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- (71) **Applicant (for all designated States except US):** **NOVO-PLANT GMBH** [DE/DE]; Am Schwabeplan 1B, 06466 Gatersleben (DE).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** **ZIMMERMANN, Jana** [DE/DE]; Turmstrasse 5, 06766 Bobbau (DE). **GIERSBERG, Martin** [DE/DE]; Lange Gasse 17, 06484 Quedlinburg (DE). **RIEHL, Marcus** [DE/DE]; Obersteigerweg 18, 06502 Thale (DE).
- (74) **Agent:** **NEUEFEIND, Regina**; Maiwald Patentanwalts GmbH, Elisenhof, Elisenstrasse 3, 80335 München (DE).
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(54) **Title:** ANTIGEN BINDING POLYPEPTIDES AGAINST F4 (K88) FIMBRIAE

(57) **Abstract:** The present invention relates to antigen-binding polypeptides, particularly antibody fragments, and especially single-chain antibodies, which specifically bind to bacterial F4 (K88)-fimbriae. The polypeptides may neutralize the attachment of F4 (K88) fimbriae to enterocytes and/or have a protective and/or curative effect on infection of mammals, especially pigs, with bacteria expressing F4 (K88)-fimbriae. Furthermore, the invention relates to polynucleotides encoding said F4 (K88)-binding polypeptides, vectors comprising these polynucleotides and prokaryotic or eukaryotic host cells comprising these polynucleotides or vectors. Also provided are transgenic plants or parts thereof and microorganisms expressing the polynucleotides of the present invention. The plants or plant parts expressing the polypeptides may be used as fodder for mammals, and especially pigs. Furthermore, the present invention provides compositions comprising said F4 (K88)-binding polypeptides, and methods of producing said polypeptides.

Antigen binding polypeptides against F4 (K88) fimbriae

The present invention relates to antigen-binding polypeptides, particularly antibody
5 fragments, and especially single-chain antibodies, which specifically bind to bacterial
F4 (K88)-fimbriae. The polypeptides may neutralize the attachment of F4 (K88)
fimbriae to enterocytes and/or have a protective and/or curative effect on infection of
mammals, especially pigs, with bacteria expressing F4 (K88)-fimbriae. Furthermore,
the invention relates to polynucleotides encoding said F4 (K88)-binding
10 polypeptides, vectors comprising these polynucleotides and prokaryotic or eukaryotic
host cells comprising these polynucleotides or vectors. Also provided are transgenic
plants or parts thereof and microorganisms expressing the polynucleotides of the
present invention. The plants or plant parts expressing the polypeptides may be used
as fodder for mammals, and especially pigs. Furthermore, the present invention
15 provides compositions comprising said F4 (K88)-binding polypeptides, and methods
of producing said polypeptides.

BACKGROUND

20 The bacterial surface structures "F4 (K88) fimbriae" are the most important and
frequent fimbrial antigens of pathogenic *E.coli* in piglets. The F4-fimbriae are
composed of two different antigenic components, resulting in three antigen variants
which are called K88ab, K88ac and K88ad (F4ab, F4ac and F4ad).

25 First step of the disease process of intestine associated *E.coli*-infections after oral
uptake of the pathogen is the attachment of *E.coli* cells to the mucosal cells of the
small intestine. The fimbriae attach to specific receptors of the enterocytes. The
E.coli cells are thus prevented to be shed from the small intestine. After attachment
bacteria multiply rapidly. The production of toxins and other pathogenic factors
30 results in damage of the host mucosa. The pathology of the illnesses caused by *E.coli*

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varies and primarily depends on the different virulence factors, surface structures and toxins (Bosworth B.T. Infect. Immun. 1996 Jan;64(1):55-60).

Fimbriae can be discriminated by immune electrophoresis according to their
5 serological activity. Different combinations of the fimbriae F4 (K88), F5 (K99), F6
(987P), F41 (Adhaesin) and F18 (F107, 2134P, 8813) and also of fimbriae with one
or more toxins are possible (Mainil J.G. Vet. Microbiol. 1998 Aug 15;62(4):291-
301). Thus frequently *E.coli* are isolated which carry F4-fimbriae and express the
heat-stable and heat-labile enterotoxins, as well as *E.coli* expressing F5- or F6-
10 fimbriae in combination with the heat-stable enterotoxins.

E.coli with F5/F6/F41 fimbriae are primarily detected in the distal jejunum and in the
ileum (Bertschinger H.U. Vet. Microbiol. 1993 May;35(1-2):79-89), while *E.coli*
with F4-fimbriae colonize the entire small intestine and the ileum
15 (Jeyasingham M.D. Vet. Microbiol. 1999 Aug 31;68(3-4):219-34).

For some of the fimbriae a genetic resistance of the host was proven. Certain pig
phenotypes exhibit a F4 (K88)-resistance which is recessively inherited, while the
susceptibility for *E.coli* carrying F4-fimbriae is dominantly inherited (Gibbons *et al.*,
20 Theor. Appl. Genet. 1977, 51 :65-70; Rapacz *et al.*, Anim. Genet. 1986, 17:305-321).

Piglets are very susceptible to F4 (K88)+ enterotoxigenic *E.coli*-induced neonatal
and post-weaning diarrhoea. Further the bacteria tend to spread within a host
population which leads to an important infectious load in the population. Thus, the
25 ability to protect animals from *E.coli* infections and/or treat infected animals is of
great importance.

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Antibodies are proteins which are produced and secreted by B lymphocytes / plasma cells and which reversely and non-covalently bind to their specific antigen. They consist of four polypeptide chains, two identical heavy (H) chains of about 50-70 kD and two identical light (L) chains of about 25 kD. Each chain consists of a constant
5 (C) and a variable (V) region. The constant region is composed of three to four constant domains in case of the heavy chain (CH₁ - CH₄) and one constant domain in case of the light chain (CL).

The variable region of the heavy chain (VH) and of the light chain (VL) are each
10 composed of one domain, consisting of about 110 amino acids. The VH and the VL region form the antigen binding site. They each comprise three complementarity determining regions (CDR1, CDR2, CDR3) which represent the most variable regions of the antibody and which confer antigen binding specificity. The framework regions in between the CDRs are relatively conserved.

15

It has been shown that fragments of a whole antibody containing the CDR regions can still perform the binding function. These smaller molecules can be produced effectively e.g. in *E.coli*, yeasts and plant cells whereas whole IgG molecules still need to be produced in mammalian cell culture. Furthermore unwanted effects
20 triggered by the constant part of the molecule are avoided, such as complement activation. However, the clearance rate is higher for antibody fragments, resulting in a short half-life upon systemic application.

An antibody "Fab" fragment (fragment antigen binding) is composed of a complete
25 light chain linked to the N-terminal part of a heavy chain, consisting of the variable region (VH) and one constant domain (CH₁). The Fab fragment may be generated e.g. by papain hydrolysis of an antibody.

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Gene technology methods such as phage display allow the production of a recombinant single-chain antibody "Fv" fragment (fragment variable) which is a truncated Fab fragment and consists of the variable region of a heavy chain and the variable region of a light chain, which are linked to each other by a synthetic peptide (scFv, single-chain fragment variable). "Phage display" is a standard technique for the presentation of distinct peptides, polypeptides or proteins on the surface of bacteriophages. It allows the identification of the exposed peptides, polypeptides or proteins via their binding properties. The technique is used for the establishment of libraries of peptide, polypeptide or protein variants such as different antibody fragments. For this, mammals such as mice are immunized against the antigen of interest and lymphocytes are isolated, e.g. from the spleen. Then, mRNA is isolated therefrom, and a cDNA library is generated by cloning the cDNA into a phage display vector.

The phage display library generated in this way contains recombinant scFv antibody fragments. The random library is screened for protein-protein interactions, e.g. for their antigen binding specificities. Interacting clones of the library are enriched, and the plasmid DNA from individual clones can be isolated and sequenced. It is an object of the present invention to provide novel antigen-binding polypeptides, particularly antibody fragments, and especially single-chain antibodies, which specifically bind to bacterial F4 (K88)-fimbriae. These polypeptides preferably neutralize the attachment of F4 (K88) fimbriae to enterocytes and/or have a protective and/or curative effect on infection of mammals, especially pigs, with bacteria expressing F4 (K88)-fimbriae. A further object of the present invention is to provide polynucleotides and/or vectors encoding these polypeptides, prokaryotic or eukaryotic host cells comprising these polynucleotides and/or vectors, transgenic plants or parts thereof or microorganisms containing the polynucleotides and/or vectors of the present invention, as well as compositions comprising said F4 (K88)-

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binding polypeptides. Furthermore, the present invention also provides a method by which said F4 (K88)-binding polypeptides may be produced.

These and further objects are achieved by the subject-matter of the patent claims,
5 which will become clear from the following description.

DESCRIPTION

In a first aspect the present invention relates to an antigen-binding polypeptide which
10 specifically binds to bacterial F4 (K88)-fimbriae. The term “antigen-binding polypeptide” as used herein means a polypeptide or protein which, according to the invention, specifically binds to bacterial F4 (K88). The term “specific binding” as used herein means that the polypeptide of the present invention does not show any significant binding to molecules other than bacterial F4 (K88) fimbriae or fragments
15 thereof. Usually this binding is reversible and/or non-covalent.

Other preferred embodiments of the present invention are defined in the respective subclaims.

20 In one preferred embodiment, the antigen-binding polypeptide of the present invention binds to a polypeptide comprising the amino acid sequence of bacterial F4 (K88) ab, F4 (K88) ac and/or F4 (K88) ad (SEQ ID NOs: 1, 2 and/or 3) and/or fragments thereof.

25 In another aspect the present invention relates to an antigen-binding polypeptide comprising a first polypeptide comprising the antigen-binding portion of the variable region of an antibody heavy chain, and a second polypeptide comprising the antigen-

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binding portion of the variable region of an antibody light chain, wherein said antigen-binding polypeptide specifically binds to bacterial F4 (K88)-fimbriae.

The term "heavy chain" or "light chain" as used herein means the conventional antibody polypeptide heavy chain (H) or light chain (L), respectively. The term
5 "variable region" as used herein means the conventional antibody variable region or variable domain or variable fragment (V or Fv) which confers antigen binding specificity. Variable regions employed in the invention may be derived from any germline or rearranged variable domain, or may be a synthetic variable domain based
10 on consensus sequences of known variable domains. Usually the variable region of the heavy chain (VH) and the variable region of the light chain (VL) each comprise three complementarity determining regions CDR1, CDR2 and CDR3.

Seven antigen-binding polypeptides which specifically bind to bacterial F4 (K88)
15 fimbriae have been generated via immunization of mice, cloned into a phage display library, tested for their F4 (K88)-binding specificity, isolated and sequenced. They carry the designations BA61, BA31, BA29, BA3, BA11, BA67 and BA5. These polypeptides are single-chain polypeptides. Their full length amino acid sequences are depicted in SEQ ID NOs: 4, 5, 6, 7, 8, 9 and 10, and their full length nucleotide
20 sequences are depicted in SED ID NOs: 11, 12, 13, 14, 15, 16 and 17.

Surprisingly it was found that these polypeptides, all comprising an antigen-binding portion of a VH chain and an antigen-binding portion of a VL chain, are characterized by similar CDR1, CDR2 and CDR3 regions, especially in the
25 VH CDR1 region (tables 1 and 2). Therefore it was possible to characterize these F4 (K88)-binding single-chain polypeptides by means of amino acid and nucleotide consensus sequences of their CDRs. The locations of the CDRs were determined by reference to Kabat E.A. *et al.* (Sequences of Proteins of Immunological Interest, 4th

Edition, U.S. Department of Health and Human Services, 1987) and updates thereof, now available on the Internet.

5

Table 1: Individual and consensus amino acid sequences of VH CDR1, VH CDR2 and VH CDR3 of the F4 (K88)-binding polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5. The most often occurring common amino acids are highlighted in bold letters. Amino acids of the consensus sequences in parentheses are optional. X₁ = N or D; X₂ = A or V; X₃ = M or I; X₄ = S or H; X₅ = E, Y or V; X₆ = S or N; X₇ = T or P; X₈ = G, A or Y; X₉ = G, N or S; X₁₀ = S, D or G; X₁₁ = H, Y, N, F or S; X₁₂ = Y, K or N; X₁₃ = P or N; X₁₄ = D, E or Q; X₁₅ = T, K or S; X₁₆ = V or F; X₁₇ = T or K; X₁₈ = G or D; X₁₉ = T or V; X₂₀ = G, D or N; X₂₁ = Y or E; X₂₂ = T, Y or N; X₂₃ = P, A or G; X₂₄ = F or M; X₂₅ = D or A; X₂₆ = Y or V; X₂₇ = G or A; X₂₈ = H, Y or N; X₂₉ = Y or V; X₃₀ = N or D; X₃₁ = V or A; X₃₂ = I or M; X₃₃ = Y or V; X₃₄ = N or S; X₃₅ = P or T; X₃₆ = N or S; X₃₇ = D or G; X₃₈ = F or S; X₃₉ = K or N; X₄₀ = E or Q; X₄₁ = K or S; X₄₂ = G or D; X₄₃ = V or T; X₄₄ = G, D or N; X₄₅ = E or Y; X₄₆ = T, Y or N; X₄₇ = P, A or G; X₄₈ = F or M; X₄₉ = A or D; X₅₀ = S or K; X₅₁ = G or D; X₅₂ = D or N; X₅₃ = Y or N; X₅₄ = A or G; X₅₅ = A or V; X₅₆ = M or I; X₅₇ = S or H; X₅₈ = E or Y; X₅₉ = S or N; X₆₀ = T or P; X₆₁ = G, A or Y; X₆₂ = G or N; X₆₃ = S or D; X₆₄ = H, Y, N or F; X₆₅ = Y or K; X₆₆ = P or N; X₆₇ = D or E; X₆₈ = T or K; X₆₉ = V or F; X₇₀ = T or K; X₇₁ = T or V; X₇₂ = Y or E; X₇₃ = D or A; X₇₄ = Y or V.

