5
3
2,5
2
1,5
1
0,5
0
21
28
35
42
days of life
group I (Fib-1)
group II (Fib-2)
group III (Hex L1)
group IV (pos. co.)
group V (neg. co.)
b. Serum neutralization test (SNT)
mean log2 titer
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
21
28
35
42
days of life
group I (Fib-1)
group II (Fib-2)
group III (Hex L1)
group IV (pos. co.)
group V (neg. co.)
c. Fib-2 ELISA
mean OD
3,5
3
2,5
2
1,5
1
0,5
0
7
11
14
21
28
35
42
days of life
group II (Fib-2)
group IV (pos. co.)
group V (neg. co.)

Fig. 3

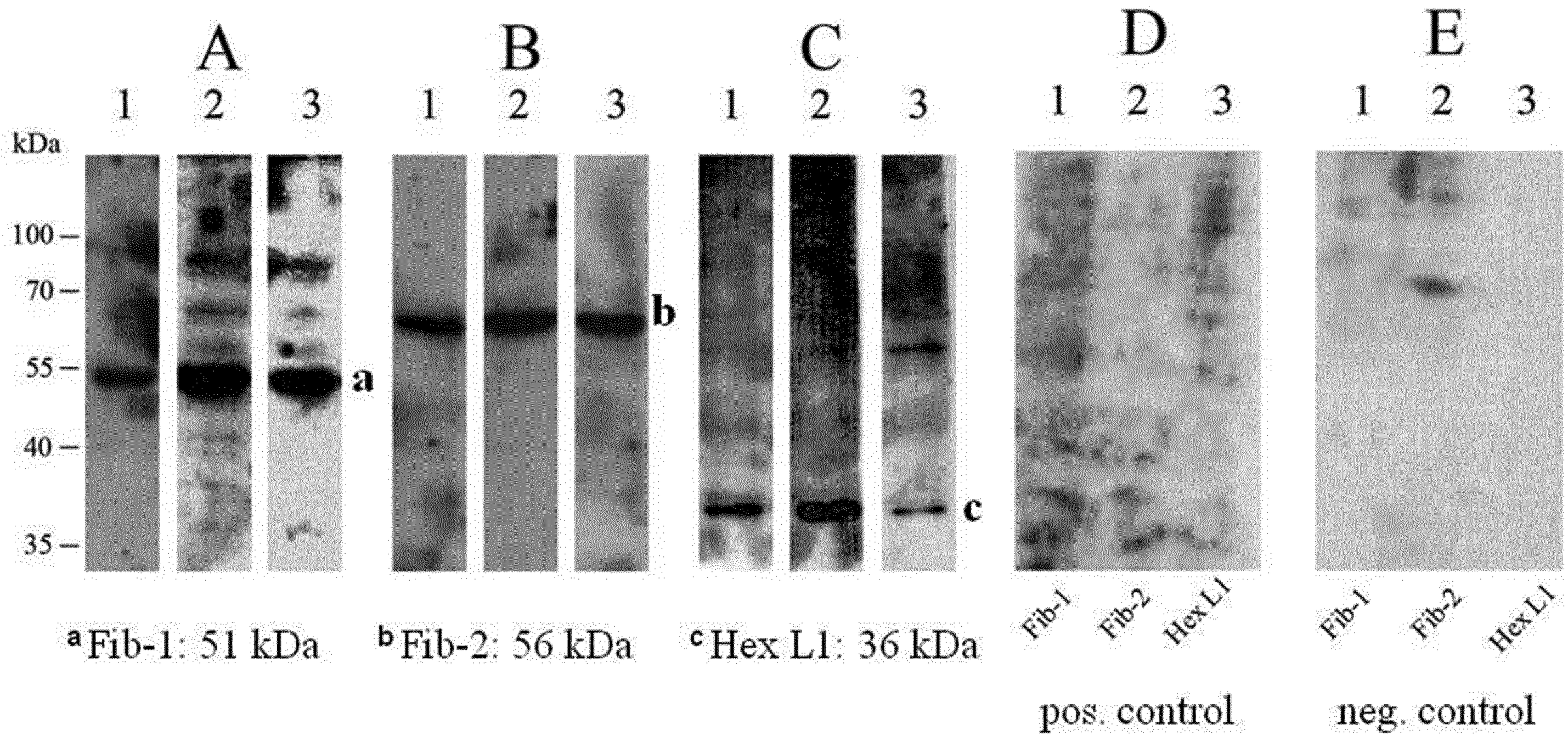

A
B
C
D
E
1 2 3
kDa
100
70
55
40
35
a
b
c
Fib-1
Fib-2
Hex L1
pos. control
neg. control
a Fib-1: 51 kDa
b Fib-2: 56 kDa
c Hex L1: 36 kDa

Fig. 4

```
                                 10        20        30        40        50        60        70        80        90       100
                                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FIBER-2_PERU53                   --------RHSENGKPETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_PERU54                   --------RHSENGKPETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_C344                     ----------SENGKPETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_K1013QT                  -------RRHSENGKPETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_K1013                    ----------SENGKPETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_K31                      MLRAPKRRHSENGKPETEAGPSP----------APIKRAKRVVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_K88-95                   --------RHSENGKPETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_IV37                     ----------------PETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_K99-97                   ------------------EAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_C2B                      MLRAPKRRHSENGQPESEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_09-584                   MLRAPKRRHSENGKPETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_09-8846                  MLRAPKRRHSENGKPETEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_09-2602                  ----------------------SP----------APIKRPKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_DA60                     --RAPKRRHSENGQPESEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_KR5                      MLRAPKRRHSENGQPESEAGPSP----------APIKRAKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_ON1_GU188428             MLRAPKRRHS------ETEAGPSP----------APIKRPKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_922-1                    MLRAPKRRHSENGQPESEAGPSP----------APIKRAKRMVRAA----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_INT4                     ----------RRHSETEAGPYP----------APIKRPKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_AG234                    MLRAPKRRHSENRHSETEAGPYP----------APIKRPKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_K388-95                  ----------------ETEAGPYP----------APIKRPKRMVRAS----------QLDLVYPFDYVADPVGG----------LNPPFLGGSGPLVDQ
FIBER-2_CELO_AC000014            ----------MADQKRKLADPD----------AEAPTGKMARAGP----------GELDLVYPFWYQVAAPTE----------ITPPFLDPNGPLYST
FIBER-2?_TADV-1_GU936707         ----------MERKRTSASGFG----------AAAPKRPRATS----------AQVDLIYPFWYQADAPSV----------NPPFLDPSGPLFDK
FIBER_A2-A_AC000013              -MAKSTPFAFSMGQHSSRKRPAD------SENTQNASKVAKTQTSATRAGVDG--NDDLNLVYPFWLQNSTSGGGGGG--SGGNPSLNPPFIDPNGPLYVQ
FIBER_HG_GU734104                -MATSTPHAFSFGQIGSRKRPAG------GDGERDASKVPKMQTPAPSATANG--NDELDLVYPFWLQNGSTGGGGGGGGSGGNPSLNPPFLDPNGPLAVQ
