Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines

Paul-Ehrlich-Institut

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Rabies Potency Testing

Regulatory Perspectives and Experiences in the Establishment of a Serological Assay

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Overview

- Paul-Ehrlich-Institut
- Rabies potency assay – NIH
- Vaccines for veterenary use: Experiences in the Establishment of a Serological Assay
- Could a multi-dose serological assay facilitate the replacement of the NIH vaccines for human use?
  - WHO Rabies feasibility study – Outcome and way forward
    (Dr Ute Rosskopf)

Our Focus is on Health

Paul – Ehrlich – Institut (PEI)

"Law Governing the Establishment of a Federal Agency for Sera and Vaccines" of 7th July 1972

An independent senior federal authority is established which is given the name Paul-Ehrlich-Institut.

It is reporting to the Federal Minister of Health.

Since 2009:
Federal Institute for Vaccines and Biomedicines

Responsibilities and Tasks

Medicinal products for

Human use

- Vaccines
- Sera like immunoglobulins and monoclonal antibodies
- Test and Therapy-allergenes
- Blood and blood products
- Tissue preparations
- ATMP

Veterinay use

- Vaccines, Sera, Immune modulators and Tuberculins

- Marketing authorisation and life cycle management
- Approvals of clinical studies
- Scientific Advice
- Inspections
- Pharmacovigilance
- Official batch release
- Research

Batch Release and Research at PEI

Official batch release of

- Vaccines
- Sera
- Allergens
- Blood products

- Number of released Batches:
  - 2015: 9.587
  - 2016: 9.182

Research Programme – Topics

- Regulatory Research & Innovative Medicinal Product Testing
- Pathogen-Host & Biomedicine-Organism Interactions
- Experimental Vaccines, Therapies & Diagnostics

National and International Integration

Federal Ministry of Health

PEI	BfArM	BzgA	DIMDI	RKI
Paul-Ehrlich-Institut	Federal Institute for Medicines and Medical Devices	Federal Centre for Health Education	German Institute for Medical Documentation and Information	Robert Koch-Institut

EMA
European Medicines Agency

HMA
Heads of Medicine Agencies

EDQM
European Directorate for the Quality of Medicines

CHMP
Committee for Medicinal Products for Human Use

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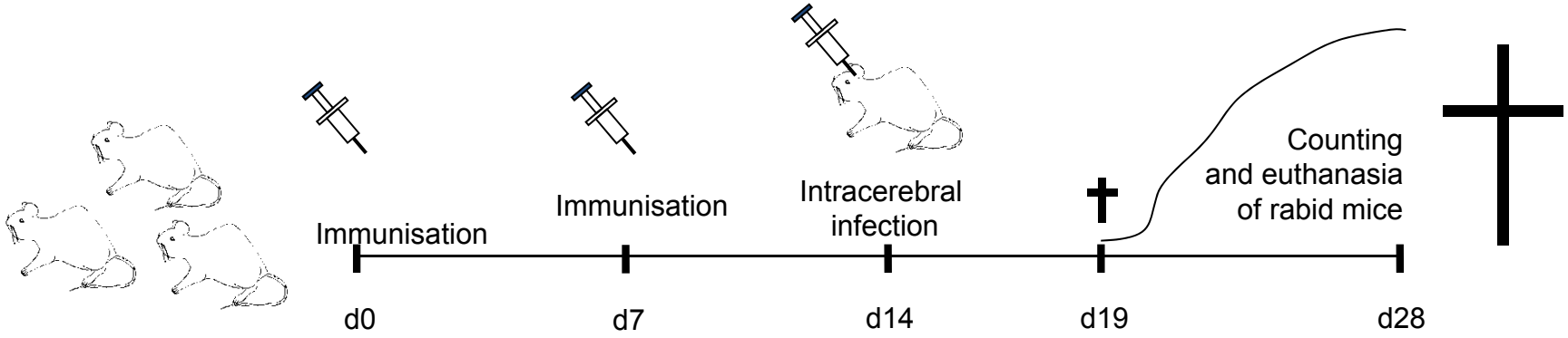
World Health Organization

- WHO Collaborating Centers at the Paul-Ehrlich-Institut
  - WHO-CC for Quality Assurance of Blood Products and in vitro Diagnostic Devices
    since 06/2005
  - WHO-CC for Standardization and Evaluation of Vaccines
    since 09/2013