25

	VH CDR1	SEQ ID No.	VH CDR2	SEQ ID No.	VH CDR3	SEQ ID No.
BA61	NYAMS	18	EISTGGSHTYYPDVTG	24	TGY-----FDY	35
BA31	NYAMS	18	EISTAGSYTYYPDVTG	25	TGY-----FDY	35
BA29	NYAMS	18	EISTAGSNTYYPDVTG	26	TGY-----FDV	36
BA3	NYAMS	18	EISTAGSNTYYPDVTG	26	TGY-----FDV	36
BA11	NYVIH	19	YINPYNDF TKYNEKFKG	27	VGE---TP FAY	37
BA67	DYAMH	20	VISTYSGSTNYNQSFKG	28	TDYPYFYAMDY	38
BA5	DYAMH	20	VISTYSGSTNYNQKFKD	29	TNYPYFNGMDY	39
cons.:						
all	X ₁ Y X ₂ X ₃ X ₄	21	X ₅ I X ₆ X ₇ X ₈ X ₉ X ₁₀ X ₁₁ TX ₁₂ Y X ₁₃ X ₁₄ X ₁₅ X ₁₆ X ₁₇ X ₁₈	30	X ₁₉ X ₂₀ X ₂₁ (PYF) (X ₂₂ X ₂₃) X ₂₄ X ₂₅ X ₂₆	40
BA61+31+29+3	NYAMS	18	EISTX₂₇GSX₂₈TYYPDVTG	31	TGYFDX₂₉	41
BA11+67+5	X ₃₀ Y X ₃₁ X ₃₂ H	22	X ₃₃ I X ₃₄ X ₃₅ Y X ₃₆ X ₃₇ X ₃₈ TX ₃₉ Y NX ₄₀ X ₄₁ FKX ₄₂	32	X ₄₃ X ₄₄ X ₄₅ (PYF) X ₄₆ X ₄₇ X ₄₈ X ₄₉ Y	42

BA67+5	DYAMH	20	VISTYSGSTNYNQX ₅₀ FKX ₅₁	33	TX ₅₂ YPYFX ₅₃ X ₅₄	43
BA61+31+ 29+3+11	NYX ₅₅ X ₅₆ X ₅₇	23	X ₅₈ IX ₅₉ X ₆₀ X ₆₁ X ₆₂ X ₆₃ X ₆₄ T X ₆₅ YX ₆₆ X ₆₇ X ₆₈ X ₆₉ X ₇₀ G	34	X ₇₁ GX ₇₂ (TP) F X ₇₃ X ₇₄	44

The amino acid sequences which are designated in claims 5 to 10 refer to the SEQ ID NOs of table 1. For the purpose of clarification of the sequence protocol, the consensus sequences of table 1 which contain amino acids in parentheses (SEQ ID

5 NOs: 40, 42 and 44) are defined in the sequence protocol as follows:

SEQ ID NO: 40 **X**₁₉**X**₂₀**X**₂₁**PYFX**₂₂**X**₂₃**X**₂₄**X**₂₅**X**₂₆

SEQ ID NO: 139 **X**₁₉**X**₂₀**X**₂₁**X**₂₄**X**₂₅**X**₂₆

SEQ ID NO: 140 **X**₁₉**X**₂₀**X**₂₁**X**₂₂**X**₂₃**X**₂₄**X**₂₅**X**₂₆

10

SEQ ID NO: 42 **X**₄₃**X**₄₄**X**₄₅**PYFX**₄₆**X**₄₇**X**₄₈**X**₄₉**Y**

SEQ ID NO: 141 **X**₄₃**X**₄₄**X**₄₅**X**₄₆**X**₄₇**X**₄₈**X**₄₉**Y**

SEQ ID NO: 44 **X**₇₁**GX**₇₂**TPFX**₇₃**X**₇₄

15 SEQ ID NO: 142 **X**₇₁**GX**₇₂**FX**₇₃**X**₇₄

Table 2: Individual and consensus amino acid sequences of VL CDR1, VL CDR2 and VL CDR3 of the F4 (K88) binding polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5. The most often occurring common amino acids are

highlighted in bold letters. Amino acids of the consensus sequences in parentheses are optional. **X**₁ = K, R or S; **X**₂ = A or G; **X**₃ = Q or S; **X**₄ = N or D; **X**₅ = V or I; **X**₆ = G or S; **X**₇ = T or N; **X**₈ = N, T, A or Y; **X**₉ = V or L; **X**₁₀ = A, N or S; **X**₁₁ = S, W, Y or D; **X**₁₂ = A, T or N; **X**₁₃ = S or N; **X**₁₄ = Y, T, N, R or K; **X**₁₅ = R or L; **X**₁₆ = Y, H or F; **X**₁₇ = S or T; **X**₁₈ = Q or G; **X**₁₉ = Q or A; **X**₂₀ = Y, G or W; **X**₂₁ = N, S or D; **X**₂₂ = S or T; **X**₂₃ = Y or L; **X**₂₄ = P or R; **X**₂₅ = Y, W or E; **X**₂₆ = T or A; **X**₂₇ = N or D; **X**₂₈ = N, T or A; **X**₂₉ = S or W; **X**₃₀ = Y, T or N; **X**₃₁ = Y or H; **X**₃₂ = S or T; **X**₃₃ = N or S; **X**₃₄ = R or S; **X**₃₅ = A or G; **X**₃₆ = Q or S; **X**₃₇ = D or N; **X**₃₈ = S or G; **X**₃₉ = L or V; **X**₄₀ = N or S; **X**₄₁ = Y or D; **X**₄₂ = T or N; **X**₄₃ = S or N; **X**₄₄ = R or K; **X**₄₅ = L or R; **X**₄₆ = H or F; **X**₄₇ = Q or G; **X**₄₈ = Q or A; **X**₄₉ = G or W; **X**₅₀ = N or D; **X**₅₁ = T or S; **X**₅₂ = P or R; **X**₅₃ = W or E; **X**₅₄ = T or A; **X**₅₅ = K or S; **X**₅₆ = N or D; **X**₅₇ =

20

25

30

5 V or I; X₅₈ = G or S; X₅₉ = T or N; X₆₀ = N, A, T or Y; X₆₁ = V or L; X₆₂ = A or N; X₆₃ = S, W or Y; X₆₄ = A or T; X₆₅ = Y, N, T or R; X₆₆ = R or L; X₆₇ = Y or H; X₆₈ = S or T; X₆₉ = Y or G; X₇₀ = N or S; X₇₁ = S or T; X₇₂ = Y or L; X₇₃ = Y or W; X₇₄ = R or K; X₇₅ = D or N; X₇₆ = I or V; X₇₇ = S or G; X₇₈ = N or T; X₇₉ = Y or N; X₈₀ = L or V; X₈₁ = N or A; X₈₂ = Y or S; X₈₃ = T or A; X₈₄ = R or Y; X₈₅ = L or R; X₈₆ = H or Y; X₈₇ = G or Y; X₈₈ = T or S; X₈₉ = L or Y; X₉₀ = W or Y.

	VL CDR1	SEQ ID No.	VL CDR2	SEQ ID No.	VL CDR3	SEQ ID No.
BA61	KASQ-NVG-TNVA	45	SASYRYS	55	QQYN-SYPYT	65
BA3	KASQ-NVG-TNVA	45	SASYRYS	55	QQYN-SYPYT	65
BA5	KASQ-NVG-TNVA	45	SASYRYS	55	QQYN-SYPYT	65
BA29	KASQ-NVG-TAVA	46	SASNRYT	56	QQYS-SYPYT	66
BA31	KASQ-DVG-TTVA	47	WASTRHT	57	QQYS-SYPYT	66
BA11	RASQ-DIS-NYLN	48	YTSRLHS	58	QQGN-TLPWT	67
BA67	SGSSSNIGNNYVS	49	DNNKRFS	59	GAWDGSLREAV	68
cons.						
all	X ₁ X ₂ S X ₃ (S) X ₄ X ₅ X ₆ (N) X ₇ X ₈ X ₉ X ₁₀	50	X ₁₁ X ₁₂ X ₁₃ X ₁₄ X ₁₅ X ₁₆ X ₁₇	60	X ₁₈ X ₁₉ X ₂₀ X ₂₁ (G) X ₂₂ X ₂₃ X ₂₄ X ₂₅ X ₂₆ (V)	69
BA61+3+ 5+29+31	KASQ X ₂₇ VGT X ₂₈ VA	51	X ₂₉ AS X ₃₀ R X ₃₁ X ₃₂	61	QQY X ₃₃ SYPYT	70
BA61+3+ 5+	KASQNVGTNVA	45	SASYRYS	55	QQYNSYPYT	65
BA11+67	X ₃₄ X ₃₅ S X ₃₆ (S) X ₃₇ I X ₃₈ (N) NYX ₃₉ X ₄₀	52	X ₄₁ X ₄₂ X ₄₃ X ₄₄ X ₄₅ X ₄₆ S	62	X ₄₇ X ₄₈ X ₄₉ X ₅₀ (G) X ₅₁ LX ₅₂ X ₅₃ X ₅₄ (V)	71
BA61+3+ 5+29+31 +11	X ₅₅ ASQ X ₅₆ X ₅₇ X ₅₈ X ₅₉ X ₆₀ X ₆₁ X ₆₂	53	X ₆₃ X ₆₄ S X ₆₅ X ₆₆ X ₆₇ X ₆₈	63	QQ X ₆₉ X ₇₀ X ₇₁ X ₇₂ P X ₇₃ T	72
BA61+3+ 5+11	X ₇₄ ASQ X ₇₅ X ₇₆ X ₇₇ X ₇₈ X ₇₉ X ₈₀ X ₈₁	54	X ₈₂ X ₈₃ S X ₈₄ X ₈₅ X ₈₆ S	64	QQ X ₈₇ N X ₈₈ X ₈₉ P X ₉₀ T	73

10 The amino acid sequences which are designated in claims 11 to 16 refer to the SEQ ID NOs of table 2. For the purpose of clarification of the sequence protocol, the consensus sequences of table 2 which contain amino acids in parentheses (SEQ ID NOs: 50, 52, 69 and 71) are defined in the sequence protocol as follows:

15 SEQ ID NO: 50 X₁X₂**S**X₃**S**X₄X₅X₆**N**X₇X₈X₉X₁₀
 SEQ ID NO: 143 X₁X₂**S**X₃X₄X₅X₆X₇X₈X₉X₁₀

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SEQ ID NO: 52 X₃₄X₃₅SX₃₆SX₃₇IX₃₈NNYX₃₉X₄₀

SEQ ID NO: 144 X₃₄X₃₅SX₃₆X₃₇IX₃₈NYX₃₉X₄₀

SEQ ID NO: 69 X₁₈X₁₉X₂₀X₂₁GX₂₂X₂₃X₂₄X₂₅X₂₆V

5 SEQ ID NO: 145 X₁₈X₁₉X₂₀X₂₁X₂₂X₂₃X₂₄X₂₅X₂₆

SEQ ID NO: 71 X₄₇X₄₈X₄₉X₅₀GX₅₁LX₅₂X₅₃X₅₄V

SEQ ID NO: 146 X₄₇X₄₈X₄₉X₅₀X₅₁LX₅₂X₅₃X₅₄

10 Thus, in a preferred embodiment, the antigen-binding polypeptide of the present invention comprises the antigen-binding portion of an antibody heavy chain variable region, which comprises a VH CDR 1, which has the consensus amino acid sequence depicted in SEQ ID NO: 21, more preferably the sequences depicted in SEQ ID
 15 SEQ ID NOS: 18, 19 or 20. Other preferred consensus amino acid sequences are depicted in SEQ ID NOS: 22 and 23. Most preferred for VH CDR1 are the individual amino acid sequences depicted in SEQ ID NOS: 18, 19 and 20.

In another preferred embodiment, the antigen-binding polypeptide of the present invention comprises the antigen-binding portion of an antibody heavy chain variable
 20 region, which comprises a VH CDR 2, which has the consensus amino acid sequence depicted in SEQ ID NO: 30, more preferably the sequences depicted in SEQ ID NOS: 27, 31 and 33. Other preferred consensus amino acid sequences are depicted in SEQ ID NOS: 32 and 34. Most preferred for VH CDR2 are the individual amino acid sequences depicted in SEQ ID NOS: 24, 25, 26, 27, 28 and 29.

25

In another preferred embodiment, the antigen-binding polypeptide of the present invention comprises the antigen-binding portion of an antibody heavy chain variable region, which comprises a VH CDR 3, which has the consensus amino acid sequence

depicted in SEQ ID NO: 40, more preferably the sequences depicted in SEQ ID NOs: 37, 41 and 43. Other preferred consensus amino acid sequences are depicted in SEQ ID NOs: 42 and 44. Most preferred for VH CDR3 are the individual amino acid sequences depicted in SEQ ID NOs: 35, 36, 37, 38 and 39.

5

In another preferred embodiment, the antigen-binding polypeptide of the present invention comprises the antigen-binding portion of an antibody light chain variable region, which comprises a VL CDR 1, which has the consensus amino acid sequence depicted in SEQ ID NO: 50, more preferably the sequences depicted in SEQ ID NOs: 48, 49 and 51. Other preferred consensus amino acid sequences are depicted in SEQ ID NOs: 52, 53 and 54. Most preferred for VL CDR1 are the individual amino acid sequences depicted in SEQ ID NOs: 45, 46, 47, 48 and 49.