FIBER_340                        -MAQ--PSGLLLGKRKRPEAPLDDSAGVSEQPLTASKMPKTHTYSSPIGFYGPTTGQLDLVYPFWFQNSSGGGGG------TVIPPVNPPLLDPAGPLYVQ

                                110       120       130       140       150       160       170       180       190       200
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FIBER-2_PERU53                   GGQLTLNVTDPIIIKNRSVDLAHDR-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_PERU54                   GGQLTLNVTDPIIIKNRSVDLAHDR-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_C344                     GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_K1013QT                  GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_K1013                    GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_K31                      GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_K88-95                   GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_IV37                     GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_K99-97                   GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_C2B                      GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_09-584                   GGQLTLNVTDPIIIKNRSVDLARDP-SLDVNAQGQLAVAVDPEGALAITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_09-8846                  GGQLTLNVTDPIIIKNRSVDLARDP-SLDVNAQGQLAVAVDPEGALAITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_09-2602                  GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALAITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_DA60                     GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_KR5                      GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_ON1_GU188428             GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_922-1                    GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALDITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_INT4                     GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALAITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_AG234                    GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALAITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_K388-95                  GGQLTLNVTDPIIIKNRSVDLAHDP-SLDVNAQGQLAVAVDPEGALAITPDGLDVKVDGVTVMVND----------------------------------------
FIBER-2_CELO_AC000014            DGLLNVRLTAPLVIIRQ----------------SNGNAIGVKTDGSITVNADG----------------------------------------------
FIBER-2?_TADV-1_GU936707         DGKLSIRLQAPVAEVNKSVGLLYDG-TMGVNNAGQLGMRINTTEGLEATGTGLAIKTDLESIGFDP----------------------------------------
FIBER_A2-A_AC000013              NSLLYVKTTAPIEVENKSLALAYDS-SLAVDAQNQLQVKVDTEGPIRISPDGLDIAVDPSTLEVDD----------------------------------------
FIBER_HG_GU734104                NNLLKVNTAAPITVANKALTLAYEPDSLELTNQQQLAVKIDPEGPLKATTEGIQLSVDPTTLEVDDV---------------------------------------
FIBER_340                        NNMLRMRTGAPIVVSNGALGLSYDT-SLGLSDQNQLQVNLEPNGPLKATDDGIELTVDPLTLEVTDWELGVKIDPAGPLDASTDGLTLRTDDTLSLGQDP
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Fig. 5a

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                                 210       220       230       240       250       260       270       280       290       300
                                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FIBER-2_PERU53                   ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_PERU54                   ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_C344                     ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_K1013QT                  ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKARGGIQA-GSSG
