

COST B28 Meeting
April 20-22, 2007, Plovdiv, Bulgaria



**COST ACTION B28
«ARRAY TECHNOLOGIES FOR BSL3 AND BSL4
PATHOGENS»**

**4th Management Committee and
WG1, WG2, WG3, WG4, WG5 Meetings**

April, 20 - 22, 2007

Meeting Venue:

**Novotel Plovdiv
Plovdiv, Bulgaria**

Local Organiser:

**Todor Kantardjiev (kantardj@ncipd.netbg.com)
National Center of Infectious and Parasitic Diseases
Department of Microbiology**

**Stefan Panaiotov (spanaiotov@yahoo.com)
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COST B28 Meeting
April 20-22, 2007, Plovdiv, Bulgaria

WORKING GROUP Meetings
20 April - Friday

17.30 - 17.45

Registration, welcome and Opening of the WG meetings

Plenary lecture: 17.45- 18.45

Janusz Paweska: Current advances in CCHFV and RVFV research

19.00 Common Dinner organised by local organiser

21 April - Saturday

Plenary lecture – 9.30-10.30

Ignacio Lopez-Goni: Construction and validation of an ORFeome-based *Brucella* DNA microarray

Coffee break 10.30 – 11.00

Working Group 1 : Array technologies

11.00-12.30

Intoduction by the WG Chair. J. Schrenzel

Presentation of the partners in WG1

- Presentation of the progress on the WG 1 Booklet
- Discussion on the collaboration scheme
- J.Kieboom, I.A.Voskamp, M.P.Broekhuijsen: Genomotyping of *Brucella*
- Antoine Huyghe, Patrice Francois, Eve-Julie Sarret, Yvan Charbonnier, Jacques Schrenzel: Microarray concepts for reliable bacterial pathogen identification applied to biological threats diagnosis.
- Erhan Piskin, Bora Garipcan, Gokhan Demirel, Memed Duman, Oguzhan Caglayan, Sinan Egri: Patterning and immobilization techniques for array platforms/technologies
- Karen Kempself, N. Silman, R. Vipond: DNA microarrays and routine laboratory diagnostics: Applications and altercations.
- Ingmar Janse: DNA microarrays for the detection and typing of biothreat agents
- Henrik Nordstrom, Kerstin Falk, Elina Rintala, Annelie Walden, Norbert Nowotny, Peter Nilsson, Ake Lundkvist : Technique for detection and identification of emerging viruse

WG1 Participants to reimburse:

1.	Henrik NORDSTROM	Sweden	To be reimbursed as WG1 member
2.	Patrice FRANCOIS	Switzerland	To be reimbursed as WG1 member
3.	Erhan PISKIN	Turkey	To be reimbursed as MC member
4.	Sinan EGRI	Turkey	To be reimbursed as WG1 member
5.	Jacques SCHRENZEL	Switzerland	To be reimbursed as MC member
6.	Antoine HUYGHE	Switzerland	To be reimbursed as WG1 member
7.	Jasper KIEBOOM	The Netherlands	To be reimbursed as WG1 member
8.	Tanja KOSTIC	Austria	To be reimbursed as WG1 member
9.	Karen KEMPESELL	UK	To be reimbursed as WG1 member
10.	Jean Luc GALA	Belgium	To be reimbursed as MC member
11.	Dimitrios FRANGOULIDIS	Germany	To be reimbursed as MC member
12.	Ruud BUSKER	The Netherlands	To be reimbursed as MC member

Working group 2: Antigenicity

12.30-13.30

Presentations of the partners in WG2

Introduction of the WG2 Chair: Claude Muller

- Discussion on the collaboration scheme
- Claude P. Muller: The spread and evolution of highly pathogenic Avian influenza (HPAI) H5N1 in Africa
- Fred Fack : Virus – host cell interactions investigated with gel based differential proteomics, ECL Plex western blot analysis and confocal imaging
- Karl Walravens, P. Michel, D. Desqueper, C. Didembourg, J. De Vriese, M. Zygmunt, D.Fretin, A. Cloeckart, J.Letesson, J. Godfroid : Typing of Brucella suis by the help of O-Polysaccharide specific monoclonal antibodies.

WG2 Participants to reimburse

1.	Claude MULLER	Luxemburg	To be reimbursed as MC member
2.	Fred FACK	Luxemburg	To be reimbursed as MC member
3.	Karl WALRAVENS	Belgium	To be reimbursed as WG 2 member

Lunch 13.30 – 14.30

Working group 3: Proteomics and glycomics

14.30-15.30

Presentations of the partners in WG 3

Introduction by the WG3 Chair Juri Stulik:

- Juraj Lenco, Martin Habalek, Marek Link, J. Stulik, A. Macela,: Application of quantitative proteomics in the study of stress responses in *F.tularensis*
- L. Skultety, L. Hernichova, E. Bereghazyova, K. Slaba R. Toman: Identification of biomarkers of *Coxiella burnetii* isolates using a mass-spectrometric approach.
- R. Toman, P. Vadovic, M. Fodorova, L. Skultety: Structural characterization of lipid A, the endotoxic center of *Piscricickettsia salmonis*

WG3 Participants to reimburse

1.	Jiri STULIK	Czech Republic	To be reimbursed as MC member
2.	Ales MACELA	Czech Republic	To be reimbursed as MC member
3.	Juraj LENCO	Czech Republic	To be reimbursed as WG3 member
4.	Rudolf TOMAN	Slovakia	To be reimbursed as MC member
5.	Ludovit SKULTETY	Slovakia	To be reimbursed as WG3 member
6.	Pavol VADOVIC	Slovakia	To be reimbursed as WG3 member

Coffee break 15.30 – 16.00

Working Group 4: Genomics

16.00 – 16.00

Presentations of the partners in WG 4

Introduction by the WG4 Chair: Stefan Panaiotov:

- Discussion on the collaboration scheme
- I. Ivanov, T. Kantardjiev. P. Padeshki, R. Nenova, S. Panaiotov, V. Levterova: MLVA typing of Bulgarian *F. tularensis* outbreak isolates
- Werner Ruppitsch : Austrian Agency for Health and Food Safety: Current work on bioterrorism relevant bacterial pathogens.
- Horacio Gil, Raquel Escudero: Simultaneous detection of *Bacillus anthracis*, *Yersinia pestis* and *Burkholderia* spp by multiplex PCR combined with Reverse Line Blot
- Anders Johansson: Analysis of canonical insertion-deletion markers for safe and rapid DNA-based typing of *Francisella tularensis*

WG4 Participants to reimburse

1.	Werner RUPPITSCH	Austria	To be reimbursed as WG4 member
2.	Horacio GIL	Spain	To be reimbursed as WG4 member
3.	Raquel ESCUDERO	Spain	To be reimbursed as MC member
4.	Stefan PANAIOTOV	Bulgaria	Not to be reimbursed
5.	Ivan IVANOV	Bulgaria	Not to be reimbursed
6.	Ingmar JANSE	Netherlands	To be reimbursed as WG4 member
7.	Ignacio LOPEZ-GONI	Spain	To be reimbursed as WG4 member
8.	Anders JOHANSSON	Sweden	To be reimbursed as WG4 member
9.	Nadia BRANKOVA	Bulgaria	Not to be reimbursed

Working group 5: Microbiology (Bacteriology, Mycology and Virology)

17.00 – 18.00

Presentations of the partners in WG 5

Introduction by the WG5 Chair: Mandy Elschner:

- Discussion on the collaboration scheme
- Manfred Weidman: Presentation of the training school
- P. Butaye - Common database for microorganisms
- Rumiana Nenova: Brucellosis - reemerging infection in Bulgaria
- Paola Pilo: Antibiotic resistance profile of Francisella tularensis in Switzerland
- Manfred Weidmann: Development of rapid field diagnostics for identification, control and management of hemorrhagic fever outbreaks (EU FP6-INCODEV3, VHF Diagnostics)
- Plamen Padeshki: Expresson library immunization and revealing of immunodominant determinants in F. tularensis

WG5 Participants

1.	Todor KANTARDJEV	Bulgaria	Not to be reimbursed
2.	Plamen PADESHKI	Bulgaria	Not to be reimbursed
3.	Mandy ELSCHNER	Germany	To be reimbursed as MC member
4.	Janusz PAWESKA	South Africa	To be reimbursed as invited expert
5.	Rumiana NENOVA	Bulgaria	Not to be reimbursed
6.	Patrick BUTAYE	Belgium	To be reimbursed as MC Chair
7.	Gernot SCHMOOCK	Germany	To be reimbursed as WG5 member
8.	Laura BRUM	Portugal	To be reimbursed as MC member
9.	Manfred WEIDMANN	Germany	To be reimbursed as MC member
10.	Paola PILO	Switzerland	To be reimbursed as WG5 member
11.	Victoria LEVTEROVA	Bulgaria	Not to be reimbursed
12.	Sybren den HOOG	The Netherlands	To be reimbursed as MC member