10

In another preferred embodiment, the antigen-binding polypeptide of the present invention comprises the antigen-binding portion of an antibody light chain variable region, which comprises a VL CDR 2, which has the consensus amino acid sequence depicted in SEQ ID NO: 60, more preferably the sequences depicted in SEQ ID NOs: 58, 59 and 61. Other preferred consensus amino acid sequences are depicted in SEQ ID NOs: 62, 63 and 64. Most preferred for VL CDR2 are the individual amino acid sequences depicted in SEQ ID NOs: 55, 56, 57, 58 and 59.

15

20

In another preferred embodiment, the antigen-binding polypeptide of the present invention comprises the antigen-binding portion of an antibody light chain variable region, which comprises a VL CDR 3, which has the consensus amino acid sequence depicted in SEQ ID NO: 69, more preferably the sequences depicted in SEQ ID NOs: 67, 68 and 70. Other preferred consensus amino acid sequences are depicted in SEQ ID NOs: 71, 72 and 73. Most preferred for VL CDR3 are the individual amino acid sequences depicted in SEQ ID NOs: 65, 66, 67 and 68.

25

According to the invention, these six different CDRs may be combined with each other in any conceivable way. The CDRs may further be carried by any antibody framework regions. Preferably these framework sequences derive from the mouse, as
5 for example in SEQ ID NOs: 4, 5, 6, 7, 8, 9 or 10.

In another preferred embodiment of the invention, the antigen-binding polynucleotide, which specifically binds to bacterial F4 (K88)-fimbriae, comprises a first polypeptide comprising the antigen-binding portion of the variable region of an
10 antibody heavy chain, a second polypeptide comprising the antigen-binding portion of the variable region of an antibody light chain, and a peptide linker linking said first and said second polypeptide into a single-chain polypeptide.

The term “single-chain polypeptide” or “single-chain antibody” or “single-chain
15 fragment” as used herein means any polypeptide comprising the variable region Fv of a heavy chain, a linking peptide and the variable region Fv of a light chain, resulting in a single chain Fv or “scFv”.

In one preferred embodiment, the single-chain antigen-binding polypeptide
20 according to the invention comprises the amino acid sequence of SEQ ID NOs: 4, 5, 6, 7, 8, 9 or 10.

The sequences in SEQ ID NOs: 4, 5, 6, 7, 8, 9 or 10 may further be altered by replacing the linker sequence (here: K L E E G E F S E A R V, SEQ ID NO: 129)
25 with any other desired linker sequence. Further, the respective amino acid sequences of the heavy chain and of the light chain of SEQ ID NOs: 4, 5, 6, 7, 8, 9 and 10 may be combined with each other to form a single-chain antigen-binding polypeptide.

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The present invention also refers to multivalent single-chain antigen-binding polypeptides, comprising two or more single-chain polypeptides according to the invention, which specifically bind to bacterial F4 (K88)-fimbriae, and wherein the two or more single-chain polypeptides are linked by a peptide linker. When two
5 single-chain polypeptides are linked by a peptide linker, the polypeptide is called “bivalent”.

The peptide linkers linking the polypeptides within a single-chain antigen-binding polypeptide or linking two or more single-chain polypeptides to a multivalent single-
10 chain antigen-binding polypeptide are described in the following.

The peptide linker may be any peptide of any length, preferably in the range of about 1 to 100 amino acids, more preferably in the range of about 12 to 25 amino acids. Preferred linkers of the present invention are e.g. the “yol linker” and the “GS
15 linker”. Preferably the peptide linker of the present invention is not susceptible to protease cleavage, particularly to proteases occurring in the gastrointestinal tract of mammals, especially of pigs.

The yol linker used in the present invention has the amino acid sequence
20 K L E E G E F S E A R V (SEQ ID NO: 129). This linker may also be truncated or elongated at the N terminus and/or at the C terminus by one or more amino acids, as described in the literature. For example, in Schmiedl A. *et al.* (Protein Eng. 2000 Oct;13(10):725-34) the yol linker is shortened by one amino acid at each terminus compared to the above described yol linker.

25 In order to improve the stability of the yol linker and to delete the two putative trypsin cleavage sites (lysine and arginine), the yol linker may be modified by exchanging lysine (K) against isoleucine (I), arginine (R) against glycine (G) and

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valine (V) against cysteine (C). The resulting linker carries the designation “my” (modified yol) and has the amino acid sequence I L E E G E F S E A G C (SEQ ID NO: 130). Any peptide linker carrying the consensus amino acid sequence X_1 L E E G E F S E A X_2 X_3 (SEQ ID NO: 131), wherein $X_1 = K$ or I , $X_2 = R$ or G , and $X_3 = V$ or C , is also preferred in the present invention.

The linker may also be a GS-linker. The GS linker used in the present invention has the amino acid sequence (GGGGS)₂ (SEQ ID NO: 132) and carries the designation “gs”. The GS-linker may also have the amino acid sequence (GGGGS)₃, as described in the literature (Huston J.S. Methods Enzymol. 1991;203:46-88). The GS-linker is a preferred linker for the linking of one or more single-chain antigen-binding polypeptides to a multivalent single-chain polypeptide of the present invention.

In one embodiment of the present invention the antigen-binding polypeptide which specifically binds to bacterial F4 (K88) fimbriae is a Fab antibody fragment. The term “Fab” (fragment antigen binding) or “Fab antibody” or “Fab fragment” as used herein means a fragment comprising a complete antibody light chain linked to the N-terminal part of a heavy chain, comprising the variable region (VH) and one constant domain (CH₁). Fab fragments are commercially available, e.g. generated by the company Morphosys, Martinsried/Planegg, Germany.

Other possible antigen-binding polypeptides of the present invention which specifically bind to bacterial F4 (K88) fimbriae are F(ab')₂ fragments (bivalent fragments comprising two linked Fab fragments; Holliger P. and Winter G. Curr. Opin. Biotechnol. 1993 Aug;4(4):446-9) and diabodies (multivalent or multispecific fragments constructed by gene fusion; WO94/13804). The generation of Fab and F(ab')₂ fragments is also described in Liddell, J.E. and I. Weeks (1995) Antibody Technology: A Comprehensive Overview, Bios Scientific: Oxford.

Also preferred in the present invention are non-immunoglobulin polypeptides which specifically bind to bacterial F4 (K88) fimbriae. These may e.g. be ANTICALINS® which are produced by Pieris Proteolab AG (Freising, Germany) or Affilin™ molecules which are produced by Scil Proteins GmbH (Halle, Germany).

ANTICALINS® are derived from lipocalins. Lipocalins are composed of a single polypeptide chain with 160 to 180 amino acid residues. They share a structurally conserved β -barrel supporting four loops at one end, which form the entrance to a binding pocket. The loops exhibit conformational differences between individual lipocalins and give rise to the variety of ligand specificities.

Affilin™ molecules are small non-immunoglobulin proteins based on human cytosolic proteins. Affilin™ molecules can be selected from two libraries, each of which is based on a different human derived scaffold protein, one of which is gamma crystalline, a human structural eye lens protein. Both human scaffolds are very small, show high temperature stability and are almost resistant to pH changes and denaturing agents. This high stability is mainly due to the expanded beta sheet structure of the proteins.

The antigen-binding polypeptide of the present invention, particularly the antibody fragment and especially the single-chain antibody, which specifically binds to bacterial F4 (K88)-fimbriae, preferably neutralizes the attachment of F4 (K88)-fimbriae to enterocytes.

One example of an *in vitro* assay which assesses the neutralizing effect of the antigen-binding polypeptides of the present invention is the “brush border adhesion test”. Brush borders are isolated from the small intestine of mammals, especially of

pigs. Under standard conditions, *E.coli* bearing F4 (K88) fimbriae attach to these brush borders. Antigen-binding polypeptides which specifically bind to bacterial F4 (K88) fimbriae are added to this system to measure their neutralizing effect on adhesion. Specific antigen-binding polypeptides protect the intestinal cells from the
5 attaching fimbriae.

In another preferred embodiment, the antigen-binding polypeptide of the present invention has a protective and/or a curative effect on infection of mammals, preferably pigs, with bacteria expressing F4 (K88)-fimbriae.

10

In order to assess the protective and/or a curative effect, mammals, especially pigs, can be infected with an *E.coli* strain bearing F4 fimbriae. The treatment group receives the antigen-binding polypeptide of the present invention with the feed. The first dose may be applied before or after infection. Thereafter, the animals receive
15 daily doses of the polypeptide, distributed over one or more daily feed applications, for several days. Animals in the control group are infected without polypeptide administration. Monitoring of the clinical symptoms, microbial examination of the feces (which is of high importance for the spread of an infection in a population) and pathological, microbiological and immunochemical analyses (monitoring the
20 presence of the polypeptides in selected organs) give information about the preventative and curative effect of the orally applied antigen-binding polypeptide.

Therefore, another aspect of the present invention is the use of the antigen-binding polypeptide of the present invention, particularly the antibody fragment and
25 especially the single-chain antibody, which specifically binds to F4 (K88)-fimbriae, for the manufacture of a medicament for the treatment and/or prevention of infections with bacteria expressing F4 (K88)-fimbriae. The medicament may be applied to mammals, especially to pigs. The administration preferably occurs orally.

The present invention further relates to the use of the F4 (K88)-binding polypeptides for detecting bacterial F4 (K88) fimbriae, especially for detecting a polypeptide comprising the amino acid sequence of SEQ ID NOs: 1, 2 and/or 3 or a fragment thereof. This detection preferably occurs through binding of the polypeptides of the present invention to the F4 (K88) fimbriae. This binding can take place *in vitro* and/or *in vivo*. For example, the polypeptides of the present invention may be used in research laboratories in cell culture and FACS assays, Western Blot analyses or the like. For the detection purposes, the F4 (K88)-binding polypeptides may be fused to other substances such as epitope tags, fluorescent molecules, enzymes and so on.

It is another object of the invention to provide a composition comprising the antigen-binding polypeptide of the present invention, particularly the antibody fragment and more particularly the single-chain antibody, which specifically binds to F4 (K88)-fimbriae, and an acceptable excipient, carrier, buffer and/or stabilizer.

The antigen-binding polypeptides of the present invention may be degraded rapidly after oral application. Special attention has to be given to the high acidity in the stomach as well as the presence of secretory proteases, particularly in the oral cavity and in the small intestine.

Therefore the excipient, carrier, buffer and/or stabilizer preferably comprises a protein source, preferably BSA, milk powder and/or pea flour. The proteins may be added alone or as a protein mixture. Additionally or alternatively, the excipient, carrier, buffer and/or stabilizer may comprise methyl-cellulose.

Furthermore, the antigen binding peptides may be formulated as acid-resistant micro-particles, or be packed into acid-resistant capsules, preferably gelatine capsules, which are preferably encased with a stabilizing liquid.

- 5 The present invention further extends to polynucleotides comprising a nucleotide sequence which encodes an antigen-binding polypeptide according to the invention, which specifically bind to bacterial F4 (K88)-fimbriae. The term "polynucleotide" as used herein is to be understood as meaning a double-stranded or single-stranded nucleic acid molecule, e.g. a DNA, cDNA, genomic DNA, RNA and/or mRNA
- 10 molecule or the like. The nucleic acid molecule can be present either as the coding strand or as the complementary strand. The polynucleotide may be of a natural source or produced by gene technological or chemical processes and synthesis methods or may have been derived therefrom.
- 15 The individual nucleotide sequences and the consensus nucleotide sequences of VH CDR1-3 and VL CDR1-3 of the seven single-chain polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5 are depicted in the following tables 3-8. Nucleotides of the consensus sequences in parentheses are optional. Abbreviations for alternative nucleotides in the nucleotide sequences (underlined> are:
- 20 R: G or A
 Y: T or C
 M: A or C
 K: G or T
 S: G or C
- 25 W: A or T
 B: T, G or C
 D: A, T or G
 H: A, T or C
 V: A, G or C
- 30 N: any

Table 3: Consensus and individual nucleotide sequences of VH CDR1 of the F4 (K88) binding polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5. The most often occurring common nucleotides are highlighted in bold letters.

	nucleotide sequence	SEQ ID No.
BA61	AAC TAT GCC ATG TCT	74
BA31	AAC TAT GCC ATG TCT	74
BA29	AAC TAT GCC ATG TCC	75
BA3	AAC TAT GCC ATG TCC	75
BA11	AAC TAT GTT ATA CAC	76
BA67	GAT TAT GCT ATG CAC	77
BA5	GAT TAT GCT ATG CAC	77
consensus:		
All	<u>RAY</u> TAT <u>GY</u> Y <u>ATR</u> <u>YMY</u>	78
BA 61 + 31 + 29 + 3	AAC TAT GCC ATG TCY	79
BA 11 + 67 + 5	<u>RAK</u> TAT <u>GY</u> T <u>ATR</u> CAC	80
BA 67 + 5	GAT TAT GCT ATG CAC	77

5

Table 4: Consensus and individual nucleotide sequences of VH CDR2 of the F4 (K88) binding polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5. The most often occurring common nucleotides are highlighted in bold letters.