FIBER-2_K1013                    ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_K31                      ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_K88-95                   ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_IV37                     ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_K99-97                   ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_C2B                      ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-GSSG
FIBER-2_09-584                   ----DWELAVKVDPAGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-GSSG
FIBER-2_09-8846                  ----DWELAVKVDPAGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-GSSG
FIBER-2_09-2602                  ----DWELAVKVDPAGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-GSSG
FIBER-2_DA60                     ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-GSSG
FIBER-2_KR5                      ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-GSSG
FIBER-2_ON1_GU188428             ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-GSSG
FIBER-2_922-1                    ----DWELAVKVDPSGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-GSSG
FIBER-2_INT4                     ----DWELAVKVDPAGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-RSSG
FIBER-2_AG234                    ----DWELAVKVDPAGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_K388-95                  ----DWELAVKVDPAGGLDSTAGGLGVSVDDTLLVDQG-------ELGVHLNQQGPITADSSGIDLEINPNMFTVN-TSTGSGVLELNLKAQGGIQA-DSSG
FIBER-2_CELO_AC000014            ------------------------------------------------ALQIGISTAGPLTTTANGIDLNIDPKTLVVD-GSSGKNVLGVLLKGQGALQS-SAQG
FIBER-2?_TADV-1_GU936707         ----TGNLQVTLDPEGPIVASADGLQLQLDGATLEVAD-----WELGVKLDPNEPIDAGSAGLKLNID-ETLLVDATGSQRSPLPNPKRPRTHRNG---TH
FIBER_A2-A_AC000013              ----EWELAVKLDPNGPLTASSAGININVDDTLLIEDDDANQAKELGVHLNPNGPITADRDGLDLEIDSQTMVVKDSGTSGGVLGVLLKPSGGLQS-SIQG
FIBER_HG_GU734104                ----DWELTVKLDPDGPLDSSATGITVRVDETLLIEDVGSGQGKELGVNLNPTGPITADDQGLDLEIDNQTLKVN-SVTGGGVLAVQLKSQGGLTA-QTDG
FIBER_340                        TTHEYELGLKLDPSGPLEASADGLNLRLDDTLLVEQDTTTQEYELGVHLNPNGPVTADENGIDLEINTDTLTVTAGAAGGGELSVLLNPQGAIHATASTG

                                 310       320       330       340       350       360       370       380       390       400
                                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FIBER-2_PERU53                   VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDTNTLAVTAGAL
FIBER-2_PERU54                   VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDTNTLAVTAGAL
FIBER-2_C344                     VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDTNTLAVTAGAL
FIBER-2_K1013QT                  VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDTNTLAVTAGAL
FIBER-2_K1013                    VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDTNTLAVTAGAL
FIBER-2_K31                      VGVSVDESLQIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDTNTLAVTAGAL
FIBER-2_K88-95                   VGVSVDESLQIVNNTLEVKPDPSG------------------P-----------------------------------------------------------L
FIBER-2_IV37                     VGVSVDESLQIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDTNTLAVTAGAL
FIBER-2_K99-97                   VGVSVDESLQIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDTNTLAVTAGAL
