



- (51) **International Patent Classification:**
C12Q 1/68 (2006.01)
- (21) **International Application Number:**
PCT/EP2016/053502
- (22) **International Filing Date:**
19 February 2016 (19.02.2016)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
62/129,293 6 March 2015 (06.03.2015) US
15174270.7 29 June 2015 (29.06.2015) EP
- (71) **Applicant (for all designated States except US):** **EVONIK DEGUSSA GMBH** [DE/DE]; Rellinghauser Straße 1-11, 45128 Essen (DE).
- (72) **Inventors; and**
- (71) **Applicants (for US only):** **IGWE, Emeka-Ignatius** [DE/DE]; Theresienstr. 120, 80333 München (DE). **WALLMEIER, Holger** [DE/DE]; CONDOR Scientific Computing & Consulting, Sossenheimer Weg 13, 65843 Sulzbach/Ts. (DE). **PELZER, Stefan** [DE/DE]; Ludwig-Uhland-Str. 33a, 33335 Gütersloh (DE). **FLÜGEL, Monika** [DE/DE]; Brandenburger Str. 34, 33803 Steinhagen (DE). **BEKEL, Thomas** [DE/DE]; Berghagen 21, 33790 Halle (Westf.) (DE).
- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

- (88) **Date of publication of the international search report:**
10 November 2016



WO 2016/142146 A3

(54) **Title:** METHOD OF DETECTING AVIAN NECROTIC ENTERITIS

(57) **Abstract:** The current invention relates to a microvesicle based method of detecting avian necrotic enteritis as well as to new markers which have been identified as suitable for detection of avian necrotic enteritis and/or avian infection by *Clostridium perfringens*.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/053502

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.
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)
C12Q

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 practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/012168 A1 (GHOSH ANIRBAN [CA]) 23 January 2014 (2014-01-23)	5,10,11
Y	paragraphs [0007], [0027], [0035], [0064], [0073] - [0074]; claim 28	1-3,6,12
Y	MCCOURT M T ET AL: "Sandwich ELISA detection of Clostridium perfringens cells and alpha-toxin from field cases of necrotic enteritis of poultry", VETERINARY MICROBIOLOGY, ELSEVIER BV, NL, vol. 106, no. 3-4, 10 April 2005 (2005-04-10), pages 259-264, XP004791652, ISSN: 0378-1135, DOI: 10.1016/J.VETMIC.2004.12.023 the whole document	1-3,6,12
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Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search 22 September 2016	Date of mailing of the international search report 06/10/2016
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Eveleigh, Anna

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2016/053502

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>ANTHONY L. KEYBURN ET AL: "Association between avian necrotic enteritis and Clostridium perfringens strains expressing NetB toxin", VETERINARY RESEARCH, vol. 41, no. 2, March 2010 (2010-03), page 21, XP055001233, ISSN: 0928-4249, DOI: 10.1051/vetres/2009069 page 1, left-hand column, paragraph 1 page 2, right-hand column, paragraph 2-3 page 7, right-hand column, paragraph 2</p> <p style="text-align: center;">-----</p>	4,6,11, 12
X	<p>WO 2008/148166 A1 (AUSTRALIAN POULTRY CRC PTY LTD [AU]; MOORE ROBERT JOHN [AU]; ROOD JULI) 11 December 2008 (2008-12-11) page 1, paragraph 3 page 2, paragraph 2 page 5, paragraph 3-8 claims 9-10 sequence 1 -& DATABASE Geneseq [Online]</p> <p>5 February 2009 (2009-02-05), "Clostridium perfringens NetB DNA SEQ ID:1.", XP002761707, retrieved from EBI accession no. GSN:AUJ86218 Database accession no. AUJ86218 sequence</p> <p style="text-align: center;">-----</p>	4,6, 11-14
X	<p>WO 2005/016962 A2 (GENENTECH INC [US]; ABBAS ALEXANDER [US]; CLARK HILARY [US]; OUYANG WE) 24 February 2005 (2005-02-24) page 8, paragraph 2 page 12, paragraph 2 claims 1,3; sequence 410 -& DATABASE Geneseq [Online]</p> <p>5 March 2009 (2009-03-05), "Human PRO nucleotide sequence SEQ ID NO:410.", XP002761706, retrieved from EBI accession no. GSN:AUZ24794 Database accession no. AUZ24794 sequence</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/--</p>	13,14