19.00 - Common Dinner organised by local organiser

Sunday, 22 April

4th Management Committee Meeting

9.30-12.30

1. **Welcome**
Patrick Butaye, the Chair of COST ACTION B28 welcomed the participants to the Forth Management Committee Meeting.
2. **Adoption of the Agenda - P. Butaye**
3. **Adoption of the Minutes of the 3rd MC – Antalya - P. Butaye**
4. **Report of the Chair of the Working Group 1 - J. Schrenzel**
5. **Report of the Chair of the Working Group 2 - C. Muller**
6. **Report of the Chair of the Working Group 3 - J. Stulik**
7. **Report of the Chair of the Working Group 4 - S. Panaiotov**
8. **Report of the Chair of the Working Group 5 - M.Elschner**
9. **Annual report - P. Butaye**
10. **Discussion on the ever absent MC members – P. Butaye**
11. **Operation of COST B28 web site <http://www.cost-b28.be/> – P. Butaye**
12. **Organisation of the training school 2007 - Manfred Weidmann, Mandy Elschner**
13. **Central database of the bacterial, fungal and viral strain collections of all the Cost Action B28 members: P. Butaye**
14. **The FP7 Health Work Programme. Possibilities for collaborations P. Butaye**
15. **STSMs : current status. R. Toman**
16. **STSM possibilities offerend by COST. An explanation on what are STSMs and how you can obtain an STSM. P. Butaye**
17. **Discussion on the programme of the Brussels meeting of June 7 and 8**
18. **Discussion on the place and programme of next meetings**
19. **Any other business**

COST ACTION B28

“Array technologies for BSL3 and BSL4 pathogens”
Registered participants for the meeting 20-22, April 2007, Plovdiv, Bulgaria
Version: 17 January, 2008

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NON-COST COUNTRIES

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SOUTH AFRICA

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TOTAL LIST OF PARTICIPANTS

count	Name	Country	Status
1	Tanja KOSTIC	Austria	To be reimbursed as WG1 member
2	Werner Ruppitsch	Austria	To be reimbursed as WG4 member
3	Jean Luc Gala	Belgium	To be reimbursed as MC member
4	Karl Walravens	Belgium	To be reimbursed as WG 1 member
5	Frank Vandenbussche	Belgium	To be reimbursed as WG5 member
6	Patrick Butaye	Belgium	To be reimbursed as MC Chair
7	Jiri STULIK	Czech Republic	To be reimbursed as MC member
8	Ales MACELA	Czech Republic	To be reimbursed as MC member
9	Juraj Lenco	Czech Republic	To be reimbursed as WG3 member
10	Bruno Garin-Bastiju	France	To be reimbursed as MC member
11	Dimitrios Frangoulidis	Germany	To be reimbursed as MC member

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12	Mandy ELSCHNER	Germany	To be reimbursed as MC member
13	Gernot Schmoock	Germany	To be reimbursed as WG5 member
14	Manfred WEIDMANN	Germany	To be reimbursed as MC member
15	Maria Sarakallos	Greece	To be reimbursed as MC member
16	Claude MULLER	Luxemburg	To be reimbursed as MC member
17	Fred Fack	Luxemburg	To be reimbursed as MC member
18	Laura Brum (Jorge Macado)	Portugal	To be reimbursed as MC member
20	Rudolf TOMAN	Slovakia	To be reimbursed as WG3 member
21	Ludovit SKULTETY	Slovakia	To be reimbursed as WG3 member
22	Pavol VADOVIC	Slovakia	To be reimbursed as WG3 member
23	Janusz Paweska	South Africa	To be reimbursed as invited Speaker
24	Horacio Gil	Spain	To be reimbursed as WG4 member
25	Raquel Escudero	Spain	To be reimbursed as WG4 member
26	Ignazio Goni	Spain	To be reimbursed as WG4 member
27	Maria NICA	Romania	To be reimbursed as MC member
28	Henrik NORDSTROM	Sweden	To be reimbursed as WG1member
30	Anders Johansson	Sweden	To be reimbursed as WG4 member
31	Iuliana APOSTOL	Romania	To be reimbursed as WG5 member
32	Patrice Francois	Switzerland	To be reimbursed as WG1member
33	Jacques SCHRENZEL	Switzerland	To be reimbursed as MC member
34	Antoine Huyghe	Switzerland	To be reimbursed as WG1member
35	Paula Pilo	Switzerland	To be reimbursed as WG5 member
36	Ingmar Janse	The Netherlands	To be reimbursed as WG4 member
37	Martien Broekhuijsen	The Netherlands	To be reimbursed as WG1member
38	Rood Busker	The Netherlands	To be reimbursed as MC member
39	Sybren de Hoog	The Netherlands	To be reimbursed as MC member
40	Erhan Piskin	Turkey	To be reimbursed as MC member
41	Sinan EGRI	Turkey	To be reimbursed as WG2 member
42	Karen Kempzell	UK	To be reimbursed as WG1 member
1	Stefan Panaiotov	Bulgaria	Not to be reimbursed
2	Ivan IVANOV	Bulgaria	Not to be reimbursed
3	Todor Kantardjev	Bulgaria	Not to be reimbursed
4	Plamen PADESHKI	Bulgaria	Not to be reimbursed
5	Rumiana Nenova	Bulgaria	Not to be reimbursed
6	Victoria Levterova	Bulgaria	Not to be reimbursed
7.	Nadia Brankova	Bulgaria	Not to be reimbursed

TOTAL NUMBER OF PARTICIPANTS : 50

TOTAL NUMBER OF PARTICIPANTS TO REIMBURSE : 44 (including invited speaker)

INVITED SPEAKER

Dr hab. JANUSZ TADEUSZ PAWESKA

SOUTH AFRICA

SPECIAL PATHOGENS UNIT-NICD, SANDRINGHAM- JOHANNESBURG: PAST AND FUTURE ROLE IN RESEARCH AND DIAGNOSIS OF VIRAL HEMORRHAGIC FEVERS IN AFRICA WITH SPECIAL REFERENCE TO RIFT VALLY FEVER AND CRIMEAN- CONGO HEMORRHAGIC FEVER

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The sudden emergence of new viruses causing viral hemorrhagic fevers (VHF) in Africa in the late sixties prompted the development of maximum security laboratories. The term “viral hemorrhagic fever” characterizes a severe multisystem syndrome associated with fever, shock, and bleeding diathesis caused by infection with several RNA viruses from the *Arenaviridae*, *Bunyaviridae*, *Flaviviridae* and *Filoviridae* families. Many viruses causing VHF are classified as “biosafety level 4” agents with a high mortality rate, person-to-person transmission, potential aerosol infectivity, and absence of immunoprophylactic and chemotherapeutic measures. Although clinical management of VHF patient is primarily supportive, early diagnosis is vital to contain the contagion and implement public health measures, especially if agents are encountered out of their natural geographic coffin. Rapid travel by humans and animals within the incubation period of these viruses presents a considerable risk for introduction into non-endemic areas.

Differential diagnostic capacity for VHF worldwide is limited to selected reference laboratories; necessitating expensive and time consuming measures for safe international transportation of specimens which may results in delayed laboratory confirmation, pose the risk of specimens deteriorating and being misplaced. A biosafety level 4 laboratory (BSL-4) was built in 1979 at the National Institute for Virology (NIV), Sandringham-Johannesburg. In 1980, the Special Pathogens Unit

(SPU) was established at the NIV with the purpose of providing diagnostic and investigatory services for VHF in southern Africa. However, capacity for laboratory diagnosis of VHF in Africa still remains a major challenge for health communities.

Studies on the natural transmission cycles of VHF are often hampered by the unavailability of validated assays, including antibody, antigen and nucleic acid detection systems in candidate reservoir species, and lack of well established long-term surveillance studies in targeted human, animal and vector populations. To address some of these challenges, in collaboration with international partners, the SPU is investigating the potential of various nucleic acid, array-based, and recombinant diagnostic technologies which could be easily and cost-effectively employed in Africa including their deployment as portable field units. The SPU is also actively involved in ecological and epidemiological studies on VHF and in the development of new generation vaccines against VHF. The SPU's past and future diagnostic, development and research activities, and especially those related to Rift Valley fever and Crimean-Congo hemorrhagic fever will be reviewed and discussed.