FIBER-2_C2B                      VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDNNTLAVTAGAL
FIBER-2_09-584                   VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDNNTLAVTAGAL
FIBER-2_09-8846                  VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDNNTLAVTAGAL
FIBER-2_09-2602                  VGVSVDESLEIVNNTLEVKPDPTG------------------PLTVSAN----------------------------------------------GLGLKYDNNTLAVTAGAL
FIBER-2_DA60                     VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDSNTLAVTAGAL
FIBER-2_KR5                      VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDSNTLAVTAGAL
FIBER-2_ON1_GU188428             VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDSNTLAVTAGAL
FIBER-2_922-1                    VGVSVDESLEIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDSNTLAVTAGAL
FIBER-2_INT4                     VGVSVDESLQIVDNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDNNTLAVTAGAL
FIBER-2_AG234                    VGVSVDESLQIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDNNTLAVTAGAL
FIBER-2_K388-95                  VGVSVDESLQIVNNTLEVKPDPSG------------------PLTVSAN----------------------------------------------GLGLKYDNNTLAVTAGAL
FIBER-2_CELO_AC000014            IGVAVDESLQIVDNTLEVKVDAAG------------------PLAVTAA----------------------------------------------GVGLQYDNTQFKVTNGTL
FIBER-2?_TADV-1_GU936707         SGRLQRRPVEIRHHTLYRHRRRAE------------------SRRTPAN-----------------------------------------LGYTS----------
FIBER_A2-A_AC000013              IGVAVADTLTITSNTVEVKTDPNGSISYSANGIAVKPDPSGPLTSSGTGLSVVTAAEGSIQSSNAGLAVKTDPSGPITSGSNGLNLSYNASDFTVSQGVL
FIBER_HG_GU734104                IQVNTQNSITVTNGALDVKVAANG------------------PLESTDT----------------------------------------------GLTLNYDPGDFTVNAGTL
FIBER_340                        IGVAVGPGLQITSNTVSVKPDPAG------------------PLTASPT----------------------------------------------GVTLNYDNSDFTITDGKL
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Fig. 5b

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                                410       420       430       440       450       460       470       480       490       500
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FIBER-2_PERU53                  TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQWNIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_PERU54                  TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQWNIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_C344                    TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQWNIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_K1013QT                 TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQWNIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_K1013                   TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQWNIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_K31                     TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQWNIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_K88-95                  TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQWNIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_IV37                    TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQ-NIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_K99-97                  TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSANAFSCAYYLQQWNIQGLLVTSLYLKLDSATMGNRPGDLNSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_C2B                     