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/053502

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>DATABASE EMBL [Online]</p> <p>5 December 2012 (2012-12-05), "Clostridium perfringens strain NE_10 plasmid pNetB-NE10, complete sequence.", XP002761708, retrieved from EBI accession no. EM STD:JQ655731 Database accession no. JQ655731 sequence</p>	13
A	<p>-----</p> <p>J. S. SCHOREY ET AL: "Exosomes and other extracellular vesicles in host-pathogen interactions", EMBO REPORTS, vol. 16, no. 1, 5 January 2015 (2015-01-05), pages 24-43, XP055211224, ISSN: 1469-221X, DOI: 10.15252/embr.201439363 the whole document</p>	1-3,5,6, 10-12
A	<p>-----</p> <p>DATABASE WPI Week 201318 Thomson Scientific, London, GB; AN 2013-A47474 XP002744039, & CN 102 697 812 A (UNIV SHANDONG AGRIC) 3 October 2012 (2012-10-03) abstract</p>	1-3,5,6, 10-12
A	<p>-----</p> <p>M. G. WISE ET AL: "Quantitative Detection of Clostridium perfringens in the Broiler Fowl Gastrointestinal Tract by Real-Time PCR", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 71, no. 7, July 2005 (2005-07), pages 3911-3916, XP055232887, US ISSN: 0099-2240, DOI: 10.1128/AEM.71.7.3911-3916.2005 the whole document</p>	1-6, 10-12
A	<p>-----</p> <p>KERRY K COOPER ET AL: "Diagnosing clostridial enteric disease in poultry", JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, vol. 25, 2013, pages 314-327, XP055211280, the whole document</p>	1-6, 10-12
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/053502

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DION LEPP ET AL: "Identification of Novel Pathogenicity Loci in Clostridium perfringens Strains That Cause Avian Necrotic Enteritis", PLOS ONE, vol. 5, no. 5, May 2010 (2010-05), page e10795, XP055232293, DOI: 10.1371/journal.pone.0010795 the whole document</p> <p style="text-align: center;">-----</p>	2-4
A	<p>CATHY STAEDEL ET AL: "MicroRNAs and bacterial infection", CELLULAR MICROBIOLOGY, vol. 15, no. 9, 11 September 2013 (2013-09-11), pages 1496-1507, XP055211213, ISSN: 1462-5814, DOI: 10.1111/cmi.12159 the whole document</p> <p style="text-align: center;">-----</p>	2-5
A	<p>HUE DINH ET AL: "Modulation of microRNAs in two genetically disparate chicken lines showing different necrotic enteritis disease susceptibility", VETERINARY IMMUNOLOGY AND IMMUNOPATHOLOGY, vol. 159, no. 1-2, May 2014 (2014-05), pages 74-82, XP055211250, ISSN: 0165-2427, DOI: 10.1016/j.vetimm.2014.02.003 cited in the application the whole document</p> <p style="text-align: center;">-----</p>	2-5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/053502

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-6, 10, 13, 14(completely); 11, 12(partially)

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3, 5, 10(completely); 6, 11, 12(partially)

A method of detecting avian necrotic enteritis comprising detecting a marker in microvesicles from an avian sample.
A method of detecting an avian bacterial infection comprising detecting an avian miRNA marker in microvesicles from an avian sample.
A method of detecting an avian infection comprising detecting a marker in microvesicles from avian excrements.
Related method of treatment.

2. claims: 4(completely); 6, 11, 12(partially)

A method of detecting avian necrotic enteritis comprising detecting a specific sequence in an avian sample.
Related method of treatment.

3. claims: 7(completely); 11, 12(partially)

A method of detecting avian necrotic enteritis comprising detecting a marker in avian fecal excrements.
Related method of treatment.

4. claims: 8(completely); 11, 12(partially)

A method of detecting avian necrotic enteritis comprising detecting a marker in avian cecal excrements.
Related method of treatment.

5. claims: 9(completely); 11, 12(partially)

A method of detecting avian necrotic enteritis comprising detecting a marker in avian blood.
Related method of treatment.

6. claims: 13, 14

Polynucleotides comprising (the complement of) polynucleotides with at least 80% sequence identity to a polynucleotide comprising 100 consecutive nucleotides of one of SEQ ID NOs:1, 3 and 7 or with at least 90% sequence identity to a polynucleotide comprising 20 consecutive nucleotides of one of SEQ ID NOs:1, 3 and 7
Related vector.

7. claim: 15

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Polypeptides comprising polypeptides with at least 80%
sequence identity to a polypeptide comprising 10 consecutive
amino acids of one of SEQ ID NOs:2, 14 and 15.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/EP2016/053502

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014012168	A1	23-01-2014	CA 2879337 A1 23-01-2014
			EP 2875348 A1 27-05-2015
			US 2015192571 A1 09-07-2015
			WO 2014012168 A1 23-01-2014

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			ES 2550685 T3 11-11-2015
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