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1. BIBLIOGRAPHICAL PARTICULARS

- 1.1 Surname:** Paweska
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1.4 Membership of Association

- 1.4.1** Polish Veterinary Science Association, 1983 -1991.
1.4.2 Polish Microbiology Association, 1984 - 1991.
1.4.3 Vet. Laboratory Diagnosticians Group of the South African Vet. Association, 1995 - 2001.
1.4.4 Southern African Society for Veterinary Epidemiology and Preventive Medicine 2005 -
1.4.5 Infectious Diseases Society of Southern Africa 2005 -

1.8 Professional Status

- 1.9.1** Registered as a veterinarian at the South African Veterinary Council: Registration No. 91/16.
1.9.2 Registered as Medical Biological Scientist at the Health Professions Council of South Africa: Registration No. MW 0007854

2. TRAINING AND QUALIFICATIONS

- 2.1** Completed university degree study in veterinary medicine, the Agricultural University, Faculty of Veterinary Medicine, Wroclaw, Poland, BVSc degree, 1982.
2.2 Completed post-graduate study and advanced research in veterinary medicine. Acquired DVSc-degree from the Faculty of Veterinary Medicine, the Agricultural University, Poland, 1989 on the ground of dissertation: "Studies of pathological and immunological properties and shedding of a modified Wroclaw-2 strain of equine arteritis virus".
2.3 Completed post-doctoral study and on the grounds of scientific attainments and the submitted habilitation dissertation on "Aspects of transmission, diagnosis and prophylaxis of orbivirus infections" admitted to the degree of doctor habilitatus in veterinary sciences in the field of epidemiology by the resolution of the Academic Board of the Faculty of Veterinary Medicine, the Agricultural University, Poland, 14 November 2006.

3. WORK EXPERIENCE

- 3.1** Researcher and academic lecturer, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Academy of Agriculture, Wroclaw, Poland: 1982-1991.
3.2 Veterinary Researcher, Department of Virology, Onderstepoort Veterinary Institute, Onderstepoort, Republic of South Africa: 25 June to 14 April 1996.
3.3 Senior Veterinary Researcher, Department of Virology, Onderstepoort Veterinary Institute, Onderstepoort, Republic of South Africa: 15 April 1996 to 31 August 1997.
3.4 Senior Veterinary Researcher, Departments of Biochemistry and Virology, Onderstepoort Veterinary Institute, Onderstepoort, Republic of South Africa: September 1997 - August 1999.
3.5 Assistant Director, Head of Department of Virology, Onderstepoort Veterinary Institute, Onderstepoort, Republic of South Africa: September 1999 - November 2001.
3.6 Chief Specialist Scientist, Special Pathogens Unit, National Institute for Communicable Diseases (former National Institute for Virology), Johannesburg, Sandringham, Republic of South Africa, December 2001 - December 2003.
3.7 Head of Special Pathogens Unit, National Institute for Virology, Johannesburg, Sandringham, Republic of South Africa, January 2004 - to date.

4. SPECIALIST FIELD

- 4.1** Viral diagnostics with special focus on development and validation of novel techniques for pathogen detection and discovery
4.2 Epidemiology of arboviruses and viral haemorrhagic fevers
4.3 Virus-host interactions

5. PRODUCTIVITY

5.1 Peer reviewed publications

- 5.1.1** KLIMENTOWSKI, S. & PAWESKA, J. (1987) The application of syncytial test for the detection of natural infections with bovine enzootic leukaemia virus. *Medycyna Weterynaryjna*, 9:556-558.

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- 5.1.2 KLIMENTOWSKI, S., PAWESKA, J. & KURYSZKO, J. (1989) The usefulness of the syncytial infectivity assay (SIA), the agar double diffusion test (AGID) and ultrastructural examination in the diagnosis of bovine enzootic leucosis (BEEL). *Medycyna Weterynaryjna*, 8:470-477.
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- 5.1.4 PAWESKA, J. (1991) Studies of pathological and immunological properties and shedding of a modified Wrocław-2 strain of equine arteritis virus. *Medycyna Weterynaryjna*, 47:55-57.
- 5.1.5 PAWESKA, J., GOLNIK, W., DZIK, W. & STARONIEWICZ, Z. (1991) Effectiveness of vaccinations in the course of enzootic equine viral abortions. *Medycyna Weterynaryjna*, 47:154-156.
- 5.1.6 STARONIEWICZ, Z., PAWESKA, J. & TORUN, T. (1991) Skuteczność unieszkodliwiania drobnoustrojów chorobotwórczych w oczyszczalniach ścieków typu BOS. *Gaz. Woda i Technika Sanitarna*. 8:170-171.
- 5.1.7 GOLNIK, W., PAWESKA, J. & DZIK, W. (1991) A stallion - a potential source of infection with infectious arteritis virus of horses. *Medycyna Weterynaryjna*, 47:459-461.
- 5.1.8 PAWESKA, J.T. & BARNARD, B.J.H. (1993) Serological evidence of equine arteritis virus in donkeys in South Africa. *Onderstepoort Journal of Veterinary Research*, 60:155-158.
- 5.1.9 PAWESKA, J.T., GERDES, T. & VAN HEERDEN, J. (1994) Serological relationship between a donkey alphaherpesvirus (Isolate M7/91) and equid herpesvirus type 1 and 4. *Journal of South African Veterinary Association*, 65:64-66.
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- 5.1.11 GOLNIK, W., PAWESKA, J. & DZIK, W. (1992) Dynamics of infections with equine arteritis virus (EAV) in the stud of horses being under the control for two years. *Medycyna Weterynaryjna*, 48: 52-54.
- 5.1.12 BARNARD, B.J.H. & PAWESKA, J.T. (1993) Prevalence of antibodies against some viruses in zebra (*Zebra burchelli*) in the Kruger National Park 1991-1992. *Onderstepoort Journal of Veterinary Research*, 60:175-179.
- 5.1.13 PAWESKA, J.T., VOLKMANN, D.H., BARNARD, B.J.H. & CHIRNSIDE, E.D. (1995) Sexual and in-contact transmission of an asinine strain of equine arteritis virus between donkeys. *Journal of Clinical Microbiology*, 33:3296-3299.
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- 5.1.16 PAWESKA, J.T., BINNS, M.M., WOODS, P.S.A. & CHIRNSIDE, E.D. (1997) A survey for antibodies to equine arteritis virus in donkeys, mules and zebra using neutralisation (VN) and enzyme linked immunosorbent assay (ELISA). *Equine Veterinary Journal*, 29:40-43.
- 5.1.17 PAWESKA, J.T. (1997) Failure to establish chronic infection of the reproductive tract of the male horse with a South African asinine strain of equine arteritis virus (EAV). *Onderstepoort Journal of Veterinary Research*, 64:17-24.
- 5.1.18 PAWESKA, J.T. (1997) Effect of the South African asinine-94 strain of equine arteritis virus (EAV) in pregnant donkey mares and duration of maternal immunity in foals. *Onderstepoort Journal of Veterinary Research*, 64:147-152.
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- 5.1.20 GOLNIK, W. & PAWESKA J. (1997) Equine arteritis virus (EAV) in pre-weaning foals. *Medycyna Weterynaryjna*, 53:654-656.
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- 5.1.23 VENTER, G.J., PAWESKA, J. T., WILLIAMS, R., & NEVILL, E.M. (1999) Prevalence of antibodies against African horse sickness and equine encephalosis in donkeys in the southern Africa. *Proceedings of the Eighth International Conference of Equine Infections Disease*, Dubai, United Arab Emirates, 23-26 March, 1998, p. 229-302.
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- 5.1.32 PAWESKA, J. T., SMITH, S.J., WRIGHT, I.M., WILLIAMS, R., COHEN, A.S., VAN DIJK, A.A., GROBBELAAR, A.A., CROFT, J.E., SWANEPOEL, R. & GERDES, G.H. (2003). Indirect enzyme-linked immunosorbent assay for the detection of antibody against Rift Valley fever in domestic and wild ruminants. *Onderstepoort Journal of Veterinary Research*, 70:49-64.
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- 5.1.36 PAWESKA, J.T., BURT, F.J., ANTHONY, F., SMITH, S.J., GROBBELAAR, A.A., CROFT, J.E., KSIAZEK, T. & SWANEPOEL, R. (2003). IgG-sandwich and IgM-capture enzyme-linked immunosorbent assay for detection of antibody to Rift Valley fever in domestic ruminants, *Journal of Virological Methods*, 113:103-112.
- 5.1.37 PAWESKA, J.T., PRINSLOO, S. & VENTER, G.J. (2003) Oral susceptibility of South African *Culicoides* species to live-attenuated serotype-specific vaccine strains of African horse sickness virus, *Medical and Veterinary Entomology*, 17:436-47.
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- 5.1.39 VENTER, G.J., GERDES, G.H., MELLOR, P.S. & PAWESKA, J.T. (2004) Transmission potential of South African *Culicoides* species for live-attenuated bluetongue virus. *Veterinaria Italiana*, 40, 198-203.
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- 5.1.48 LEROY, E.M., KUMULUNGUI B., POURRUT X., ROUQUET P., HASSANIN A., YABA P., DELICAT A., PAWESKA J.T., GONZALEZ J-P., SWANEPOEL R. (2005). Fruit bats as reservoirs of Ebola virus. *Nature*, 438, 575-576.
- 5.1.49 PAGAMJAV OCHIR, SAKATA TOHRU, IBRAHIM EL-SAYED M., SUGIMOTO CHIHIRO, TAKAI SHINJI, PAWESKA JANUSZ T., YAMAGUCHI TSUYOSHI, YASUDA JUN, FUKUSHI HIDETO (2005). Detection of novel gammaherpesviruses in wild animals of South Africa. *Journal of Veterinary and Medical Science*, 67, 1185-1188.
- 5.1.50 STADEJEK, T., MITTELHOLZER, Ch., OLEKSIEWICZ, M.B., PAWESKA J., BELAK, S. (2006). Highly diverse type of equine arteritis virus (EAV) from the semen of a South African donkey. *Acta Veterinaria Hungarica*, 54, 263-270.
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- SWANEPOEL, R., FELDMAN, H., JAHRLING, P.B., LIPKIN, W.I. (2006). MassTag polymerase chain reaction for differential diagnosis of viral hemorrhagic fevers. *Infectious Emerging Diseases*, 12, 692-695.
- 5.1.54** NIEDRIG, M., SONNENBERG, K., STEINHAGEN, K., PAWESKA, J.T. (2007). Comparison of ELISA and immunoassays for measurement of IgG and IgM antibody to West Nile virus in human sera against virus neutralization. *Journal of Virological Methods*, 139, 103-105.
- 5.1.55** ZHAI JUNHUI, PALACIOS GUSTAVO, TOWNER JONATHAN S., JABADO OMAR, KAPOOR VISHAL, VENTER MARIETJIE, GROLLA ALLEN, BRIESE THOMAS, PAWESKA JANUSZ, SWANEPOEL ROBERT, FELDMANN HEINZ, NICHOL STUART T., LIPKIN IAN W. (2007). Rapid molecular strategy for filovirus detection and characterization. *Journal of Clinical Microbiology*, 45, 224-226.
- 5.1.56** JANSEN VAN VUREN PETRUS, POTGIETER ABRAHAM C., PAWESKA JANUSZ T., VAN DIJK ALBIE A. (2007). Preparation and evaluation of a recombinant Rift Valley fever virus N protein for the detection of IgG and IgM antibodies in humans and animals by indirect ELISA. *Journal of Virological Methods*, 140, 106-114.
- 5.1.57** FATEINE JOSE MANUEL, TIJHAAR EDWIN, PAWESKA, JANUSZ T., NEVES LUIS C.B.G. HENDRINGS JUDITH, SWANEPOEL ROBERT, COETZER J.A.W., EGBERING HERMAN F., RUTTEN VICTOR P.M.G. (2007). Cloning and expression of Rift Valley fever virus nucleocapsid (N) protein and evaluation of a N-protein based indirect ELISA for the detection of specific IgG and IgM antibodies in domestic ruminants. *Veterinary Microbiology*, in press.
- 5.1.58** VENTER, G.J., KOEKEMOER J.J.O., PAWESKA, J.T. (2006) Investigations on African horse sickness outbreaks in the diseases surveillance zone of South Africa, *OIE Scientific and Technical Review*, in press.
- 5.1.59** VENTER G.J., MELLOR P.S., WRIGHT I., PAWESKA J.T. (2007). Replication of live-attenuated vaccine strains of bluetongue virus in orally infected South African *Culicoides* species, *Medical and Veterinary Entomology*, submitted.
- 5.1.60** LUDOLFS DIANA, LINCKH STEFAN, NIEDRICH MATTHIAS, PAWESKA JANUSZ T., SCHMITZ HERBERT (2007). Reverse ELISA for the detection of anti West Nile IgG antibodies in humans. *Clinical Microbiology and Infection*, submitted.
- 5.1.61** SABARZO A., PAWESKA J.T., HERRMAN S., AMIR T., MARKS R.S., LOBEL L. (2007). Optical fiber immunosensor for the detection of IgG antibody to Rift Valley fever virus in humans. *Journal of Virological Methods*, submitted.
- 5.1.62** BURT FELICITY J., PAWESKA JANUSZ T., SWANEPOEL ROBERT. Crimean-Congo hemorrhagic fever in South Africa (2007), submitted.
- 5.1.63** VENTER G.J., PAWESKA J.T. (2007). Virus recovery rates for wild-type and live-attenuated vaccine strains of African horse sickness virus serotype 7 in orally infected South African *Culicoides* species. *Medical and Veterinary Entomology*, submitted.
- 5.1.64** WÖLFEL ROMAN, PAWESKA JANUSZ T., PETERSEN NADINE, GROBBELAAR ANTOINETTE A. LEMAN PATRICIA A., HEWSON ROGER, GEORGES-COURBOT MARIE-CLAUDE, PAPA ANNA, GÜNTER STEPHAN, DROSTEN CHRISTIAN (2007). Virus detection and monitoring of viral load in Crimean-Congo hemorrhagic fever patients, submitted.