TVVGGGSISTPIATFVSGSASLNAYNARMVNSSAHAFSCAYYLQQWNIQGLLFTSLYLKLDSATMGNRPGDNNSVNAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_09-584                  TVVGGGSVSTPIATFVSGSPSLDAYNATTVNSSAHPFSCAYYLQQWNVQGLLFTSLYLKLDSTTMGTRPGDWNSVNAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_09-8846                 TVVGGGSVSTPIATFVSGSPSLDAYNATTVNSSAHPFSCAYYLQQWNVQGLLFTSLYLKLDSTTMGTRPGDWNSVNAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_09-2602                 TVVGGGSVSTPIATFVSGSPSLDAYNARMVNSSAHPFSCAYYLQQWNVQGLLFTSLYLKLDSTTMGNRPGDWNSVNAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_DA60                    TVVGGGSVSTPIATFVSGSPSLNTYNATIVNSSSHPFSCAYYLQQWNVQGLLFTSLYVKLDSTTMGTRPGDNSSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_KR5                     TVVGGGSVSTPIATFVSGSPSLNTYNATIVNSSSHPFSCAYYLQQWNVQGLLFTSLYVKLDSTTMGTRPGDNSSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_ON1_GU188428            TVVGGGSVSTPIATFVSGSPSLNTYNATIVNSSSHPFSCAYYLQQWNVQGLLFTSLYVKLDSTTMGTRPGDNSSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_922-1                   TVVGGGSVSTPIATFVSGSPSLNTYNATIVNSSAHPFSCAYYLQQWNVQGLLFTSLYVKLDSTTMGTRPGDNSSANAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_INT4                    TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSAHAFSCAYYLQQWNIQGLLFTSLYLKLDSTTMGTRPGDNSSVNAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_AG234                   TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSAHAFSCAYYLQQWNIQGLLFTSLYLKLDSTTMGTRPGDNSSVNAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_K388-95                 TVVGGGSVSTPIATFVSGSPSLNTYNATTVNSSAHAFSCAYYLQQWNIQGLLFTSLYLKLDSTTMGTRPGDNSSVNAKWFTFWVSAYLQQCNPSGIQAGT
FIBER-2_CELO_AC000014           QLYQAPTSSV--AAFTSGTIGLSSPTGNFVSSSNNPFNGSYFLQQINTMGMLTTSLYVKVDTTTMGTRPTGAVNENARYFTVWVSSFLTQCNPSNIGQGT
FIBER-2?_TADV-1_GU936707        ----------ISYVSGSTSLNSNTAEIVNSSNNSFKCSYYVKQVNCMGMLFTSLYIKLDSATMGTRPTGNTNVNAKWFNFIVSSYLTDFNPSQMDTGT
FIBER_A2-A_AC000013             NIIRN-PSTLPDAYLESGTNYLNNFTAQAENSSVFKFNCAYFLQSWYSNGLVTSSLYLKIDRAQFSNMPTGQSAENARYFTFWVPTY-ESLNLSRVSTPT
FIBER_HG_GU734104               SIIRD-PALVANAYLTSGASTLQQFTAKSENSSQFSFPCAYYLQQWLSDGLVLSSLYLKLDRAQFTNMPTGANYQNARYFTFWVGAG-TSFNLSTLTEPT
FIBER_340                       TLYKT-PAVTSDAYLTSGNSAMTTYTAFFGNSSNYRFKCSYFLQQWLRDRLVITSLYIKLDRSQLENLSSDASAQNARYFTFWVSSN-ALMNLSGIDEPV

                                510       520       530       540       550       560       570       580       590       600
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FIBER-2_PERU53                  VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVTAS---GDRYTLLCYSLQCTNSSIFN-
FIBER-2_PERU54                  VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVTAS---GDRYTLLCYSLQCTNSSIFN-
FIBER-2_C344                    VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVTAS---GDRYTLLCYSLQCTNSSIFN-
FIBER-2_K1013QT                 VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVTAS---GDRYTLLCYSLQCTNSSIFN-
FIBER-2_K1013                   VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVTAS---GDRYTLLCYSLQCTNSSIFN-
FIBER-2_K31                     VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVSAS---GERYTLLCYSLQCTNASIFN-
FIBER-2_K88-95                  VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVSAS---GERYTLLCYSLQCTNASIFN-
FIBER-2_IV37                    VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVSAS---GERYTLLCYSLQCTNASIFN-
FIBER-2_K99-97                  VSPSTAT-LTDFEPMANRSVTSPWTYSAN-----GYYEPSIGEFQVFSPVVTGAWN------PGNIGIRVLPVPVSAS---GERYTLLCYSLQCTNASIFN-