10

	nucleotide sequence	SEQ ID No.
BA61	GAA ATT AGT ACT GGT GGT AGT CAC ACC TAC TAT CCA GAC ACT GTG ACG GGC	81
BA31	GAA ATT AGT ACT GCT GGT AGT TAC ACC TAC TAT CCA GAC ACT GTG ACG GGC	82
BA29	GAA ATT AGT ACT GCT GGT AGT AAC ACC TAC TAT CCA GAC ACT GTG ACG GGC	83
BA3	GAA ATT AGT ACT GCT GGT AGT AAC ACC TAC TAT CCA GAC ACT GTG ACG GGC	83
BA11	TAT ATT AAT CCT TAC AAT GAT TTT ACT AAA TAC AAT GAG AAG TTC AAA GGC	84
BA67	GTG ATT AGT ACT TAC TCT GGT AGT ACA AAC TAC AAC CAG AGC TTT AAG GGC	85
BA5	GTT ATT AGT ACT TAC TCT GGT AGT ACA AAC TAC AAC CAG AAG TTT AAG GAC	86
consensus:		
All	KWD ATT ART MCT <u>KVY</u> <u>DVT</u> <u>RRT</u> <u>HDY</u> <u>ACH</u> <u>WAM</u> <u>TAY</u> MMH <u>SAS</u> <u>AVB</u> <u>KTB</u> <u>AMR</u> <u>GRC</u>	87
BA 61 + 31 + 29 + 3	GAA ATT AGT ACT GST GGT AGT HAC ACC TAC	88

	TAT CCA GAC ACT GTG ACG GGC	
BA 11 + 67 + 5	<u>KWK</u> ATT <u>ART</u> <u>MCT</u> TAC <u>WMT</u> <u>GRT</u> <u>WKT</u> <u>ACW</u> <u>AAM</u> TAC AAY <u>SAG</u> ARS <u>TTY</u> AAR GRC	89
BA 67 + 5	GTK ATT AGT ACT TAC TCT GGT AGT ACA AAC TAC AAC CAG <u>ARS</u> TTT AAG GRC	90

5 **Table 5:** Consensus and individual nucleotide sequences of VH CDR3 of the F4 (K88) binding polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5. The most often occurring common nucleotides are highlighted in bold letters.

	nucleotide sequence	Seq ID No.
BA61	ACT GGT TAC --- --- --- --- --- TTT GAC TAC	91
BA31	ACT GGT TAC --- --- --- --- --- TTT GAC TAC	91
BA29	ACG GGG TAC --- --- --- --- --- TTC GAT GTC	92
BA3	ACG GGG TAC --- --- --- --- --- TTC GAT GTC	92
BA11	GTC GGG GAA --- --- --- ACC CCC TTT GCT TAC	93
BA67	ACG GAC TAC CCT TAT TTC TAT GCT ATG GAC TAC	94
BA5	ACA AAC TAC CCT TAT TTC AAT GGT ATG GAC TAC	95
consensus:		
All	<u>RYN</u> <u>RRB</u> <u>KAM</u> (CCT TAT TTC) (<u>WMY</u> <u>SSY</u>) <u>WTB</u> <u>GMY</u> <u>KWC</u>	96
BA 61 + 31 + 29 + 3	ACK GGK TAC --- --- --- --- --- TTY GAY <u>KWC</u>	97
BA 11 + 67 + 5	<u>RYV</u> <u>RRS</u> <u>KAM</u> (CCT TAT TTC) <u>WMY</u> <u>SSY</u> <u>WTK</u> <u>GMY</u> TAC	98
BA 67 + 5	ACR <u>RAC</u> TAC CCT TAT TTC <u>WAT</u> <u>GST</u> ATG GAC TAC	99

Table 6: Consensus and individual nucleotide sequences of VL CDR1 of the F4 (K88) binding polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5. The most often occurring common nucleotides are highlighted in bold letters.

5

	nucleotide sequence	SEQ ID No.
BA61	AAG GCC AGT CAG --- AAT GTG GGT --- ACT AAT GTA GCC	100
BA3	AAG GCC AGT CAG --- AAT GTG GGT --- ACT AAT GTA GCC	100
BA5	AAG GCC AGT CAG --- AAT GTG GGT --- ACT AAT GTA GCC	100
BA29	AAG GCC AGT CAG --- AAT GTG GGT --- ACT GCT GTA GCC	101
BA31	AAG GCC AGT CAG --- GAT GTG GGT --- ACT ACT GTA GCC	102
BA11	AGG GCA AGT CAG --- GAC ATT AGC --- AAT TAT TTA AAC	103
BA67	TCT GGA AGC AGC TCC AAC ATT GGA AAT AAT TAT GTC TCC	104
consensus:		
all	<u>WVK</u> GSM AGY <u>MRS</u> (TCC) <u>RAY</u> <u>RTK</u> <u>RGH</u> (AAT) AMT <u>DMT</u> <u>KTM</u> <u>DMC</u>	105
BA61+3+5+29+31	AAG GCC AGT CAG --- <u>RAT</u> GTG GGT --- ACT <u>RMT</u> GTA GCC	106
BA61+3+5	AAG GCC AGT CAG --- AAT GTG GGT --- ACT AAT GTA GCC	100
BA11+67	<u>WSK</u> <u>GSA</u> AGY <u>MRS</u> (TCC) <u>RAC</u> <u>ATT</u> <u>RGM</u> (AAT) AAT TAT <u>KTM</u> <u>WMC</u>	107
BA61+3+5+29+31+11	<u>ARG</u> <u>GCM</u> AGT CAG --- <u>RAY</u> <u>RTK</u> <u>RGY</u> --- AMT <u>DMT</u> <u>KTA</u> <u>RMC</u>	108
BA61+3+5+11	<u>ARG</u> <u>GCM</u> AGT CAG --- <u>RAY</u> <u>RTK</u> <u>RGY</u> --- AMT <u>WAT</u> <u>KTA</u> <u>RMC</u>	109

Table 7: Consensus and individual nucleotide sequences of VL CDR2 of the F4 (K88) binding polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5. The most often occurring common nucleotides are highlighted in bold letters.

10

	nucleotide sequence	SEQ ID No.
BA61	TCG GCA TCC TAC CGG TAC AGT	110
BA3	TCG GCA TCC TAC CGG TAC AGT	110
BA5	TCG GCA TCC TAC CGG TAC AGT	110
BA29	TCG GCA TCC AAT CGG TAC ACT	111
BA31	TGG GCA TCC ACC CGG CAC ACT	112
BA11	TAC ACA TCA AGA TTA CAC TCA	113
BA67	GAC AAT AAT AAG CGA TTC TCA	114
consensus:		
all	<u>KVS</u> <u>RMW</u> <u>WMH</u> <u>WVN</u> <u>YKR</u> <u>YWC</u> <u>WSW</u>	115
BA61+3+5+29+31	TSG GCA TCC WMY CGG YAC AST	116
BA61+3+5	TCG GCA TCC TAC CGG TAC AGT	110

BA11+67	<u>KAC</u> <u>AMW</u> <u>WMW</u> <u>ARR</u> <u>YKA</u> <u>YWC</u> TCA	117
BA61+3+5+29+31+11	T <u>V</u> S <u>R</u> C<u>A</u> T<u>C</u>M <u>W</u><u>V</u><u>H</u> <u>Y</u><u>K</u><u>R</u> Y<u>A</u>C <u>W</u><u>S</u><u>W</u>	118
BA61+3+5+11	T <u>M</u> S <u>R</u> C<u>A</u> T<u>C</u>M <u>W</u><u>R</u><u>M</u> <u>Y</u><u>K</u><u>R</u> Y<u>A</u>C <u>W</u><u>S</u><u>W</u>	119

Table 8: Consensus and individual nucleotide sequences of VL CDR3 of the F4 (K88) binding polypeptides BA61, BA31, BA29, BA3, BA11, BA67 and BA5. The most often occurring common nucleotides are highlighted in bold letters.

5

	nucleotide sequence	SEQ ID No.
BA61	CAG CAA TAT AAC --- AGC TAT CCT TAC ACG	120
BA3	CAG CAA TAT AAC --- AGC TAT CCT TAC ACG	120
BA5	CAG CAA TAT AAC --- AGC TAT CCT TAC ACG	120
BA29	CAG CAA TAT AGC --- AGC TAT CCG TAC ACG	121
BA31	CAG CAA TAT AGC --- AGC TAT CCG TAC ACG	121
BA11	CAA CAG GGT AAT --- ACG CTT CCG TGG ACG	122
BA67	GGA GCA TGG GAT GGC AGC CTG CGT GAA GCG GTA	123
consensus:		
all	SRR SMR <u>KRK RRY</u> (GGC) ASS YWK CSK KRV RCG (GTA)	124
BA61+3+5+29+31	CAG CAA TAT ARC --- AGC TAT CCK TAC ACG	125
BA61+3+5	CAG CAA TAT AAC --- AGC TAT CCT TAC ACG	120
BA11+67	SRA SMR <u>KGK RAT</u> (GGC) ASS CTK CSK KRR RCG (GTA)	126
BA61+3+5+29+31+11	CAR CAR KRT ARY --- ASS YWT CCK TRS ACG	127
BA61+3+5+11	CAR CAR KRT AAY --- ASS YWT CCK TRS ACG	128

Thus, in a preferred embodiment, the polynucleotide of the present invention comprises the nucleotide sequence encoding the antigen-binding portion of an antibody heavy chain variable region, which comprises a VH CDR 1, which is encoded by the nucleotide sequence depicted in SEQ ID NO: 78, more preferably by the sequences depicted in SEQ ID NOs: 76, 77 and 79. Another preferred consensus nucleotide sequence is depicted in SEQ ID NO: 80. Most preferred for encoding VH CDR1 are the individual nucleotide sequences depicted in SEQ ID NOs: 74-77.

10

15

Also preferred in the present invention is a polynucleotide which comprises a

nucleotide sequence encoding the VH CDR1 depicted in the amino acid sequences SEQ ID NOs: 18-23, preferably 18-20.

5 In another preferred embodiment, the polynucleotide of the present invention comprises the nucleotide sequence encoding the antigen-binding portion of an antibody heavy chain variable region, which comprises a VH CDR 2, which is encoded by the nucleotide sequence depicted in SEQ ID NO: 87, more preferably by the sequences depicted in SEQ ID NOs: 84, 88 and 90. Another preferred consensus nucleotide sequence is depicted in SEQ ID NO: 89. Most preferred for VH CDR2 are
10 the individual nucleotide sequences depicted in SEQ ID NOs: 81-86. Also preferred in the present invention is a polynucleotide which comprises a nucleotide sequence encoding the VH CDR2 depicted in the amino acid sequences SEQ ID NOs: 24-34, preferably 24-29.

15 In another preferred embodiment, the polynucleotide of the present invention comprises the nucleotide sequence encoding the antigen-binding portion of an antibody heavy chain variable region, which comprises a VH CDR 3, which is encoded by the nucleotide sequence depicted in SEQ ID NO: 96, more preferably by the sequences depicted in SEQ ID NOs: 93, 97 and 99. Another preferred consensus
20 nucleotide sequence is depicted in SEQ ID NO: 98. Most preferred for VH CDR3 are the individual nucleotide sequences depicted in SEQ ID NOs: 91-95. Also preferred in the present invention is a polynucleotide which comprises a nucleotide sequence encoding the VH CDR3 depicted in the amino acid sequences SEQ ID NOs: 35-44, preferably 35-39.

25 In another preferred embodiment, the polynucleotide of the present invention comprises the nucleotide sequence encoding the antigen-binding portion of an antibody heavy chain variable region, which comprises a VL CDR 1, which is

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5 encoded by the nucleotide sequence depicted in SEQ ID NO: 105, more preferably by the sequences depicted in SEQ ID NOs: 103, 104 and 106. Other preferred consensus nucleotide sequences are depicted in SEQ ID NOs: 107-109. Most preferred for VL CDR1 are the individual nucleotide sequences depicted in SEQ ID NOs: 100-104. Also preferred in the present invention is a polynucleotide which comprises a nucleotide sequence encoding the VL CDR1 depicted in the amino acid sequences SEQ ID NOs: 45-54, preferably 45-49.

10 In another preferred embodiment, the polynucleotide of the present invention comprises the nucleotide sequence encoding the antigen-binding portion of an antibody heavy chain variable region, which comprises a VL CDR 2, which is encoded by the nucleotide sequence depicted in SEQ ID NO: 115, more preferably by the sequences depicted in SEQ ID NOs: 113, 114 and 116. Other preferred consensus nucleotide sequences are depicted in SEQ ID NOs: 117-119. Most preferred for VL CDR2 are the individual nucleotide sequences depicted in SEQ ID NOs: 110-114. Also preferred in the present invention is a polynucleotide which comprises a nucleotide sequence encoding the VL CDR1 depicted in the amino acid sequences SEQ ID NOs: 55-64, preferably 55-59.

20 In another preferred embodiment, the polynucleotide of the present invention comprises the nucleotide sequence encoding the antigen-binding portion of an antibody heavy chain variable region, which comprises a VL CDR 3, which is encoded by the nucleotide sequence depicted in SEQ ID NO: 124, more preferably by the sequences depicted in SEQ ID NOs: 122, 123 and 125. Other preferred consensus nucleotide sequences are depicted in SEQ ID NOs: 126-128. Most preferred for VL CDR3 are the individual nucleotide sequences depicted in SEQ ID NOs: 120-123. Also preferred in the present invention is a polynucleotide which

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comprises a nucleotide sequence encoding the VL CDR1 depicted in the amino acid sequences SEQ ID NOs: 65-73, preferably 65-68.

The polynucleotides may also comprise sequences which hybridize to a
5 complementary strand of the above mentioned nucleotide sequences or are a degenerate of the above mentioned nucleotide sequences.

In another preferred embodiment, the polynucleotide comprising a nucleotide sequence which encodes an antigen-binding polypeptide according to the invention,
10 which specifically binds to bacterial F4 (K88)-fimbriae, is selected from the group consisting of

- a) polynucleotides comprising a nucleotide sequence which encodes the amino acid sequence identified in SEQ ID NOs: 4, 5, 6, 7, 8, 9 or 10,
- b) polynucleotides comprising the nucleotide sequence identified in
15 SEQ ID NOs: 11, 12, 13, 14, 15, 16 or 17,
- c) polynucleotides comprising a nucleotide sequence which hybridizes to a complementary strand of the nucleotide sequence of a) or b),
- d) polynucleotides comprising a nucleotide sequence which is degenerate to a nucleotide sequence of c),
- 20 e) polynucleotides which represent a derivative, analogue, part or fragment of a nucleotide sequence of a), b), c) or d).