5.2 Scientific projects and programmes

5.2.1 Project & programme manager of the following projects

- 5.3.1.1 Project Manager: 1993-1995
"Equine viral arteritis in donkeys in South Africa" Joint ARC-OVI/ Animal Health Trust, Newmarket, UK.
- 5.3.1.2 Project Manager/Project local coordinator: 1996-1998
"Bluetongue viruses and *Culicoides* vectors in the USA and South Africa: Regional comparison" Collaborative research project with Arthropod-borne Animal Diseases Research Laboratory, Laramie, WY. Supported by a grant from US Department of Agriculture, Foreign Agricultural Service, Office of International Cooperative Development (Specific Cooperative Agreement No.58-5410-6-F108).
- 5.3.1.3 Project Manager: November 1997 to March 2000
"OIE Reference Laboratory for African horse sickness and bluetongue".
- 5.3.1.4 Project Manager: July 1998 to April 2001.
"Serodiagnostic Unit"
- 5.3.1.5 Programme Manager: 31st March 2000 -2001
"Epidemiology of arboviral diseases" It consisted of the following projects:
- OIE Reference Laboratories for African Horse Sickness, Bluetongue, Lumpy Skin Disease and Rift Valley Fever
- Molecular epidemiology
- Serological surveillance of viral diseases
- Veterinary geographical information
- Geographic and seasonal distribution of *Culicoides* species and their systematic
- Capacity of *Culicoides* as vectors of arboviral diseases in livestock
- 5.3.1.6 Project Manager/Project Local Coordinator: 2000 - 2001
"Development of safe, efficacious bluetongue vaccination strategy for Europe" Three-year international (UK, France, Greece, Bulgaria, RSA) collaborative research project funded by European Union (Contract No. QLRT-2000-01722).

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- 5.3.1.7 Project Manager: 2001
“Investigation of the 2000/2001 African horse sickness outbreak in the Eastern Cape Province”. Joint ARC-OVI/NDA ECP project founded by the National Department of Agriculture”.
- 5.3.1.8 Project Manager: 2003 - to date
“Novel immunodiagnostic for viral haemorrhagic fevers based on recombinant antibodies obtained from a semi-synthetic combinatorial library”, project funded by the Poliomyelitis Research Foundation.
- 5.3.1.9 Project Manager: 2005 – to date
“Cloning and expression of recombinant antigens of important and re-emerging viruses for the development of new diagnostic and research tools”, project funded by the Poliomyelitis Research Foundation.
- 5.3.1.10 Project Manager: 2005 – to date
“Loop-mediated isothermal amplification of RNA genomes for improved laboratory diagnosis and field response to outbreaks of viral haemorrhagic fevers”, project funded by the Poliomyelitis Research Foundation.

6. RECOGNITION

6.1 Awards and grants received for postgraduate study

- 6.1.1 In 1994 I was granted the UK Wellcome Trust’s fellowship award for supporting my collaborative research with the Animal Health Trust, New Market, England, on equine arteritis virus in the Republic of South Africa.
- 6.1.2 In 2001 I obtained grant award from the Department of Agriculture to investigate the 2001 outbreak of African horse sickness in the Eastern Cape, South Africa.
- 6.1.3 In 2003, 2005, 2006 I obtained grant awards from the Poliomyelitis Research Foundation for various research projects focusing on the development and validation of diagnostic immunoreagents and assays for diagnosis of viral haemorrhagic fevers.