FIBER-2_C2B                     VSPSTAT-LADFEPMANRSVSSPWTYSAN-----GYYQPPSGEFQLFTPVVTGAWT------PGNIGIRVLPVPVSAS---GDRYTLLCYSLQCTNASIFN-
FIBER-2_09-584                  LSPSTAT-LADFEPMANRSVSSPWTYSAN-----AYYEPSSGEFQTFTPVVTGAWN------PGNIGIRVLPVSVSAS---GDRYTLLCYSLQCTNASIFN-
FIBER-2_09-8846                 LSPSTAT-LADFEPMANRSVSSPWTYSAN-----AYYEPSSGEFQTFTPVVTGAWN------PGNIGIRVLPVSVSAS---GDRYTLLCYSLQCTNASIFN-
FIBER-2_09-2602                 LSPSTAT-LADFEPMANRSVSSPWTYSAN-----AYYEPSSGEFQTFTPVVTGAWN------PGNIGIRVLPVSVSAS---GERYTLLCYSLQCTNASIFN-
FIBER-2_DA60                    VSPSTAA-LADFEPMANRSVSSPWTYSAN-----AYYQPSSGEFQVFTPVVTGAWN------PGNIGIRVLPVPVTAS---GDRYTLLCYSLQCTNSSIFN-
FIBER-2_KR5                     VSPSTAA-LADFEPMANRSVSSPWTYSAN-----AYYQPSSGEFQVFTPVVTGAWN------PGNIGIRVLPVPVTAS---GDRYTLLCYSLQCTNSSIFN-
FIBER-2_ON1_GU188428            VSPSTAA-LADFEPMANRSVSSPWTYSAN-----AYYQPPSGEFQVFTPVVTGAWN------PGNIGIRVLPVPVTAS---GDRYTLLCYSLQCTNSSIFN-
FIBER-2_922-1                   VSPSTAA-LADFEPMANRSVSSPWTYSAN-----AYYQPSSGEFQVFTPVVTGAWN------PGNIGVRVLPVPVSAS---GDRYTLLCYSLQCTNASIFN-
FIBER-2_INT4                    VSPSTAT-LTDFEPMANRSVSSSWTYSAN-----AYYQPSSGEFQVFTPVVTGAWN------PGNIGVRVLPVPVSAS---GDRYTLLCYSLQCTNASIFN-
FIBER-2_AG234                   VSPSTAT-LTDFEPMANRSVSSSWTYSAN-----AYYQPSSGEFQVFTPVVTGAWN------PGNIGVRVLPVPVSAS---GDRYTLLCYSLQCTNASIFN-
FIBER-2_K388-95                 VSPSTAT-LTDFEPMANRSVSSSWTYSAN-----AYYQPSSGEFQVFTPVVTGAWN------PGNIGVRVLPVPVSAS---GDRYTLLCYSLQCTNASIFN-
FIBER-2_CELO_AC000014           LEPSNIS-MTSFEPARNPISPPVFNMNQN---IPYYASRFGVLESYRPIFTGSLN------TGSIDVRMQVTPVLATN--NTTYNLIAFTFQCASAGLFN-
FIBER-2?_TADV-1_GU936707        LNPAVSNGMTYMEPAPNRTLPSNWDADTN-----TYYEPSSGVSQSLTAVLTGSWA------PGNITVVAMPVIAQKN---QERYTVLCFSFRCTNGGLFN-
FIBER_A2-A_AC000013             ITPNTVQ-WGAFSPAQNCSGNPAFQYNLTQPPSIYFEPKSGSVQTFQPVLTGAWNTDTYNPGTVQVCILPQTVVG--GQSTFVNMTCYNFRCQNPGIFKV
FIBER_HG_GU734104               ITPNTTQ-WNAFAPALDYSGAPPFIYDASSVVTIYFEPTSGRLESYLPVLTDNWS-QTYNPGTVTLCVKTVRVQLR-SQGTFSTLVCYNFRCQNTGIFNS
FIBER_340                       VTPSTVT-WSKFLPDVNYTNPPTFNFNTS-----LYFEPPSGEILTFNPVTTGDWS-TTYSPGTVSVCVLPVNVRASSGTGTLQTLLCFNFRCANTGLFKT
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Fig. 5c

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                          610       620
                     ....|....|....|....|....|.
FIBER-2_PERU53       PANSGTMIVGPVLYSCPAASV-----
FIBER-2_PERU54       PANSGTMIVGPVLYSCPAASVP----
FIBER-2_C344         PANSGTMIVGPVLYSCPAASVP----
FIBER-2_K1013QT      PANSGTMIVGPVLYSCPAASV-----
FIBER-2_K1013        PANSGTMIVGPVLYSCPAAS------
FIBER-2_K31          PNNSGTMIVGPVLYSCPAGSLP----
FIBER-2_K88-95       PNNSGTMIVGPVLYSCPAASLP----
FIBER-2_IV37         PNNSGT--------------------
FIBER-2_K99-97       PNNSGT--------------------
FIBER-2_C2B          PNNSGTMIVGPVLYSCPAGSLP----
FIBER-2_09-584       PANSGTMIVGPVLYSCPAGSLP----
FIBER-2_09-8846      PANSGTMIVGPVLYSCPAGSLP----
FIBER-2_09-2602      PANSGT--------------------
FIBER-2_DA60         PANSGTMIVGPVLYSCPAASV-----
FIBER-2_KR5          PANSGTMIVGPVLYSCPAASVP----
FIBER-2_ON1_GU188428 PANSGTMIVGPVLYSCPAASVP----
FIBER-2_922-1        PANSGTMTVGPVLYTCPAASVP----
FIBER-2_INT4         PANSGTMIVGPVLYSCPAASV-----
FIBER-2_AG234        PANSGTMIVGPVLYSCPAASVP----
FIBER-2_K388-95      PANSGTMIVGPVLYSCPAASVP----
FIBER-2_CELO_AC000014 PTVNGTVAIGPVVHTCPAARAPVTV-
FIBER-2?_TADV-1_GU936707 PSVQGTATIGPVNYICEASQSPNVVP
FIBER_A2-A_AC000013  AASNGTFTIGPIFYSCPTNELTRPT-
FIBER_HG_GU734104    NATAGTMTLGPIFFSCPALSTANAP-
FIBER_340            AATTGTFYVGPIVYSCPGNPLI----
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Fig. 5d