Rabies Potency Assay - NIH



Developed by Seligmann EB Jr. in 1966

- **Pros:** Worldwide used release test, specification ≥2.5 IU per single human dose
  - Ph.Eur. 0216, WHO TRS 941

- **Cons:**
  - Huge mouse number involved (multi-dose test, *in vivo* titration)
  - Severe distress for mice
  - Emotionally challenging for lab staff
  - Highly variable, high costs, time consuming
  - → Currently PEI only OMCL performing NIH in Europe

Replacement of the NIH assay ?

- Disadvantages of the NIH
- The 3R concept
  - Refinement:
    less suffering for animals
  - Reduction:
    less animals involved
  - Replacement:
    animal-free test

  → Worldwide acceptance and willingness to implement 3R approaches

- Alternative method is really needed
- Ph.Eur. 0216: *„Alternatively, in the interest of animal welfare, a validated serology potency assay or an immunochemical assay for a native glycoprotein content is recommended."*
- Ph.Eur. 5.2.14
  - New general Chapter to facilitate the transition from *in vivo* to *in vitro* methods (referenced in the general monograph „Vaccines for humane use, but is not mandatory)

Vaccines for veterenary use: Serology Assay

The rapid fluorescent focus inhibition test is a suitable method for batch potency testing of inactivated rabies vaccines. Developed by Krämer B in 2009

- Single-dose serological assay prescibed for batch potency testing (Ph.Eur. 0451)
  - Semi-quantitativ results (pass/fail)
  - Groups of 10 mice
  - Single immunisation with 1/5th of recommended dose
    - Vaccine to be tested
    - Biological Reference Preparation (BRP) diluted to minimum potency requirement (1 IU)
  - Comparison of neutralising antibodies *in vitro*

Vaccines for veterenary use: current stage of serology

- Single-dose assay (SDSA) for batch potency testing
- NIH still needed
  - Establishment of new standard material
  - Within stability studies
- Could a multi-dose serological assay (MDSA) facilitate the replacement of the NIH?
  - Use of four doses to calibrate the vaccine against the reference
  - BSP115
- MDSA in current form not suitable for rabies potency quantification
  - Require improvement and more animal tests for a few occasions
- Serology was considered as an intermediate step until animal test can be fully replaced
  - *In vitro* ELISA has been developed
  - One manufacturer has already implemented the ELISA on routine basis
  - Currently, for veterenary rabies vaccines the MDSA approach will not be pursued.

Could a multi-dose serological assay facilitate the replacement of the NIH for vaccines for human use?

World Health Organization

WHO Rabies feasibility study – Outcome and way forward

Dr Ute Rosskopf

WHO Rabies serology feasibility project

Study directors

- WHO: Dr Ute Rosskopf, Technical assistance and laboratory services (TAL) group
- PEI: Dr Beate Krämer

Participating laboratories

- Institute of Biological Products (IBP), Thai national control laboratory; Focal point: Ms Teeranart Jivapaisarnpong (director IBP) Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand
- Paul-Ehrlich-Institut (PEI), German national control laboratory; Focal point: Dr Beate Kraemer (head of laboratory for product testing of immunological medicinal products for veterinary use), Langen, Germany

Statistical evaluation

- Dr Kay-Martin Hanschmann, PEI
- Dr Stan Deming, Deming Statistics

Outline

1) Background

2) Rationale Serological potency assay

3) Rationale Feasibility study

4) Test principle

5) Study design

6) Differences in testing of vaccines for human and for veterinary use

7) Test results

8) Study outcome

9) Influencing factors

10) Conclusions

11) Points for discussion

Background

- Currently in use: mouse protection test, so called NIH (National Institute of Health) potency test

- Established in the 1950ies in absence of GMP

- NIH test performed by a limited number of national control laboratories (animal test, high safety requirements)

- Hugh number of mice involved

- Intracerebral injection: severe distress for mice, emotionally challenging for lab staff

- Highly variable

- High costs and time consuming

Background (cont.)