6.2 Awards and distinction received from scientific organizations

- 6.2.1 Obtained the 1986 Group Award from the Agricultural Academy for display the research, teaching and training achievements of the Faculty of Veterinary Medicine commemorating the 40th Anniversary of Polish Science in Wroclaw.
- 6.2.2 Obtained the 1991 Individual Award from the Agricultural Academy in Wroclaw for achievements in research but principally for my DVSc dissertation:” Studies of pathological and immunological properties and shedding of a modified Wroclaw-2 strain of equine arteritis virus”.
- 6.2.3 In 1992 I was given the Annual Scientific Award from the Polish Association of Veterinary Sciences for research on equine viral arteritis.
- 6.2.4 Obtained the 1998 Agricultural Research Council Executive Management’s Award for excellent performance as a member of a multi-disciplinary team on diagnostic services.
- 6.2.5 Obtained the 1999 OVI’s Director Award.

6.3 Election to scientific societies, executive boards, advisory working groups

- 6.3.1 Member of Technical Committee of the SA Horse and Export Council 1998-2000.
- 6.3.2 Onderstepoort Veterinary Institute-ARC representative on African horse sickness for the South African Developing Countries (SADC) member’s states, from 1999 to 2001.
- 6.3.3 The Office International Des Epizooties (World Organization for Animal Health) designated expert on Bluetongue and African Horse sickness, 1999-2002.
- 6.3.4 Member of the Biological Weapons Working Committee (BWWC) of the South African Council for the Non-Proliferation of Weapons of Mass Destruction, from 2003.
- 6.3.5 Member of Global Outbreak Alert and Response Network Steering Committee, from 2004.
- 6.3.6 Member of International High Security Laboratory Network, from 2004.
- 6.3.7 Member of the Inter-Departmental Non-Proliferation and Arms Control Steering Committee, from 2005.

8. CURRENT DUTIES AT SPECIAL PATHOGENS UNIT, NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES, SANDRINGHAM-JOHANNESBURG

- Manage the operation of bio-safety level 4 laboratory (laboratory specialized particularly in research & diagnostic work on Ebola, Marburg, Hanta, Crimean Congo haemorrhagic fever & Rift Valley fever viruses).
- Training and overseeing staff in the operation at the maximum-security laboratory and in diagnostics methods for the highly hazardous haemorrhagic fever diseases.
- Assist and advise clinicians throughout South Africa in diagnosis and managing cases of viral haemorrhagic fever and other hazardous infections.
- Planning, executing, conducting and supervising research on the above-mentioned diseases.

COST B28 Meeting
April 20-22, 2007, Plovdiv, Bulgaria

- Development and validation of diagnostic and epidemiological tools.
- Quality control of in-house produced diagnostic kits and proficiency panels with special focus on kits for the diagnosis of Rift Valley fever and Crimean Congo haemorrhagic fever.
- Assisting in the planning, implementation, and co-ordination of a national strategy for the management of haemorrhagic fever cases.
- Training undergraduates and postgraduates.
- Advise and inform officials of the Department of Health, the media and the public as necessary on viral haemorrhagic fevers and rabies, particularly during outbreaks.
- Contribute to publication of research and diagnostic achievements.
- Refereeing of scientific articles and reviewing of research project proposals
- Contribution to the general and strategic management of the NICD

9.4 International Research Expeditions & outbreak response missions

- 9.4.1 Member of International Team for conducting ecological investigations at the scene of Ebola fever outbreaks in Ogooue-Ivindo Province, Gabon, February 2002.
- 9.4.2 Member of WHO team for controlling the 2005 Marburg disease outbreak in Uige Province, northern Angola, April-March 2005.
- 9.4.3 Invited by the Kenyan Ministry of Health and the Kenyan Medical Research Institute/CDC to assist in establishing diagnostic capacity for monitoring and controlling of the 2006-2007 RVF outbreak in Kenya, 02 – 12 January 2007.

INVITED SPEAKER

IGNACIO LÓPEZ-GOÑI

SPAIN

CONSTRUCTION AND VALIDATION OF AN ORFEOME-BASED *BRUCELLA* DNA MICROARRAY

Department of Microbiology and Parasitology,
University of Navarra, Pamplona, Spain.

The bacteria of the *Brucella* genus are responsible for one of the most common zoonosis worldwide called brucellosis. *Brucellae* belong to the α -proteobacteria group, are mainly intracellular pathogens and the molecular mechanisms of their virulence are still poorly understood. The complete genome sequences of three *Brucella* species (*B. abortus*, *B. melitensis* and *B. suis*) are now available and confirm the high degree of similarity of the genomic sequences and synteny among them. Using the complete genome sequence of *B. melitensis*, De Bolle and collaborators (1) have generated a database of protein-coding open reading frames (ORFs) and constructed an ORFeome library of 3258 Gateway Entry clones, each containing a defined ORF (ORFeomes are comprehensive collections of predicted coding sequences or ORFs of a given organism). This first version of the *Brucella* ORFeome provides the coding sequences in a user-friendly format amenable to high-throughput functional genomic and proteomic experiments. One of the multiples applications of the ORFeome is the generation of low-cost DNA microarrays by amplification of the ORFs with a single pair of primers, as all ORFs are inserted in the same vector (pDONR201).

The 3258 recombinant Gateway Entry clones were isolated and purified by the Plasmid Miniprep 96 System (Millipore); each *Brucella* ORF was amplified with the IQ SuperMix (Bio-Rad) using pDONR201 specific primers (attl1 5'-CAAGTTTGTACAAAAAAGCAGGC-3' and attl2 5'-CCACTTTGTACAAGAAAGCTGG-3'); PCR product was purified using the Montage PCR \square 96 Cleanup System (Millipore), and was visually scored

for presence, purity and size after agarose gel electrophoresis. Subsequently, the products were dried, resuspended in 50% DMSO, and arrayed into 384-well plates for printing. For the construction of the DNA microarray, the PCR products were printed in duplicated onto UltraGAPs Coated Slides (Corning Life Sciences) using MicroGrid II 610 Robotic System (Genomic Solutions). The microarray also included internal controls elements: negative control spots without DNA, negative controls with PCR-amplified *Arabidopsis thaliana* gene (*porB*, *protochlorophyllide oxidoreductase B*), and positive expression controls with PCR-amplified *Brucella* translation initiation factor IF-1 gene (constitutively expressed). The microarray has a total of 7680 spots and represents over 96.4% of the complete coding sequences assigned to *B. melitensis*.

Brucella mRNA for microarray analysis was purified. First, total RNA was extracted using RNeasy Mini System (Qiagen) in combination with the RNase-Free DNase Set (Qiagen) and the Protect Bacteria Reagent (Ambion). Then, *Brucella* mRNA was enriched using MICROBExpress Kit (Ambion), and antisense amino-allyl dUTP marked RNA was obtained by the MessageAmp II-Bacteria RNA Amplification Kit (Ambion), an ultra sensitive amplification system of bacterial mRNA for microarray analysis. Antisense RNA was labelled with Cy3 dye (Amersham Bioscience) and the microarrays were hybridised in standard conditions. Fluorescent images of the microarrays were generated by scanning the slides using a GenePix 4100A microarray scanner (Amersham Bioscience), and the results were normalised and statistically analysed by using the BRB-Array Tools software package. This microarray of PCR-amplified products was used to analyse the global genetic expression in *Brucella* strains with disruption of different regulatory genes. Each experiment included the information provided by three biological replicas and two technical replicas. Preliminary results will be presented.

(1) Dricot A, et al. 2004. Generation of the *Brucella melitensis* ORFeome version 1.1. Genome Res. 14(10B):2201-6.

GENOMOTYPING OF *BRUCELLA*

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Rapid biotyping of potential biological weapons such as *Brucella* is of great importance for protection against these agents. The efficacy of medical countermeasures is partly dependent on such rapid biotyping and identification. By means of molecular biology tools it is possible to identify the *Brucella* species, in some cases even to the biovar level. However, not all known biovars can be identified by means of a single rapid molecular biology method yet. To overcome this problem, whole genome microarray experiments were used to identify new genomic markers, in the genomotyping *Brucella*.

Genomotyping basically uses hybridization of the fluorescently labeled genomic DNA of a strain of interest to the microarray, along with the fluorescently labeled genomic DNA of a reference strain. The latter is typically the strain of which the genome sequence was employed to establish the array. By comparing the ratios of the signal intensities, obtained from the spots on the microarray, one can predict whether a given gene is present or 'divergent' in the strain of interest. Here, 'divergent' refers to either absence of a gene or to a gene that has poor hybridization properties. Thus, the genome of one organism is screened in reference to the genome of a chosen reference organism. A number of studies have investigated genome composition using DNA microarrays. Strain, such as *Francisella tularensis* [1], *Campylobacter jejuni* [2], *Helicobacter pylori* [5], *Staphylococcus aureus* [4], *Vibrio cholerae* [3], and *Streptococcus* [6], have been examined by the DNA microarray genomotyping technique.