The terms "to hybridize" or "hybridization" describe the process by which a single-stranded polynucleotide enters into base-pairing with a complementary
25 polynucleotide strand. In the context of the present invention the term "hybridization" means a hybridization under conventional hybridization conditions, preferably under stringent conditions, for example as described in Sambrook et al.

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(1989), Molecular Cloning: A Laboratory Manual, 2. Ed., Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY.

Suitable stringent conditions include salt solutions of about 0.9 molar at temperatures
5 of 35°C to 65°C. Stringent hybridization conditions preferably comprise the following conditions:

Hybridization buffer:	7% SDS
	250 mM NaCl
10	250 mM K-phosphate buffer pH 7.0
	1 mM EDTA
Hybridization temperature:	58°C to 60°C
Hybridization time:	overnight
Washing buffer:	I 2 x SSC 0.1% SDS
15	II 0.2 x SSC 0.1% SDS
Washing temperature and time:	each 2 x 30 min at 55°C to 60°C

The degeneration of the genetic code offers one skilled in the art among other things
the possibility of adapting the nucleotide sequence to the codon preference of the
20 target host cell, thereby optimising the expression of the desired antigen-binding polypeptide of the present invention.

The above-mentioned polynucleotides also comprise fragments, derivatives, analogues or parts of the polynucleotide sequences described above, which code for a
25 polypeptide, particularly an antibody fragment, and especially a single-chain antibody, having the biological characteristics of a polypeptide which specifically binds to bacterial F4 (K88)-fimbriae. The fragments, derivatives, analogues or parts may also be both naturally occurring variations or mutations, wherein these mutations may have occurred naturally or have been introduced e.g. by targeted
30 mutagenesis. Moreover, the variations can further comprise synthetic sequences.

The polypeptide being encoded by the fragments, derivatives, analogues or parts of the polynucleotide sequences described above is preferably characterized by “conservative” amino acid substitutions, for instance substitution of one hydrophobic residue for another or the substitution of one polar residue for another. At certain
5 positions non-conservative substitutions are allowable.

The term “fragments” is to be understood as parts of the polynucleotide sequence that are sufficiently long to encode one of the described polypeptides. The term “derivative” in this context means that the sequences differ from the polynucleotide
10 sequences described above at one or several position(s), but have a high degree of homology to these sequences. Homology here means a sequence identity of at least 40%, particularly an identity of at least 60% or 70%, preferably of at least 80%, 82%, 84%, 86% or 88%, and particularly preferably of at least 90%, 92%, 94%, 96% or 98%. Variations from the nucleotide sequences described above may be caused, for
15 example, by deletion, substitution, insertion or recombination.

The proteins encoded by these polynucleotide sequences show a sequence identity to the amino acid sequences identified in SEQ ID NOs: 4, 5, 6, 7, 8, 9 or 10, respectively, of at least 40%, particularly an identity of at least 60% or 70%,
20 preferably of at least 80%, 82%, 84%, 86% or 88%, and particularly preferably of at least 90%, 92%, 94%, 96% or 98%. The differences to the above described polynucleotide sequences may be the result of, for example, deletion, substitution, insertion or recombination.

25 In order to determine the percentage of homology (= identity) between two amino acid or nucleotide sequences, the two sequences are aligned, and the amino acids or nucleotides at each position are compared. If one position within the sequences is occupied by the same amino acid or the same nucleotide, then the molecules at this

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position are homologous (= identical). The percentage of homology between the two sequences is a function of the number of common positions that are identical (i.e. homology = number of identical positions per total number of positions x 100).

- 5 The homology is calculated over the total amino acid or nucleotide sequence area. In order to compare different sequences a variety of programs based on different algorithms are available to the person skilled in the art. The algorithms of Needleman and Wunsch or Smith and Waterman provide especially reliable results. For the sequence alignments and comparisons, the programs "PileUp" (J. Mol. Evolution.,
10 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989: 151-153) or "Gap" and "BestFit" [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981)], which are enclosed in the GCG Software-Packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], can be used. The sequence homology values
15 mentioned above as percentages can be determined by means of the "Gap" program over the total sequence area with the following adjustments: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 und Average Mismatch: 0.000. These adjustments can be used as standard adjustments for sequence homology analyses.
- 20 The polynucleotides comprising the nucleotide sequence identified in SEQ ID NOs: 11, 12, 13, 14, 15, 16 or 17 may be altered by replacing the linker encoding nucleotide sequence (here: AAG CTT GAA GAA GGT GAA TTT TCA GAA GCA CGC GT, SEQ ID NO: 133) with any other desired linker encoding nucleotide sequence. Further, the nucleotide sequences encoding the heavy chain and the
25 nucleotide sequences encoding the light chain of SEQ ID NOs: 11, 12, 13, 14, 15, 16 or 17 may be combined with one another in any conceivable way.

Preferably, the polynucleotide of the present invention comprising a nucleotide sequence encoding the antigen-binding polypeptide, which specifically binds to bacterial F4 (K88)-fimbriae, is under control of regulatory sequences which provide for a specific transcription. This can be achieved by conventional cloning methods
5 (e.g. Sambrook et al. 1989, *Molecular Cloning: A Laboratory Manual*, 2. Ed., Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY). Thus, the present invention further provides a recombinant polynucleotide molecule, which comprises

- a) a promoter region,
- b) a polynucleotide according to the invention as described above, which
10 is operatively linked to the promoter region, and
- c) optionally, regulatory sequences operatively linked thereto, which may act as transcription, termination and/or polyadenylation signals, enhancer sequences and/or sequences coding for leader signals.

15 Using routine molecular biological techniques (see for example Sambrook *et al.*, *vide supra*) it is possible to prepare and to produce the desired constructs for the transformation of any prokaryotic or eukaryotic cell. Methods for cloning, mutagenesis, sequence analysis, restriction analysis and other biochemical/molecular biological methods are well known to the person skilled in the art and are
20 conventionally used for gene technological manipulation.

Thus, not only may suitable polynucleotide constructs containing the desired fusion of a promoter sequence and an antigen-binding polypeptide sequence of the present invention and optionally further regulatory sequences be produced, but rather the
25 person skilled in the art may further, if desired, introduce various mutations, deletions, fusions or insertions of natural or artificial nucleotide sequences into the nucleotide sequence encoding the antigen-binding polypeptide according to the invention using routine techniques. For the fusion of the constructs with each other

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adapters or linkers may be attached to the constructs where necessary. Additionally, appropriate restriction sites may be provided or removed using enzymatic and other manipulation techniques. This may result in the synthesis of a protein with altered biological properties, e.g. stronger binding properties (affinity, avidity, time period)
5 or higher stability, e.g. temperature stability, pH stability and/or stability against protease cleavage, or resulting in the synthesis of proteins with altered expression properties, e.g. at the transcription and/or translation level, or with altered purification capacities.

10 The present invention also refers to vectors comprising the polynucleotide which encodes the antigen-binding polypeptide of the present invention, particularly the antibody fragment and more particularly the single-chain antibody, which specifically binds to bacterial F4 (K88)-fimbriae. The term "vector" describes a polynucleotide which is used for introducing exogenous polynucleotides into host
15 cells. A vector contains a nucleotide sequence which encodes one or more polypeptides. Preferably the vector of the present invention is a phage display vector, a bacterial expression vector, a yeast expression vector, a fungus expression vector, an algae expression vector or a plant expression vector. Vectors which are able to control the expression of the genes which they contain are termed "expression
20 vectors".

In order to prepare the introduction of foreign genes in higher plants a variety of vectors are available which contain replicating signals for *E.coli* and a marker gene for selecting transformed bacterial cells. Examples for such vectors are pBR322,
25 pUC series, M13mp series, pACYC184, pBlueSfi and the like. The desired sequence may be introduced in the vector at a suitable restriction site. The resulting plasmid is then used for the transformation of *E.coli* cells. Transformed *E.coli* cells are cultivated in a suitable medium and then harvested and lysed, and the plasmid is

recovered. In order to characterize the produced plasmid DNA in general restriction analysis, gel electrophoresis and further biochemical and molecular biological methods are used as analytic method. After each manipulation step the plasmid DNA may be cleaved and the obtained DNA fragments may be linked to other DNA
5 sequences.

Several known techniques are available for introducing DNA in a plant host cell, and the person skilled in the art will not have any difficulties in selecting a suitable method. These techniques comprise the transformation of plant cells with T DNA by
10 use of *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as the transforming agent, the fusion of protoplasts, the direct gene transfer of isolated DNA into protoplasts, the electroporation of DNA, the introduction of DNA by means of the biolistic method as well as further possibilities.

15 During the injection and electroporation of DNA into plant cells no specific requirements for the used plasmids are necessary per se. The same is true for the direct gene transfer. Plain plasmids such as pUC and pBlueScript derivatives may be used. The presence of a selectable marker gene is necessary if entire plants are to be regenerated from such transformed cells. The person skilled in the art is familiar with
20 these gene selection markers and will not have any problems in selecting a suitable one.

Further DNA sequences may be required depending on the introduction method for desired genes into the plant cell. If, for example, the Ti or Ri plasmid is used for the
25 transformation of the plant cell, at least the right border, however more often both, the right and the left border of the T DNA in the Ti or Ri plasmid, has to be linked as flanking region to the genes to be introduced.

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If agrobacteria are used for the transformation, the DNA to be introduced has to be cloned into special plasmids, either into an intermediate or into a binary vector.

Intermediate vectors may be integrated into the Ti or Ri plasmid of the agrobacteria by homologous recombination via sequences which are homologous to sequences in the T DNA. This also contains the *vir* region which is required for T DNA transfer.

5

Intermediate vectors are not able to replicate in agrobacteria. With the aid of a helper plasmid the intermediate vector may be transferred to *Agrobacterium tumefaciens* (conjugation). Binary vectors are able to replicate in *E.coli* as well as in agrobacteria.

They contain a selection marker gene, and a linker or polylinker framed by the right and left T DNA border region. They may be transformed directly into agrobacteria.

10

The agrobacterial host cell should contain a plasmid with a *vir* region. The *vir* region is required for the transfer of the T DNA into the plant cell. Additional T DNA may be present. Such a transformed agrobacterial cell is used for the transformation of plant cells.

15

The use of T DNA for the transformation of plant cells has been studied intensively, and has been sufficiently described in generally known reviews and plant transformation manuals.

20

For transfer of the DNA into the plant cell, plant explants may be cultivated for this purpose together with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*.

From the infected plant material (e.g. leaf pieces, stem segments, roots, but also protoplasts or suspension-cultivated plant cells) whole plants may be regenerated in a suitable medium which may contain antibiotics or biocides for selection of

25

transformed cells. Regeneration of plants may take place according to conventional regeneration methods by use of known growth media. The resulting plants may be analysed for the presence of the introduced DNA. Other possibilities for the

introduction of foreign DNA by use of biolistic methods or protoplast transformation are well known, and as have been described extensively.

5 Once the introduced DNA has been integrated into the plant cell genome it is generally stable there and is maintained in the progeny of the originally transformed cell as well. Usually it contains a selection marker which allows the selection of transformed cells from those lacking the introduced DNA. This selection marker endows the transformed plant cells with resistance to a biocide or an antibiotic, such as kanamycin, G418, bleomycin, hygromycin, methotrexate, glyphosate,
10 streptomycin, sulfonylurea, gentamycin or phosphinotricin and others. But also nutritive markers and screening markers (such as GFP, green fluorescent protein) are being used as alternatives to antibiotics resistance markers. It is also possible to perform this selection procedure without any selection marker, however this requires a quite high screening expenditure.

15 The resulting plants may be cultivated normally, and may be crossbred with plants having the same transformed hereditary disposition or other predispositions. The resulting hybrids will have the pertinent phenotype characteristics. Seeds may be obtained from the plant cells.

20 Two or more generations should be generated to ensure that the phenotypic feature is stably maintained and inherited. Additionally, seeds should be harvested to verify the maintenance of the respective phenotype or other features.

25 In a preferred embodiment, the vector comprising the polynucleotide which encodes the antigen-binding polypeptide of the present invention, particularly the antibody fragment and more particularly the single-chain antibody, which specifically binds to bacterial F4 (K88)-fimbriae, contains specific regulatory elements, either constitutive

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or inducible (which may be either by biotic or abiotic stimuli), especially promoters, specific for the expression of the cloned construct in the prokaryotic or eukaryotic host cell, e.g. in bacteria, yeast or plant cells. For the expression of the polypeptides of the present invention in a plant cell or a microorganism cell, in principal any
5 promoter can be considered which is active in the respective host cells. The promoter may be homologous or heterologous to the cell to be transformed.

For plant expression, the promoter is preferably tissue specific, but it may also be development-specific. The promoter may e.g. be active in tissues that are used as
10 fodder such as seed, leaf and fruit tissues. The most preferred promoters for plant expression are seed specific promoters.

Useful promoters for seed specific expression are the USB promoter, preferably the long (Zakharov et al. 2004, J Exp Bot., Jul;55(402):1463-71. Epub 2004 Jun 4) or the
15 short USB promoter (Bäumlein et al. 1991, Mol. Gen. Genet. 225:459-467). Other promoters which can be used in the present invention are the NOS-promoter, the Vicilin promoter, the Phaseolin promoter, the Actin promoter, the Arcelin promoter, the Hordein promoter (Brandt et al. 1985, Carlsberg Res. Commun. 50:333-345) and the Napin promoter, the ACP promoter as well as the FatB3- and FatB4 promoters,
20 the 35S RNA promoter of the Cauliflower Mosaic Virus, the Ubiquitin promoter from maize, or the LeB4 promoter. The following publication summarizes most of the useful promoters: Zakharov et al. 2004, J Exp Bot., Jul;55(402):1463-71. Epub 2004 Jun 4.