- High need for an alternative to the current potency test

(expressed by European convention for the protection of vertrebrate animals, manufacturers, European OMCLs, WHO)

In principle, potency determination of rabies vaccine may be performed by

- mouse protection - challenge with the virus (*in vivo*)
- measurement of serum response (*in vivo*)
- antigen quantification (*in vitro*)

Rationale – Serological potency testing

- Method already available for veterinary rabies vaccines' batch release (*Ph.Eur.*)

- Test is based on serum neutralization (SNT) and allows quantitative information on the individual serum response / no dead – alive interpretation

- Less animals needed

- Faster

- Avoidance of intracerebral challenge and suffering from rabies

Rationale – Feasibility study

INVESTIGATION

if the assay is suitable for the potency determination
of rabies vaccines for human use
- quantitative information

INCLUSION

of a heat-treated (sub-potent) rabies vaccine

TRANSFERABILITY

of test protocol to other laboratory

Serological potency testing - Principle

Comparison of the dose dependent antibody production in animals immunized with test and reference preparation.
Antibodies are assessed by inhibition of virus growth in cell culture.

- Twofold immunization of mice (Day 0 and Day 7)

- Blood withdrawal on day 28 (days post 1st immunization) and preparation of sera

- Methodology: Rapid fluorescent focus inhibition test (RFFIT) – modified through use of micro titre plates
  Virus is neutralized → no immunofluorescence
  Virus not neutralized → immunofluorescence

- Use of 2 International Standards:
  - 6th WHO Int. Standard (vaccine)
  - 2nd WHO Int. Standard – Rabies Immunoglobulin (RAI)

Study design

- Two participating laboratories

- Panel of three vaccines:
    1) Pitman Moore strain (Vaccine 1)
    2) Flury LEP strain (Vaccine 2)
    3) Heat-treated vaccine (sub-potent vaccine), Flury LEP strain (Vaccine 3)

tested twice (six test runs) by each of the participating laboratories using the serum neutralization test

- Performance of multi-dilution assay (4 dilutions for test vaccine and Int. Standard)

- Performance of single-dilution assay (cut-off test): Fifth dilution of Int. Standard (equivalent to minimum required potency (2.5 IU/mL)
  → prove of single-dilution approach

Study design (continuation)

- Use of 10 mice per vaccine dilution

- Investigation of individual sera and pooled sera

- One NIH test performed by the labs to investigate the heat-treated (sub-potent) vaccine in addition to the serology

- Statistical evaluation of the data by PEI, Dr Kay Hanschmann and Dr Stan Deming (Statistical Designs, Houston)

Differences related to testing of human and veterinary vaccines (serological assay)

Human	Veterinary
- Vaccines not adjuvanted	- Vaccines adjuvanted
- Tested against WHO Standard	- Tested against BRP Standard
- Mouse age/weight: 6 weeks / 29-32 g	- Mouse weight: 18-20 g
- 2 Immunizations: day 0 and day 7	- 1 Immunizations: day 0
- 4 vaccine dilutions administered: quantitative test results	- 1 vaccine dilution administered: cut-off test
- Minimum titer required: 2.5 IU/ml = dose	- Minimum titer required: 1 IU per dose

Study outcome - summary

- Dose-dependent immune response of rabies antibody titres could be shown

- Estimates of potency based on the individual sera agree quite well

- Use of pooled sera to estimate the relative potencies might be possible

- Use of a single dilution approach (cut-off test) is not feasible

- Differences in replicate estimates of the vaccine observed: within the laboratories and between them

- Results of the NIH test (manufacturer's results) only partly confirmed by the serological assay (difference: survival rates versus antibody responses)

- Heat treated (sub-potent) vaccine correctly classified by applying the NIH test and by the serological assay using the individual sera determination. For the serum pools the sup-potent quality was correctly assigned in 3 out of 4 cases.