The microarray used in our genomotyping study was designed and constructed on the basis of the *Brucella melitensis* 16M and *Brucella suis* 1330 genome sequence. The protocols for microarray spotting, labeling of genomic DNA, DNA hybridization, microarray washing, and

microarray scanning were optimized. In addition, some initial experiments were performed using several *Brucella suis* biovars. From these initial experiments, some unique oligo-DNA sequences could already be identified that allowed preliminary biotyping of *Brucella suis*, although, additional experiments are required to validate the presence of these markers in other *Brucella suis* strains.

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MICROARRAY CONCEPTS FOR RELIABLE BACTERIAL PATHOGEN IDENTIFICATION APPLIED TO BIOLOGICAL THREATS DIAGNOSIS

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CHARBONNIER Yvan, SCHRENZEL Jacques.

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We report the use of two novel microarray design approaches applied to the characterization of the genetic signature of three *Bacillus anthracis* strains.

The first microarray strategy is based on a hierarchical probe design where each of the ~10'000 probes matches a different level of the 16S phylogenetic tree (domain, phylum, class, order, family, genus, specie), for the large majority of bacterial pathogen, commensal and environmental strains. The second approach relies on a probe design scheme that allows targeting multiple organisms without having prior knowledge of their genomic content. This strategy named Non-Cognate Hybridization System (NCHS) is based on 13-mer probes providing all combinations of only two nucleotides. This oligonucleotide length provides an optimal ratio between the number of required NCHS probes and the number of probes actually able to discriminate different targets. Hybridization of 3 different *B. anthracis* strains using the aforementioned strategies yielded in a probe pattern common to these strains. Both approaches should prove useful in the rapid diagnosis of microorganisms potentially involved in bio-threats.

PATTERNING AND IMMOBILIZATION TECHNIQUES FOR ARRAY PLATFORMS/TECHNOLOGIES

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Abstract

The design and production of advanced array technologies, referred also “biochips”, (commonly known DNA- and protein chips) have attracted tremendous interest for the scientific and engineering communities because of the broad application of these materials especially in the life sciences, for detection of several targets of biologically originated. Patterning of substrate surfaces is one of the major steps for preparation of the arrays. Several techniques for surface patterning have been described in the related literature. Here, the basic information about soft lithography, photolithography, robotic printing (micro-spotting and ink-jet printing), and lithography with AFM (dip-pen lithography, conducting AFM lithography, nanoshaving and nanografting) is presented by also giving some interesting literature reports.

Immobilization of the probe molecules, or referred also as “bio-ligands” (e.g., oligonucleotides, oligopeptides/proteins, etc.) effectively and actively on the array platforms is one of the most important steps in array technologies. Glass slides, silicon wafers, gold-coated slides and gold particles, quantum dots, polymer coated slides, polymeric membranes/films/coatings and particles are utilized as substrates both in

surface- and suspension-based array platforms. Several physical and chemical techniques are applied immobilizations of probes onto these substrate surfaces, usually after a surface functionalization step. Here, both substrate materials and immobilization approaches are briefly described.

DNA MICROARRAY TECHNIQUE FOR DETECTION AND IDENTIFICATION OF EMERGING VIRUSES

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*Presenting author.

The microarray technique allows simultaneous screening for many different nucleic acid fragments based on hybridization between similar sequences in a sample and on a microarrayslide. This has a promising potential for development of broad tests for virus detection and identification. For emerging viruses, rapid and correct virus identification is important, making this type of screening assays useful. Virus species of interest belongs to Flaviviridae, Filoviridae, Arenaviridae, Bunyaviridae but also Influenza virus of Orthomyxoviridae. In addition to the multiplex capacity the microarray technique have a potential to better discover new strains with new sequence variation frequently encountered for RNA viruses.

We have combined amplification of viral nucleic acid from a sample and hybridisation to microarrays with virus-specific probes. Microarrays have been designed for Hantaviruses, Flaviruses, a group of hemorrhagic fever viruses and Influenza A virus. Different, more or less random strategies, for amplification of the sample material, were applied for the various assays. Both long 500-nucleotide probe fragments as well as short 50mer oligonucleotides were attached to the microarray slides.

The capacity of the long probe fragments to tolerate mismatch by new strains was evaluated on Hantaviruses and Flaviviruses while the combination of different probe lengths was tested for Human Influenza A virus. For flaviviruses the microarray method demonstrated a lower limit of detection comparable to standard RT-PCRs and it was successfully run

on samples from Dengue infected patients. A protocol for broad screening including random amplification allowed successful detection and identification of cellcultured Sin Nombre virus, Lassa virus, Crimean Congo Hemorrhagic Fever virus and Ebola Zaire virus.

The results are promising and the tests on clinical samples demonstrated the potential and usefulness of virus identification methods based on microarray technique.

THE SPREAD AND EVOLUTION OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) H5N1 IN AFRICA

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A.A. Owoade⁶

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In Africa HPAI H5N1 virus was first detected in Northern Nigeria and since then in 7 other African countries: Niger, Egypt, Cameroon, Burkina Faso, Côte d'Ivoire, Sudan and Djibouti. The study of the Nigerian-Luxembourg Poultry Virus Network at the University of Ibadan showed high seroprevalences for some poultry viruses but antibodies against AIV were not found in farms in the Southwest of Nigeria at least until May 2004. Phylogenetic analysis and substitution rates of complete genome sequences of Nigerian strains from the South-West and the North showed that three sublineages were present in Nigeria as early as February 2006 and that three independent introductions of H5N1 into the country are likely. (*MF Ducatez et al. Avian flu: multiple introductions of H5N1 in Nigeria. Nature 442, 37, 2006*). These three sublineages include all African strains and by now have a distinct geographic distribution: sublineage A (southwest Nigeria, Lagos, Niger), B (southwest Nigeria, Lagos, Egypt, Djibouti) and C (Northern Nigeria, Burkina Faso, Sudan, Côte d'Ivoire) within Africa. Probable non-African ancestors within the West-Asian/Russian/European lineage distinct from the Southeast Asian lineages were identified for each sublineage. In 2006 all reported human cases in Africa (Egypt, Djibouti) were caused by sublineage B with a distinct but poorly understood amino acid signature. In 2006, this sublineage B has been found also in at least one farm in/near Lagos. Genetic analysis will reveal whether the same strain caused the recent human case in Lagos. We have also characterized the first avian influenza

strains from Burkina Faso and the first strains from African wild birds (sublineage C). Between February and June 2006, 48 hooded vultures (*Necrosyrtes monachus*) were found dead or sick throughout Ouagadougou. We present here the first sequences from African wild birds and compare them to strain found in Burkina poultry. The sequences clustered with strains found in Ivory Coast, Northern Nigeria and The Sudan (sublineage C). We showed that the infection of scavenger birds in Africa is likely to cause spill-backs from poultry to wild birds, which is rarely seen in other countries and has important consequences for surveillance in Africa and beyond. As they scavenge on many dead species, they may also function as conspicuous sentinels in the African continent, similar to raptors or swans in Europe or cats in Indonesia. Proper disposal of infected carcasses must be carefully enforced on affected farms to avoid primary infections of carrion feeders.

**VIRUS - HOST CELL INTERACTIONS INVESTIGATED WITH GEL
BASED DIFFERENTIAL PROTEOMICS, ECL PLEX WESTERN BLOT
ANALYSIS AND CONFOCAL IMAGING.**

FACK, F., PIRROTTE, P., KREMER, J., REVETS, D., KESSLER, J.,
AMMERLAAN, W., and MULLER, C. P.

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Measles virus (MV) induced immune suppression is only partially understood and accounts for many complications caused by co-infections with other pathogens.

We investigated the cellular proteome of a human monocyte/macrophage cell line (THP1) infected by MV vaccine strain to better understand virus-host interactions and molecular mechanisms responsible for differences in virulence among different wild type viruses which need to be further characterized. We used a 2D-gel based comparative proteomics approach (2D-DIGE) based on the specific and differential fluorescence labeling of mock- and virus-infected protein extracts before co-electrophoresis in a same 2D gel. This method reduces experimental variations and ensures efficient identifications of biological variations in the compared proteomes. To apply this method to limited samples, we have used small 2D gels for DIGE studies. These gels were also used for the preparation of 2D Western blots, using a novel antigene detection system, based on fluorescence labeled secondary antibodies. (ECL Plex technology). The proportion of infected cells was monitored by flow cytometry of viral antigens on the host cells. Beyond 36 hours post-infection cell mortality and protein degradation of viral proteins increased. Differentially expressed protein spots were trypsin-digested and identified by MALDI-TOF mass spectrometry on an Ultraflex I TOF/TOF instrument (Bruker Daltonics). Based on these identifications the localization of cellular MxA and viral nucleoproteins was made by confocal microscopy and completed by quantitative PCR.