25 Leader sequences may also be introduced into the vector, e.g. in order to achieve secretion of the polypeptides of the present invention into the medium. For example, the amino acid sequence "EAYA" is a cleavage site at the C terminus of a leader

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sequence. The "KDEL" sequence at the C terminus of a polypeptide sequence is an endoplasmatic reticulum (ER) retention signal in plants.

Useful promoters for the expression in microorganisms are e.g. the Lac
5 Operon/promoter, the T3/T7 promoter, and the AOX promoter.

These promoters are well known to a person skilled in the art of molecular biology. In any case the skilled person can find suitable promoters in the literature, e.g. in relevant scientific journals and gene databases, or can isolate them from any desired
10 organism using standard methods.

Another aspect of the present invention is to provide prokaryotic or eukaryotic host cells which comprise a polynucleotide encoding an antigen-binding polypeptide of the present invention or a vector comprising said polynucleotide. Preferably, the host
15 cells are stably or transiently transformed with the above-described vectors of the present invention. Still another aspect of the present invention is to provide transgenic plants or parts thereof as well as microorganisms containing an above-described polynucleotide or an above-described vector of the present invention.

20 Preferably, the host cells or the plants or plant parts or microorganisms of the present invention express and synthesize the antigen-binding polypeptide of the present invention. These host cells may be any prokaryotic or eukaryotic cells, preferably microorganismic cells or plant cells, more preferably bacterial, yeast, fungus, algae and plant cells. Most preferred among the microorganismic cells are *Escherichia coli*
25 cells, *Streptomyces* cells, *Pichia pastoris* cells, *Schizosaccharomyces* cells, especially *Schizosaccharomyces pombe* cells, *Aspergillus* cells, and *Chlamydomonas* cells.

The plant cells and plants of the present invention may be in principle any plant cells or plants. Preferably, it is a monocotyledonous or dicotyledonous crop plant and more preferably a fodder plant. The most preferred plants are a *Nicotiana spec.*, especially *Nicotiana benthamiana* and *Nicotiana tabacum*, *Pisum sativum*, *Hordeum*
5 *vulgare*, *Zea mays*, *Cucumis sativus* and *Linum usitatissimum* and rapeseed. Other plants which may be used are e.g. flax, poppy, olive, cocoa, barley, wheat, almond, sesame, mustard, castor oil plant, sunflower, soybean, peanut, coconut, turnip seed, cotton, cucumber, tobacco and oil palm trees.

10 The invention also relates to propagation material and agricultural products of the plants according to the invention, for example fruits, seeds, tubers, rootstocks, seedlings, cuttings and the like.

Further, the invention relates to the use of the plants, plant parts or microorganisms
15 of the present invention as fodder for mammals, especially for pigs.

This fodder preferably has a preventive or therapeutic effect on mammals, especially pigs, that are exposed to bacteria expressing F4 (K88)-fimbriae or that are already infected with those bacteria (see Example 11). The fodder, i.e. the plants, plant parts
20 or microorganisms according to the invention, can be fed exclusively or in combination with "normal" (wild type) fodder or any other fodder additive. The mixture can for example contain 1 %, 2 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 % or 90 % of the plants, plant parts or microorganisms according to the invention. It can be administered at every time of the day and over
25 any desired period of time to animals that are or are not infected with bacteria expressing F4 (K88)-fimbriae.

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Another object of the invention is to provide a method of producing an antigen-binding polypeptide of the present invention, particularly an antibody fragment and more particularly a single-chain antibody, which specifically binds to F4 (K88)-fimbriae, comprising:

- 5 - providing an expression vector which comprises a polynucleotide encoding said antigen-binding polypeptide, and
- expressing said vector in a prokaryotic or eukaryotic host cell.

The expression vector, the polynucleotide and the host cell used for this method are described above.

10

EXAMPLES

The following examples are intended to illustrate the present invention and are in no way to be understood as limiting.

5

Example 1: Generation of a phage display library

Fimbriae were isolated from an *E.coli* strain bearing F4 (K88) fimbriae, purified and separated on an SDS gel (fig. 1; molecular weight: 27,6 kDa). Mice were immunized with this antigen, Blood samples showed high antibody titers in the antigen ELISA. The spleens of the mice were prepared and RNA isolated therefrom. After mRNA purification single stranded DNA was prepared. DNA quality was tested by PCR amplification of the immunoglobulin genes. An aliquot of the cDNA was cloned into a phage display vector. The phage display library contained recombinant antibody fragments (scFv = single chain fragment variable).

For library screening the F4 antigen was immobilized on Maxisorp immunoplates and the phage display library was cycled three times for binding selection. Wells were blocked with BSA. Clones were selected randomly and assayed using a phage ELISA to compare binding to target antigen (F4 fimbriae) and several non-relevant proteins for preventing non-specific binding. Positive clones (only binding to F4) were further analyzed by restriction analysis, sequencing, western blot and ELISA. The following clones were isolated (table 9):

25 Table 9: scFv antibodies obtained from a screening with F4-fimbriae.
"Screened clones" are the clones which were analyzed by ELISA (576). 133 gave an ELISA signal up to 0,8 and 319 an ELISA signal up to 0,2. From the 576 clones analyzed by ELISA only 96 were further analyzed (protein expression, western blot, DNA extraction, restriction analysis). Unique scFv are scFv which have the same amino acid sequence. Max. 3 amino acids (10 amino acids) are different.

30

antigen	nomenclature	screened clones	positive clones ELISA >0,2 (>0,8)	analyzed clones	unique scFv > 3 amino acids (> 10 amino acids)	in cell culture positive tested medium (strong)
F4 (K88) <i>E.coli</i>	BAxx	576	319 (133)	96	35 (25)	6 (3)

Three of the isolated scFv antibodies against F4-fimbriae showed a strong neutralizing effect (BA61, BA3 and BA11). Among the antibodies tested “medium” are BA5, BA29, BA31 and BA67.

For the ELISA assay, Maxisorp-plates (Nunc) were coated over night at 4°C with 0,5 µg / well F4 antigen (in PBS pH 7,4). Plates were washed three times with PBS and blocked two hours at room temperature (RT). After three additional washes the BA11 antibody (blocked for 30min with 6% BSA-PBS at RT) was added for two hours. Plates were washed once with PBS/0,05%Tween and four times with PBS before adding the primary anti-His antibody (Sigma H1029) diluted 1:3000 in 2 % BSA-PBS for one hour at RT. After washing once with PBS/0,05%Tween and four times with PBS the secondary anti-mouse-HRP-conjugated antibody (Sigma A4312) diluted 1:8000 in 2% BSA-PBS was incubated for one hour at RT. Plates were washed again once with PBS/0,05%Tween and four times with PBS before TMB/H₂O₂ was added. After 30min the reaction was stopped by adding 1M sulfuric acid and the OD_{450nm} was measured. The cell culture assay is described in example 3.

20

Example 2: Expression of scFv antibodies

As expression systems (production systems) for the scFv antibodies *E.coli* and *Pichia pastoris* were used. Antibody binding peptides were cloned into specific expression vectors.

25

The production of the scFv BA11 in *Pichia pastoris* was optimized starting from a 20ml expression.

- 5 Expression rate: 25-50 mg/l culture medium
Purification loss: < 15%
Purity: > 95%
Time needed: maximum two weeks for pure protein
Storage stability: lyophilized and stored at 4°C for several months (most
10 probably longer)

Fig. 2A shows a 12% SDS-gel with a dilution series of scFv-BA11 produced in *Pichia pastoris*.

- 15 BA11 expression in *E.coli*: The BA11 was cloned into the pET16b vector (Novagen) and transformed into BL21 cells (Stratagene). Cells were cultured until $OD_{600} = 0,6$ at 37°C in LB-medium. After induction with 100µM IPTG cells were grown for one hour at 37°C in LB-medium resulting in the expression of the BA11 construct. Cells were harvested by centrifugation and analyzed on a 12% SDS-gel (fig. 2B).

20

Example 3: In vitro testing of antibodies (brush-border adhesion and adhesion-inhibition assay)

- A brush border adhesion test was established to assess the neutralizing effect of the
25 isolated scFv antibodies. Brush borders were isolated from the small intestine of pigs. Under standard conditions *E.coli* bearing F4 fimbriae attach to these brush borders. Antibodies were added to this system to measure their neutralizing effect on adhesion (Fig. 3). The strongest neutralization effect was shown by the scFv antibodies BA11, BA61 and BA3. None of the negative controls or antibodies
30 against other pathogens showed an inhibitory effect.

An *in vitro* brush-border adhesion assay was performed as described by Van den Broeck W. *et al.* (Vaccine. 1999 Apr 9;17(15-16):2020-9) and Dean-Nystrom E.A. *et al.* (Vet Microbiol. 1993 Oct;37(1-2):101-14). Subsequently after brush border
 5 isolation 4×10^8 F4ac⁺ *E.coli* were added to an average of 100 villi in 60µl PBS buffer in a microtiter plate (Nunc) and incubated for 2 hours at room temperature. Adhesion was examined by phase contrast microscopy at magnification x 600.

For adhesion-inhibition assay bacteria (4×10^8 F4ac⁺) were incubated 20min at room
 10 temperature with scFv antibody (serial dilutions, starting at 0,2mg/ml) before adding the brush-borders (100/60µl).

Table 10: ScFv antibodies tested in the brush border adhesion assay. BB= antibodies
 15 against F5-fimbriae, BC= antibodies against F41-fimbriae.

scFv	neutralization		controls	neutralization
BA 11	strong		no scFv	none
BA 61	strong		boiled BA 3	none
BA 3	strong		boiled BA 61	none
BA 5	medium		BB 2	none
BA 29	medium		BB 15	none
BA 31	medium		BC 14	none
BA 67	medium			none

Example 4: Modification of the BA11 linker

20 Two putative trypsin cleavage sites could be detected at the lysine and at the arginine position in the yol linker (KLEEGEFSEARV, SEQ ID NO: 129). In order to improve the stability of scFv BA11, the linker was modified: The lysin (K) was exchanged against isoleucin (I), arginin (R) against glycine (G) and the valin (V) against cystein (C). The resulting anti F4 scFv carries the designation myBA11 (BA11 with

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modified yol linker). The nucleotide and the amino acid sequence of myBA11 are depicted in SEQ ID NOs: 134 and 135.

In a second example the yol linker was replaced by a GS-linker (GGGGS)₂. The
5 resulting antiF4 scFv carries the designation gsBA11. The nucleotide and the amino acid sequence of gsBA11 are depicted in SEQ ID NOs: 136 and 137. In a third approach two BA11 scFv fragments were coupled by a “double” GS-linker to form a bivalent BA11 (bvBA11). The amino acid sequence of bvBA11 is depicted in SEQ ID NO: 138. For gsBA11 and myBA11 similar expression rates were obtained
10 in *E.coli* (up to 500µg/l).

Example 5: Stability studies

In order to examine the stability of the antibody fragments in the gastro-intestinal
15 (GI) tract from pigs the antibody fragments were incubated with contents from stomach and small intestine. After incubation samples were analyzed by Western Blot to quantify the remaining antibody fragments. Best results were obtained with bvBA11 and myBA11 where the antibody fragment was stable for up to 240 min (fig. 4). In further experiments milk powder and/or BSA (1%) were added to the
20 samples. In all cases this led to a stabilization of the antibody fragments in the GI probes.

Example 6: Encapsulation test

25 In order to delay the degradation of scFv BA11 in stomach material from feasted animals, acid-resistant capsules were used to achieve a delayed release of the antibody fragments. Conventional gelatin capsules were encased with a special liquid

(cellulose acetate phthalate, CAP). The more often the capsules were coated with this liquid, the longer the antibody fragment was protected from low pH values (fig. 5).

Example 7: Feeding study with antibody fragments against K88 (F4) fimbriae in
5 piglets

Post-weaning piglets were infected with a high dose of an *E.coli* strain bearing F4
fimbriae. The treatment group (five animals) received scFv BA11 with the feed. The
first dose was applied ca. 12 hrs before infection, thereafter the animals received a
10 dose of 10mg/kgBW/day, distributed over three daily feed applications, for five days.
Animals in the control group (seven animals) were infected without antibody
fragment administration. All animals were sacrificed on day 4 and on day 5
respectively.

15 Infection with a highly concentrated *E.coli* broth was successful in all animals as
shown by microbial examination of the feces. Clinical symptoms were observed only
in one animal of the antibody group and in four animals of the control group.

All sick animals in the control group showed symptoms either immediately after
20 infection or one day after whereas the one sick animal in the treatment group showed
effects only late, i.e. on day 3. This animal was the only one with reduced body
temperature, suggesting an increased sensitivity over all other animals included in
this study.

25 Macroscopic analysis of the feces showed a more consistent quality in the treatment
group (1/5 animals with lesser quality) compared to the control (6/7 animals with
lesser quality). This was confirmed by determination of the dry weight which also
showed marked higher values in the treatment group.

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These data demonstrate that the single-chain antibody, BA11, has a protective and also a curative effect on *E.coli* infections. The number of infected animals was lower in the treatment group as was the pre-symptomatic effect as demonstrated by the
5 analysis of fecal matter.

Another important aspect is the reduction of *E.coli* in the feces. While this is not directly correlated to the clinical aspects, it is of high importance for the spread of an infection in a population. A lower microbial burden means a lower infectious
10 pressure, i.e. the risk for infections of healthy animals in a stable by an infected one is markedly reduced by application of the antibody fragment.

Samples from feces, urine, blood, muscle and intestine lymph nodes were taken and subjected to ELISA assays. The antibody fragment could not be detected in any of
15 these samples.