Influencing factors

- Heat treatment of the vaccine by the testing laboratories

- Use of different mice strains (ICR & NMRI)

- One additional washing step (before adding the FITC)

- Different tubes to treat and to store the sera

- RFFIT performed by only one person (6 h for neutralization and fixation and 7 h for staining

Conclusions

- Routine testing of individual sera does not seem appropriate:
  - high number of plates
  - meeting validity criteria

- Testing of serum pools preferable

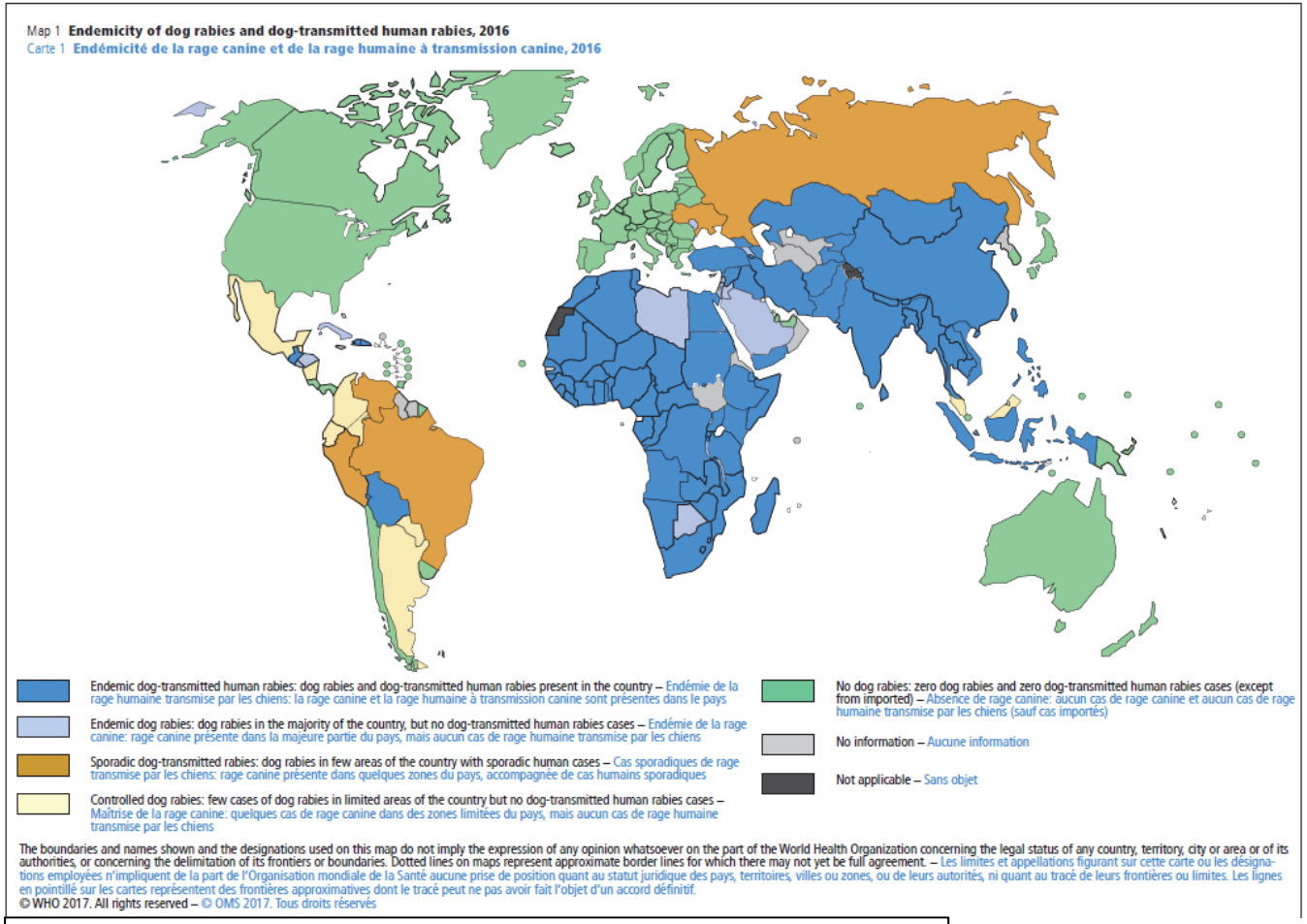
- First feasibility study performed – potency determination of vaccines for human use to be further proven

Points for discussion

- Will the Serology assay or the Glycoprotein assay replace NIH test performed by manufacturers?

- Could sera generated by the manufacturer be retested by releasing NRA / NCL?

Thank you



WHO. Weekly epidemiological record. 17 FEBRUARY 2017, 92th YEAR (No 7, 2017,92, 77-88)