Our results illustrate that this 2D mini-gel based western blot applies to 1-10microgram protein extracts and that high resolution fluorescence imaging allows to exploit these dense protein patterns to yield information

on specific pathogen and host cell proteins, including information on the diversity of post-translational modified forms.

TYPING OF *BRUCELLA SUIIS* BY THE HELP OF O- POLYSACCHARIDE SPECIFIC MONOCLONAL ANTIBODIES

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The species *Brucella suis* is currently divided into five biovars, each having its own host preference, geographical distribution and virulence properties. They are subdivided in biovars, based on the sensitivity to dyes (thionine and basic fuchsine), agglutination with mono-specific sera, lysis by phages and H₂S production. These techniques are poorly standardized and needed relatively long analysis time. Cloeckaert and coworkers (Cloeckaert A, Weynants V, Godfroid J, Verger JM, Grayon, M, Zygmunt MS. Clin Diagn Lab Immunol. 1998 Nov;5(6):862-70) described the distinction between *Brucella suis* biovars according to their reactivity with a panel of O-chain specific monoclonal antibodies (Mabs) in ELISA. One Mab (12B12) was able to bind to type strains (species and biovars) and field strains representative of all the smooth *Brucella* species with the striking exception of *B. suis* biovar 2.

The analysis of a broad collection of *Brucella suis* strains, shows that all *B. suis* biovar 2 strains can be clearly distinguished from the other *B. suis* biovars. This differentiation was also confirmed via the classical phenotypic typing of *Brucella suis* biovars. This work confirms that reactivity with O-Polysaccharides specific Mab 12B12, can be used as an

additional tool for the unequivocal identification of *Brucella suis biovar 2 strains*.

Development of multiplex typing technique based on the use of these Mabs coupled on the surface of colored beads (Bead array) is under way and will be discussed.

APPLICATION OF QUANTITATIVE PROTEOMICS IN THE STUDY OF STRESS RESPONSES IN *FRANCISELLA TULARENSIS*.

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Pathogens encounter a number of different environments during their life. In order to survive and further proliferate the bacterial pathogens must regulate gene expression in response to the environment within the host organism. The phenotype adaptation is modulated using signals specific for host environment such as higher temperature or iron-sequestration. Modern proteomic technologies enable to investigate hundreds of proteins in one single experiment. That is why proteomics represents very suitable tool for studying differences of phenotype in pathogenic bacteria cultivated under conditions mimicking the host environment. Here we present two different technologies for investigating relationship of proteins to a particular environmental signal. Using classical approach (combination of two-dimensional gel electrophoresis and MALDI-TOF MS) we investigated protein profile of *Francisella tularensis* LVS grown in iron-restricted medium. In the second study we used iTRAQ quantitative technology and LC-MS/MS for searching of genes regulated by temperature shifts or nutrient starvation.

IDENTIFICATION OF BIOMARKERS OF *COXIELLA BURNETII* ISOLATES USING A MASS-SPETROMETRIC APPROACH

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Specific spectral markers were detected in aqueous acetonitrile extracts of the *Coxiella burnetii* isolates RSA 493, BUD, and Priscilla using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The measurements revealed noticeable differences in the ion profiles of the isolates in the mass range of 3-18 kDa. The number of characteristic ions for RSA 493, BUD, and Priscilla was 24, 15, and 7, respectively. The specific markers were compared against the *C. burnetii* database using the Tag-Ident proteomics tool. Eleven potential biomarkers for RSA 493, five for Priscilla, and three for BUD have been identified.

STRUCTURAL CHARACTERIZATION OF LIPID A THE ENDOTOXIC CENTER OF *PISCIRICKETTSIA SALMONIS*

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Lipid A from the *Piscirickettsia salmonis* lipopolysaccharide was investigated for its composition and structure using chemical analysis, gas chromatography/mass spectrometry, and electrospray ionization combined with the tandem mass spectrometry. It appears that at least two molecular species are present in the lipid A. Both share the classical backbone of D-glucosamine disaccharide in which one molecular species carries six 3-hydroxytetradecanoic fatty acids while the major one bears five 3-hydroxytetradecanoic fatty acids and one 3-hydroxyhexadecanoic fatty acid. The results have shown that the structural features of the investigated lipid A resemble the classical form of enterobacterial lipid A with a strong endotoxic potency. This fact could be one of the reasons for the observed high endotoxic activity of the *P. salmonis* bacterium.

**MULTIPLE-LOCUS VARIABLE-NUMBER TANDEM REPEAT
ANALYSIS (MLVA) FOR GENOTYPING OF *FRANCISELLA
TULARENSIS* AND ITS APPLICATION TO CLINICAL SPECIMENS**

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V. Levterova

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Key words: *Francisella tularensis*, multiple-locus variable-number tandem repeat analysis (MLVA),

More than 290 tularemia cases were registered during the outbreak in Pernik and Slivnica vicinities in Bulgaria in 1997-2005. The need for characterization of the isolated strains led to the development and introduction of appropriate high-resolution genotyping systems.

The typing methods based on the analysis of multiple variable tandem repeats (VNTRs) loci represent the most discriminatory ones and are particularly informative when explicitly monomorphic genomes are involved.

The hereby study describes a modification of a six-locus MLVA strategy and its application for typing of 30 *F. tularensis* strains. The six loci from each strain or clinical sample were amplified simultaneously in a single hexaplex-PCR. This modification significantly reduced the overall turnaround time and cost of the analysis.

An attempt for extending its typeability for clinical specimens was also performed with different types of samples from tularemia patients.



AUSTRIAN AGENCY FOR HEALTH AND FOOD SAFETY: CURRENT WORK ON BIOTERRORISM RELEVANT BACTERIAL PATHOGENS

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Introduction: As annexed within the Austrian law for Health and Food Safety § 8 (3) 7, the Austrian Agency for Health and Food Safety (AGES) has to provide for the laboratory infrastructure in crisis and emergency situations. The Agency has 3 BSL3 laboratories and a BSL4 laboratory is planned. Our duty in a bioterrorism event is the rapid identification of relevant dangerous bacteria. This includes also sequence analysis of the 16S rRNA genes of culture isolates. The aim of a recent study was to assess the usefulness of partial 16S rRNA gene sequence analysis and the suitability of diverse databases for identifying dangerous bacterial pathogens.

Methods and Results: For rapid identification purposes a 500 basepair fragment of the 16S rRNA gene of 28 isolates comprising *Bacillus anthracis*, *Brucella melitensis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Francisella tularensis*, *Yersinia pestis*, and eight genus-related and unrelated control strains was amplified and sequenced. The obtained sequence data were submitted to three public and two commercial sequence databases for species identification. The most

common reason for incorrect identification was the lack of the respective 16S rRNA gene sequences in the database.

Conclusions: Sequence analysis of a 500 basepair 16S rDNA fragment allows the rapid identification of dangerous bacterial species. However, for discrimination of closely related species sequencing of the entire 16S rRNA gene, additional sequencing of the 23S rRNA gene or sequencing of the 16S-23S rRNA intergenic spacer is essential.

Significance and Impact: This work provides comprehensive information on the suitability of partial 16S rDNA analysis and diverse databases for rapid and accurate identification of dangerous bacterial pathogens.

Future Work: The relevant dangerous bacteria and especially closely related species not differentiable by 16S rRNA sequence analysis will be compared by amplified fragment length polymorphism (AFLP). Possibly obtained species specific DNA fragments will be analyzed by sequencing. This species specific DNA sequences may be included in the design of a microarray or for the development of a multiplex-PCR that should allow the detection of most of the relevant dangerous bacteria.

SIMULTANEOUS DETECTION OF *BACILLUS ANTHRACIS*, *YERSINIA PESTIS* AND *BURKHOLDERIA* SPP. BY A MULTIPLEX PCR COMBINED WITH REVERSE LINE BLOT

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Introduction: In the recent years, the number of cases of exotic diseases in travelers, immigrants or NGO workers is increasing. Moreover, the threat of biological weapons in a potential bioterrorist attack recommends setting up a program for rapid response at the laboratory level. The diversity of agents that can be used in a potential bioterrorist attack and the lack of specificity in the initial symptoms that they can cause, require the design of diagnostic tools for the simultaneous detection of these pathogens.

Objective: Development of a molecular method for the simultaneous and specific detection of *Yersinia pestis*, *Bacillus anthracis*, *Burkholderia mallei*, *B. pseudomallei* and *B. thailandensis*.