In conclusion this infection trial with post-weaning piglets strongly indicates a preventative and also a curative effect of the orally applied single-chain antibody, BA11. Besides its direct effect on both clinical and pre-symptomatic symptoms,
20 BA11 lessened the *E.coli* burden in the feces of infected animals and was thus able to reduce the infectious load in a population. Moreover it is safe as no scFv could be detected outside the GI tract, either in the animal itself nor in the feces.

Example 8: Transient antibody fragment expression in plants

25

About 6 % scFv BA11 was purified from *Nicotiana benthamiana* from the total soluble protein after infection with *Agrobacterium* (MagnIcon®, IconGenetics GmbH, Halle, Germany). For protein extraction whole plants were used. Fig. 6

shows a freeze dried BA11 protein separated on a 12% SDS gel. After extraction the antibody fragment was tested by the brush border adhesion test. The results were similar to those obtained with BA11 expressed in yeast (fig. 7).

5 Example 9: Stable plant transformation

The gene for scFv BA11 was transformed into tobacco under control of either the short or the long USB promoter. 89 lines were produced. BA11 was detected in 68 % of the 39 lines transformed with the short USB promoter. Using the long USB
10 promoter only 10% were in the range of the detection limit. Five independent lines with the long and the short USB promoter are available which are being further characterized and propagated.

Example 10: Stability tests of the pea line R46

15

Pea seeds containing the scFv antibody BA11 were milled and the meal stored for 6 months at room temperature. Antibody fragment activity (binding to the F4 antigen) was determined by ELISA. Fig. 8 represents stability analysis of the pea line R46 after 6 months.

20

25mg pea powder of the line R46-18 (BA11 with long USB promoter) were incubated in 250µl stomach contents of pigs. The antibody fragment concentration in the pea was about 2µg BA11/mg pea. scFv BA11 was not degraded in the stomach fluid for up to 120min as shown by Western blot (fig. 9). Thus pea offers a distinct
25 protection for the antibody fragment in stomach fluid.

50mg flour of pea line R46-9/2 (BA11 with long USB promoter) were incubated with 100µl PBS buffer. At different time points the mixture was centrifuged and the

- 46 -

supernatant was analyzed by ELISA. The pellet was then incubated again with 100 μ l PBS. The procedure was repeated up to 240min. During incubation with the PBS buffer the antibody fragment was slowly released from the pea flour (fig. 10).

5 Example 11: Experimental infection of piglets with Enterotoxigenic *E. coli* (ETEC): effects of feeding with pea seeds containing antibody fragments against K88 (F4) fimbriae

10 Six post-weaning piglets were fed with a conventional starter diet containing 10% wild type (WT) pea seeds for 10 days. Starting one day before infection the treatment group (three animals) received a feed containing 10% pea seeds with scFv antibody BA11 instead of WT pea. On day 1 all piglets were infected with a high dose of an *E.coli* strain bearing F4 fimbriae. Infection was successful in all animals as shown by microbial examination of the feces. All animals were sacrificed and necropsied on
15 day 4 and day 5 after infection.

During infection feces samples were taken in regular intervals and assayed for dry weight, chloride, sodium, pH and excretion of the strain used for experimental infection. Microbiological examination was based on rectal swaps. Feeding
20 antibodies to experimentally infected piglets results in a lower rate of clinical symptoms, faster elimination of the applied *E. coli* strain and by increased fecal dry weight (Fig. 11).

After the trial organ samples were taken during necroscopy. In all samples analyzed
25 by ELISA assays (liver, spleen, muscle, heart, lymph nodes, tonsils, kidney, urine, feces and blood) no antibody could be detected.

- 47 -

In conclusion this animal study shows evidence that feeding antibodies to experimentally infected piglets has protective effects. Antibodies applied with the feed are solely confined to the GI tract and are not taken up systemically.

5

DESCRIPTION OF THE FIGURES

- Fig. 1: Purified *E.coli* F4-fimbriae on a 15% SDS gel.
- Fig. 2A: 12% SDS gel showing a dilution series of scFv-BA11 isolated from *Pichia*
5 *pastoris*.
- Fig. 2B: BA11 expressed in *E.coli* (12% SDS gel).
- Fig. 3: Brush border adhesion test. With scFv = neutralization; the fimbriae of the bacteria are not able to attach to the brush borders (left). Without scFv = Fimbriae-bearing *E.coli* attach to the brush borders (right).
- 10 Fig. 4: Stability of some BA11-variants in small intestine content.
- Fig. 5: Acid stability of gelatin capsules with increasing number of coatings.
- Fig. 6: Freeze dried scFv BA11 purified from *Nicotiana benthamiana* and separated on a 12% SDS gel.
- Fig. 7: Comparison of the inhibitory activity of scFv BA11 expressed in yeast and
15 scFv BA11 transiently expressed in tobacco.
- Fig. 8: Stability analysis of the pea line R46 after 6 months.
- Fig. 9: Stability of the pea line R46 after 1 to 120min in stomach fluid.
- Fig. 10: Release of the scFv antibody BA11 from pea flour in PBS at different time points.
- 20 Fig. 11: Faeces dry matter of piglets during animal trial.

CLAIMS

1. An antigen-binding polypeptide which specifically binds to F4 (K88)-
5 fimbriae.
2. The antigen-binding polypeptide of claim 1, which specifically binds to a polypeptide comprising the amino acid sequence of SEQ ID NOs: 1, 2 and/or 3.
- 10 3. An antigen-binding polypeptide of claims 1 or 2, comprising:
a first polypeptide comprising the antigen-binding portion of the variable region of an antibody heavy chain, and
a second polypeptide comprising the antigen-binding portion of the variable region of an antibody light chain.
- 15 4. The antigen-binding polypeptide of claim 3, which further comprises a peptide linker linking said first and said second polypeptides into a single-chain polypeptide.
- 20 5. An antigen-binding polypeptide of claims 3 or 4, wherein said variable region of an antibody heavy chain comprises a VH CDR1, wherein the VH CDR1 has the amino acid sequence depicted in SEQ ID NO: 21.
6. The antigen-binding polypeptide of claim 5, wherein the VH CDR1 has the
25 amino acid sequence depicted in SEQ ID NOs: 18, 19 or 20.
7. An antigen-binding polypeptide of any one of claims 3 to 6, wherein said variable region of an antibody heavy chain comprises a a VH CDR2, wherein the VH CDR2 has the amino acid sequence depicted in SEQ ID NO: 30.

8. The antigen-binding polypeptide of claim 7, wherein the VH CDR2 has the amino acid sequence depicted in SEQ ID NOs: 27, 31 or 33.
9. An antigen-binding polypeptide of any one of claims 3 to 8, wherein said
5 variable region of an antibody heavy chain comprises a VH CDR3, wherein the VH CDR3 has the amino acid sequence depicted in SEQ ID NO: 40.
10. The antigen-binding polypeptide of claim 9, wherein the VH CDR3 has the amino acid sequence depicted in SEQ ID NOs: 37, 41 or 43.
- 10
11. An antigen-binding polypeptide of any one of claims 3 to 10, wherein said variable region of an antibody light chain comprises a VL CDR1, wherein the VL CDR1 has the amino acid sequence depicted in SEQ ID NO: 50.
- 15
12. The antigen-binding polypeptide of claim 11, wherein the VL CDR1 has the amino acid sequence depicted in SEQ ID NOs: 48, 49 or 51.
13. An antigen-binding polypeptide of any one of claims 3 to 12, wherein said
20 variable region of an antibody light chain comprises a VL CDR2, wherein the VL CDR2 has the amino acid sequence depicted in SEQ ID NO: 60.
14. The antigen-binding polypeptide of claim 13, wherein the VL CDR2 has the amino acid sequence depicted in SEQ ID NOs: 58, 59 or 61.
- 25
15. An antigen-binding polypeptide of any one of claims 3 to 14, wherein said variable region of an antibody light chain comprises a VL CDR3, wherein the VL CDR3 has the amino acid sequence depicted in SEQ ID NO: 69.

16. The antigen-binding polypeptide of claim 15, wherein the VL CDR3 has the amino acid sequence depicted in SEQ ID NOs: 67, 68 or 70.
17. The single-chain antigen-binding polypeptide of claim 4 comprising the amino acid sequence of SEQ ID NOs: 4, 5, 6, 7, 8, 9 or 10.
18. An antigen-binding polypeptide of any one of claims 4 to 16, as far as they relate to a single-chain polypeptide, wherein said peptide linker is not susceptible to protease cleavage.
19. An antigen-binding polypeptide of any one of claims 4 to 16, as far as they relate to a single-chain polypeptide, wherein said peptide linker has an amino acid sequence of SEQ ID NO: 131.
20. An antigen-binding polypeptide of any one of claims 4 to 16, as far as they relate to a single-chain polypeptide, wherein said peptide linker is selected from the group consisting of a GS-linker, a yol-linker or a linker with the amino acid sequence of SEQ ID NO: 130.
21. A multivalent single-chain antigen-binding polypeptide, comprising two or more single-chain polypeptides of any one of claims 4 to 20, wherein the single-chain polypeptides are linked by a peptide linker.
22. The multivalent single-chain antigen-binding polypeptide of claim 20, wherein the peptide linker is a GS-linker.
23. An antigen-binding polypeptide of any one of claims 1 to 3 or 5 to 16, which is a Fab antibody fragment.

24. An antigen-binding polypeptide of claims 1 or 2, which is a non-immunoglobulin polypeptide.
- 5 25. A polynucleotide comprising a nucleotide sequence which encodes an antigen-binding polypeptide of any one of the preceding claims.
26. The polynucleotide of claim 25 comprising the nucleotide sequence of SEQ ID NOs: 11, 12, 13, 14, 15, 16 or 17.
- 10 27. A vector comprising the polynucleotide of claims 25 or 26.
28. The vector of claim 27 which is a phage display vector, a bacterial expression vector, a yeast expression vector, a fungus expression vector, an algae expression vector or a plant expression vector.
- 15 29. The plant expression vector of claim 28 comprising a seed specific promoter.
30. The plant expression vector of claim 29 wherein the promoter is the short or
20 the long USB promoter.
31. A host cell comprising a polynucleotide of claim 25 or 26 or a vector of any one of claims 27 to 30.
- 25 32. The host cell of claim 31, which is a bacterial cell, a yeast cell, a fungus cell, an algae cell or a plant cell.

33. The host cell of claim 32, which is an *Escherichia coli* cell, a Streptomyces cell, a *Pichia pastoris* cell, a Schizosaccharomyces cell, an Aspergillus cell, a Chlamydomonas cell, a *Nicotiana spec.* cell, a *Pisum sativum* cell, a *Hordeum vulgare* cell, a *Zea mays* cell, a rapeseed cell, a *Cucumis sativus* cell or a *Linum usitatissimum* cell.
5
34. Transgenic plants or parts thereof or microorganisms containing a polynucleotide of claim 25 or 26 or a vector of any one of claims 27 to 30.
- 10 35. An antigen-binding polypeptide of any one of claims 1 to 24, which neutralizes the attachment of F4 (K88)-fimbriae to enterocytes.
36. An antigen-binding polypeptide of any one of claims 1 to 24, which has a protective and/or a curative effect on infection of mammals with bacteria expressing
15 F4 (K88)-fimbriae.
37. A composition comprising the antigen-binding polypeptide of any one of claims 1 to 24, 35 and 36 and an acceptable excipient, carrier, buffer and/or stabilizer.
20
38. The composition of claim 37, wherein the excipient, carrier, buffer and/or stabilizer comprises a protein source, preferably BSA, milk powder and/or pea flour; micro-particles and/or gelatine capsules, which are preferably encased with a stabilizing liquid.
25
39. Use of an antigen-binding polypeptide of any one of claims 1 to 24, 35 and 36 for the manufacture of a medicament for the treatment and/or prevention of infections with bacteria expressing F4 (K88)-fimbriae.

40. Use of an antigen-binding polypeptide of any one of claims 1 to 24, 35 and 36 for detecting bacterial F4 (K88) fimbriae.
- 5 41. Use of the plants, plant parts or microorganisms of claim 32 as fodder for mammals.
42. Use according to claim 41, wherein the mammals are pigs.
- 10 43. A method of producing an antigen-binding polypeptide of any one of claims 1 to 24, 35 and 36, comprising:
providing an expression vector of any one of claims 27 to 30, and
expressing said vector in a host cell.