Material and methods:

The following described targets were selected: the capsule gene (*capC*) and the lethal factor (*lef*) for *B. anthracis*, the gene coding the plasminogen activator, (*pla*) for *Y. pestis*, two genes related with the type III secretion system (*orf11* and *BpSCU2*) for *B. pseudomallei* and *B. thailandensis*, respectively, and finally *bimA* for *B. mallei*. The method consisted in a multiplex PCR and a reverse line blot with specific probes for the mentioned targets. The sensitivity was tested with different amounts of genomic DNA (10 - 10^3 genomic equivalents) of the proposed species, and other species of the genus *Burkholderia*, *Bacillus* and *Yersinia*, were used to test the specificity.

Results and Conclusions:

We are presenting a method for detecting warfare biological agents. The sensitivity of the method ranges between 10 and 10^3 genomic equivalents. Moreover, this method allows a specific and simultaneous amplification of the proposed targets. No cross-hybridizations with other bacteria species tested were observed. This assay represents a good diagnostic tool for a rapid and specific identification of bacteria in a potential bioterrorist attack. This work has been supported by the Ministry of Defense, program DN8836.

ANALYSIS OF CANONICAL INSERTION-DELETION MARKERS FOR SAFE AND RAPID DNA-BASED TYPING OF *FRANCISELLA TULARENSIS*

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Francisella tularensis is a potent human pathogen and a putative bioterrorist agent. Rapid and accurate typing methods are needed. Current medical practice does not rely upon subspecies or subpopulation identification, although this information may have predictive value for clinical outcome. We combined analysis of insertion/deletion (Indel) markers with multiple-locus variable-number tandem repeat analysis (MLVA). From five representative *F. tularensis* genome sequences, 38 distantly localized Indel markers were selected. To avoid markers with a propensity for homoplasy, only Indels with two allelic variants and being short of repeated sequences were included. The MLVA was based on well-known sequences. By the combined procedure, subspecies division, differentiation of clades A.I and A.II of subspecies *tularensis*, and differentiation of Japanese strains from other strains of subspecies *holarctica* was enabled. The present strategy affords reliable sorting at subspecies level resultant from the use of Indels with canonical properties and fine-grained intraspecies discrimination from the use of MLVA. It allows work with killed bacteria and, due to its utilization of one single analytic method, is time and cost effective.

BRUCELLOSIS – REEMERGING INFECTION IN BULGARIA

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Brucellosis in Republic of Bulgaria has been officially eradicated since 1958. Despite this, the closeness of the Mediterranean region, endemic for this zoonotic infection and also the yearly presence of human cases in the neighbor countries creates a real possibility for importing this infection in Bulgaria.

In this study we present data about an outbreak of brucellosis among Bulgarian citizens, who have been worked in a several countries which are endemic for the disease.

For a period of two years (2005-2006) according to the epidemiological and clinical data 112 patients suspected for brucella infection were tested and 48 of them proved to be positive.

This data indicate that we should be on the alert for this reemerging in Bulgaria infection.

Brucella, outbreak, laboratory diagnosis

ANTIBIOTIC RESISTANCE PROFILE OF *FRANCISELLA TULARENSIS* IN SWITZERLAND

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The facultative intracellular bacterium *Francisella tularensis* is a highly virulent microorganism capable of infecting many mammalian species (Grunow, 2000) and is the causative agent of the zoonotic disease tularemia. This species includes four distinct subspecies: *tularensis*, *holartica*, *novicida* and *mediaasiatica*. Only the two first are medically relevant. *F. tularensis* is widely distributed in the Northern Hemisphere. *F. tularensis* subsp. *holartica* is found throughout the Northern Hemisphere while *F. tularensis* subsp. *tularensis* is essentially limited to North America. *F. tularensis* subsp. *holarctica* strains are divided into erythromycin sensitive (biovar I) and erythromycin resistant (biovar II) isolates. A predominance of biovar I was observed for Western Europe, Eastern Siberia and the Far East, as well as North America, whereas biovar II prevailed in central Europe, the European part of ex-USSR, especially the south, and western Siberia. This antibiotic is generally not used to treat tularemia but can be an interesting epidemiological marker.

Recently concern of public health officials regarding tularemia has increased. This has been due in part to the terrorism threat, the high infectivity rate and multiple routes of infection of *F. tularensis*. Moreover, tularemia has emerged or re-emerged in different European countries. In order to be efficiently and accurately prepared for a natural or intentional manifestation of the disease, biological expertise and epidemiologic surveillance plans were set up by public health officials in Switzerland.

On average in Switzerland two human serological cases are registered per year. Over the last five years, different cases in zoo monkeys, born and raised in Switzerland, have give cause for concern about a potential outbreak.

To organize the Swiss epidemiologic surveillance of tularemia, we have collected all Swiss *F. tularensis* isolates originating from animal and human tularemia cases and performed antibiograms to characterize them. All isolates were susceptible to the antibiotics normally used to treat tularemia and were all erythromycin sensitive. Hence the strains that were isolated in Switzerland over the last five years, all belong to *F. tularensis* subsp. *holartica* biovar I.

DEVELOPMENT OF RAPID FIELD DIAGNOSTICS FOR IDENTIFICATION, CONTROL AND MANAGEMENT OF HAEMORRHAGIC FEVER OUTBREAKS (EU FP6-INCODEV3, VHF DIAGNOSTICS)

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Background: To improve the early detection of Viral Haemorrhagic Fever (VHF) cases, adequate tools are needed for the basic conditions of African hospitals. Specialised outbreak management also needs onsite tools such as viral genome detection. We will develop a) line assays (LA) for antibody detection as an easy to use frontline detection assay for health care workers in local hospitals, and b) fluorescent RT-PCR (F-RTPCR) assays to be used by specialised mobile outbreak investigation teams. Both assays will cover Ebola-Virus (EBOV), Marburg Virus (MARV), Crimean-Congo-Virus (CCHFV) Lassa virus (LASV), Rift Valley Fever Virus (RVFV), Yellow Fever virus (YFV) and Dengue virus 1-4 (DENV). Methods: (i) To develop LA, purified recombinant proteins of the VHF viruses will be expressed in the in-vitro RTS-500 system and sprayed onto immunoblot strips. Validation of the LA will be achieved by using available sera in the consortium of laboratories, centralised in a repository for VHF diagnostics development. (ii) We will validate F-RT-PCRs for field use by testing their sensitivity and specificity using RNA standards and recent isolates of each aetiological agent, and patient and/or rodent samples provided by the collaborating laboratories. We will adapt the extraction of nucleic acids from blood samples to field conditions. We will develop lyophilised ready to use PCR mixes to allow field PCR without the need for refrigeration facilities. Conclusions: (i) We expect to be able to

produce the envisioned line assay and to be able to test its applicability in local hospitals in Mali and Guinea. We hope to prove that an easy to use frontline test can reduce alert time in the case of an outbreak. (ii) The integrated F-RT-PCR-toolbox may enable outbreak investigation to perform initial differential diagnostics and to follow-up patients during the containment of an outbreak.

EXPRESSON LIBRARY IMMUNIZATION AND REVEALING OF IMMUNODOMINANT DETERMINANTS IN *F. TULARENSIS*

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Abstract:

So far no *Francisella tularensis* proteins that can be used for induction of T cell protective immunity have been identified. The main goal of the study is to identify *Francisella tularensis* (*Ft*)-derived proteins that can serve for developing a safe, acellular vaccine against *Ft*. The Expression-Library Immunization (ELI) technique was used to take advantage of the relatively small genome of *Ft* and the already established protocols for plasmid DNA immunization in rodents. Two separate representative genomic libraries were created and tested. The first encoding secreted products that will be processed via the class II MHC presentation pathway and stimulating CD4, CD8 T cell and humoral immunity. Alternatively, products from the second one targeted to the proteasome and processed via the class I MHC presentation pathway for primarily CD8 T cell stimulation. Immunization studies were conducted in a DBA mouse model and the sub-libraries were screened for protection against *Francisella tularensis* – a clinical strain Gal. Mice were immunized with expression libraries containing the entire genome of the pathogen. Complete or partial (prolongation of survival) protection from *Ft* -induced disease in immunized animals identifies sub-libraries that contain plasmids encoding for reactive antigenic epitopes. In this way, expression library immunization provides an unbiased, systemic approach for isolating vaccine candidates. Briefly, animals were immunized intramuscularly (i.m.) with the respective DNA sublibrary and inoculated intraperitoneally (i.p.) 14 days later with 10 colony forming units (c.f.u.) of Schu4 and survival were monitored and compared with control mice that have been injected with PBS or with “empty” vector. Clones that are protective will be identified, sequenced and characterized.