FIGURES

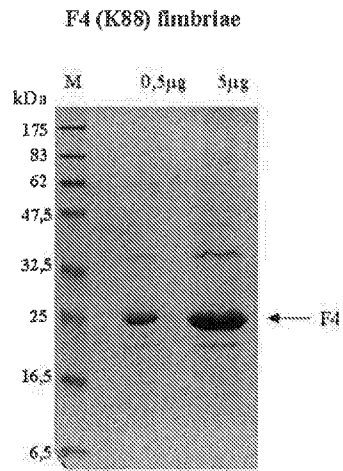


Fig.1

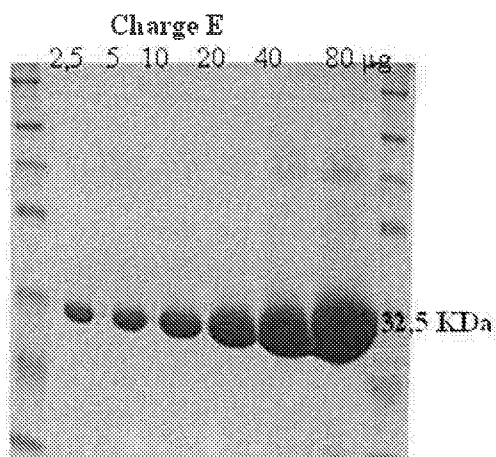


Fig. 2A

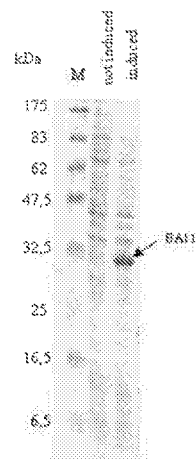


Fig. 2B

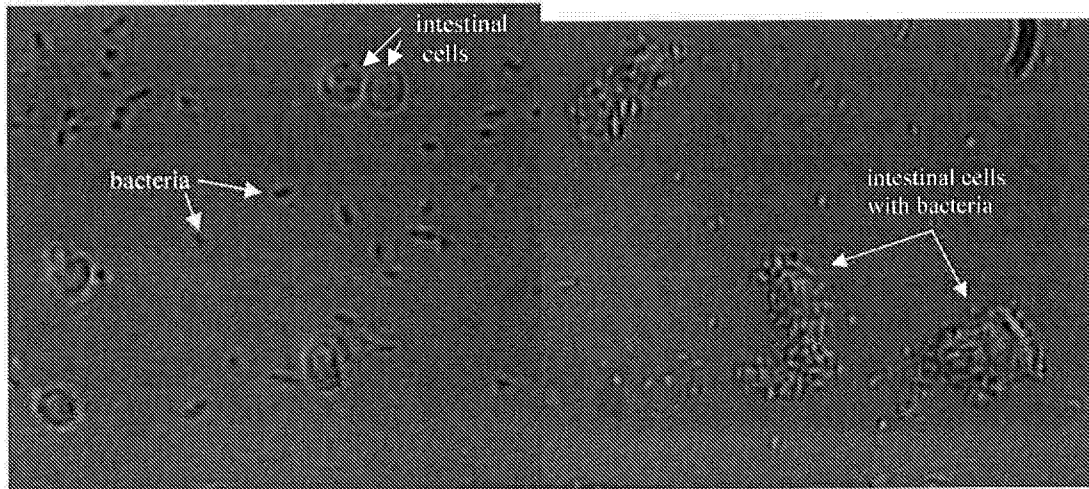


Fig. 3

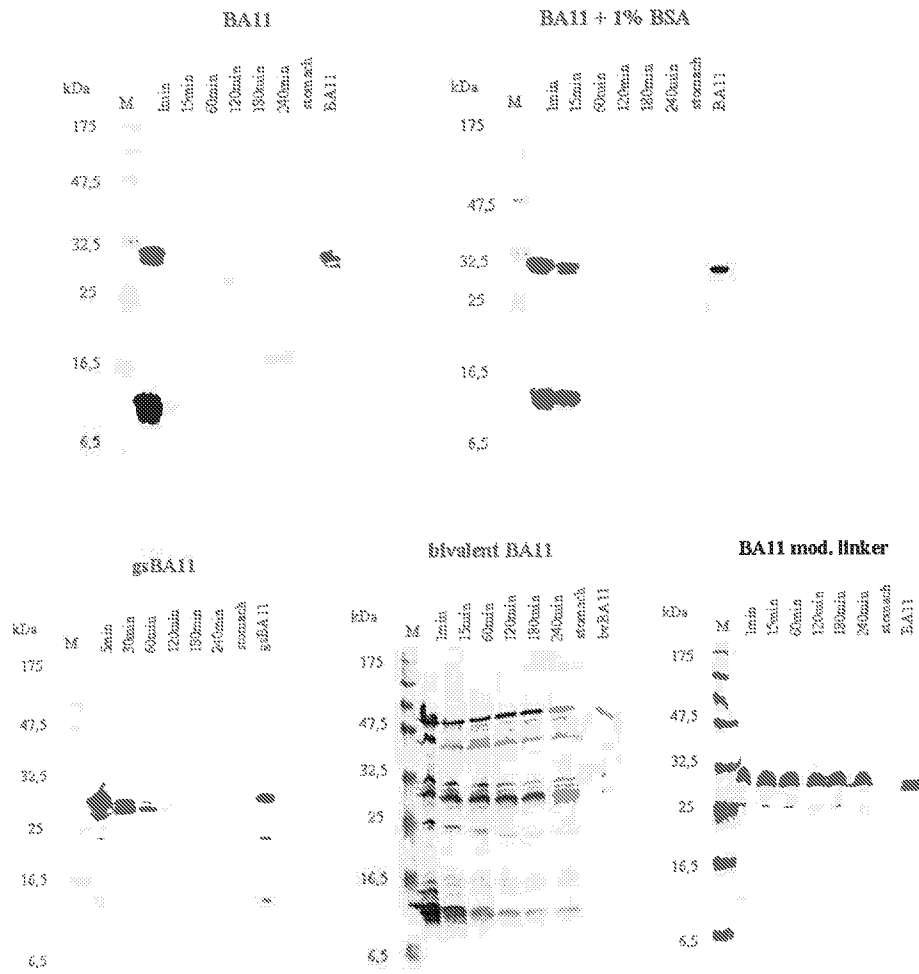


Fig. 4

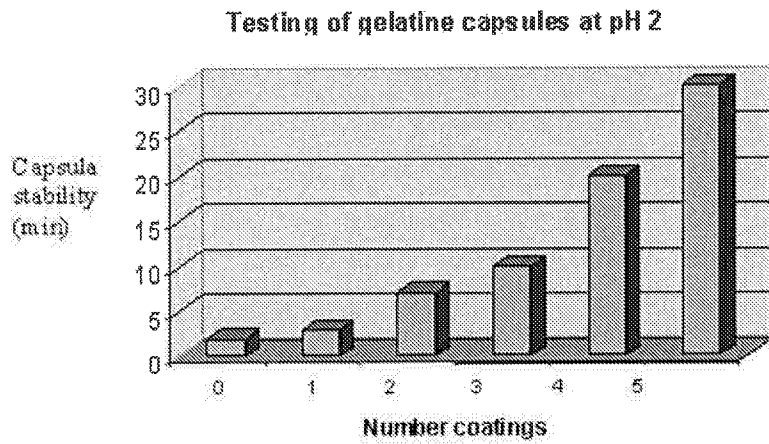


Fig. 5

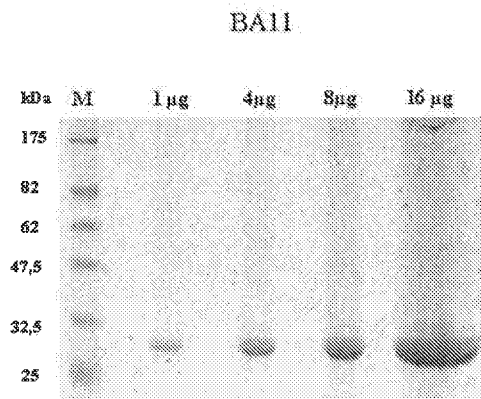


Fig. 6

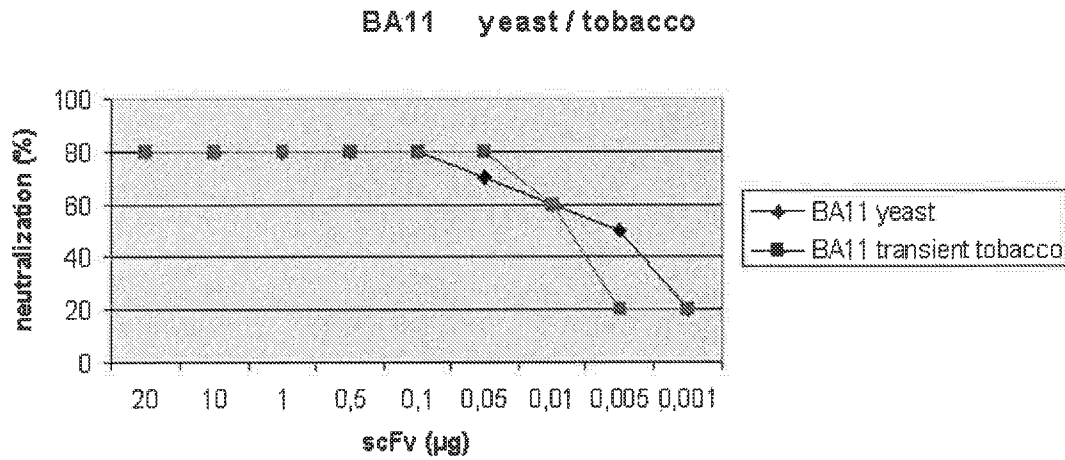


Fig. 7

**stability of the scFv antibody BA11 in pea flour
(6 month RT)**

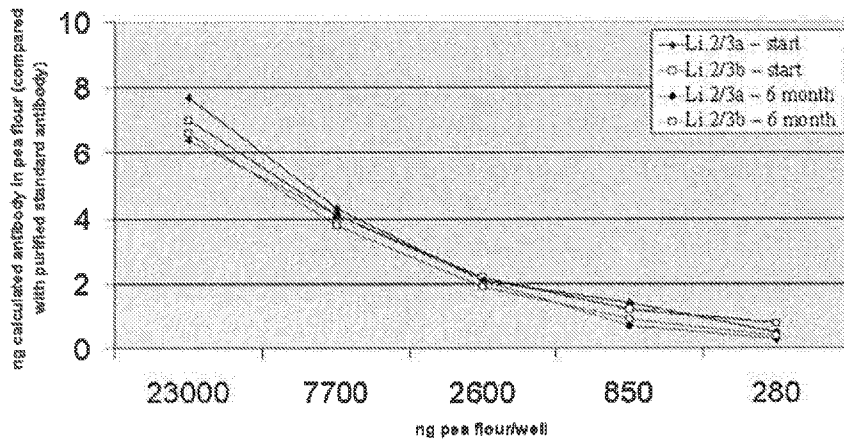


Fig. 8

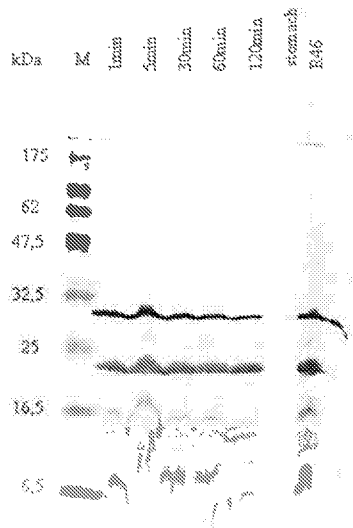


Fig 9

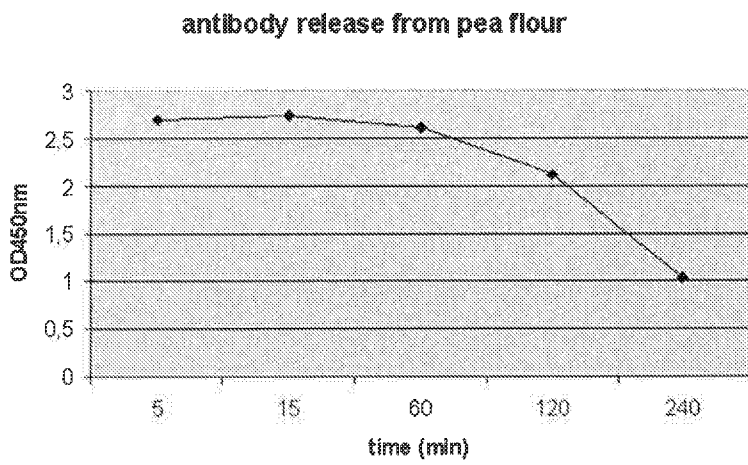


Fig. 10

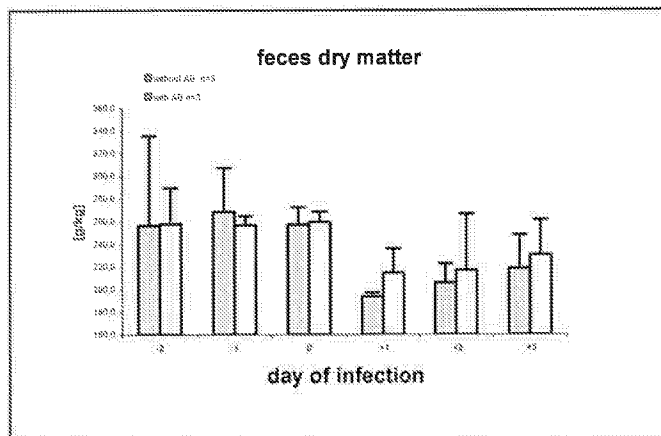


Fig. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/064514

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/12 C12N15/82 A61K39/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/074491 A (SYNETA PARTICIPATIONS AG; BROWN, DEVON; CAMPOS, MANUEL; DALMIA, BIPIN) 2 September 2004 (2004-09-02) page 4 - page 12 page 44	1-43
X	DE GEUS B ET AL: "Prevention of diarrhoea using pathogen specific monoclonal antibodies in an experimental enterotoxigenic E. coli infection in germfree piglets" VETERINARY QUARTERLY, vol. 20, no. SUPPL. 3, June 1998 (1998-06), pages S87-S89, XP009052434 ISSN: 0165-2176 the whole document	1-43

Further documents are listed in the continuation of Box C.

See patent family annex.

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Date of the actual completion of the international search

7 November 2006

Date of mailing of the international search report

07/12/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Wimmer, Georg

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/064514

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>VAN ZIJDERVELD F G ET AL: "EPITOPE ANALYSIS OF THE F4 K88 FIMBRIAL ANTIGEN COMPLEX OF ENTEROTOXIGENIC ESCHERICHIA-COLI BY USING MONOCLONAL ANTIBODIES" INFECTION AND IMMUNITY, vol. 58, no. 6, 1990, pages 1870-1878, XP002341332 ISSN: 0019-9567 the whole document</p> <p style="text-align: center;">-----</p>	<p>1-3, 31, 35, 36, 40, 43</p>

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2006/064514

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004074491 A	02-09-2004	AU 2004213554 A1	02-09-2004
		CA 2516286 A1	02-09-2004
		CN 1761754 A	19-04-2006
		EP 1597372 A2	23-11-2005
