Réseau National CLAT

EAZWV

Tuberculose(s) & Parcs Zoologiques

AFVPZ

Dr Alexis Lécu, DVM Paris Zoo
Chair EAZWV TB Working Group

MUSEUM NATIONAL D'HISTOIRE NATURELLE

Journée INTERCLAT PARIS 06 juin 2013

Introduction-1

Préhistoire de la tuberculose sur l'ancien et le nouveau continent

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- Préhistoire de la tuberculose sur l'ancien et le nouveau continent
- Prévalence en Europe :
  - Animaux de rente
  - Faune sauvage

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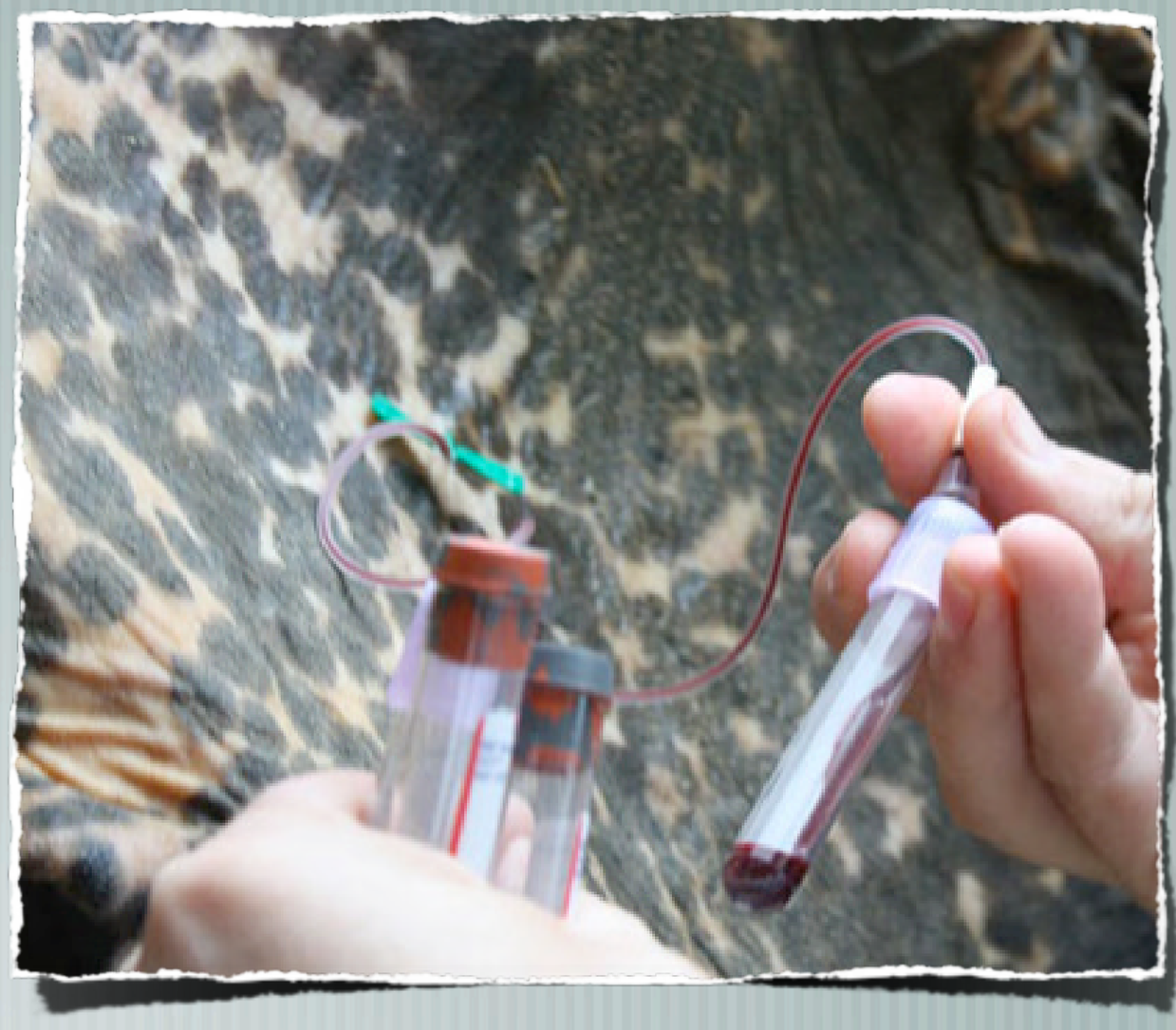
  - Faune sauvage captive :
    - Cirques : différence avec USA !



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TB outbreak forces zoo to kill animals

NEWPORT NEWS (AP)—The Peninsula SPCA petting zoo has been closed and 100 of its animals are to be destroyed in an effort to halt the spread of a tuberculosis bacterium found in some of the zoo's animals.

Health officials said the outbreak does not threaten humans.

State veterinarians first discovered a type of tuberculosis, Mycobacterium bovis, during routine tests of a fallow deer in April, said Robert D. Whiting, chief of veterinary services for the Virginia Department of Agriculture. The animal, which had broken its leg, was destroyed.

At the state's request, eight other fallow deer, two potbellied pigs, a goat and a sheep were destroyed. Tests by the National Veterinary Services lab in Ames, Iowa, confirmed Thursday that three of those animals also were infected with the tuberculosis bacterium.

The Peninsula Society for the Prevention of Cruelty to Animals closed the petting zoo, which attracts about 50,000 visitors a year, on Friday.

"This is such a heart-wrenching thing," Sarah Forbes, president of the Peninsula SPCA, said Monday. "The petting zoo was created for children to see the animals, feed them corn, to allow them contact with animals."

The Newport News Health Department tested nine SPCA employees, said Linda Rose, a public health nurse. One tested positive for tuberculosis in a skin test. Nothing showed up on a subsequent chest X-ray, and it is unlikely the man picked up the bacteria from an infected animal, she said.

"I don't think there is any serious threat to human health," said William Sims, state veterinarian and director of the Division of Animal Health.

The animals, including the zoo's only llama, four African and Asian antelope, 20 goats, peacocks, chickens, geese and turkeys will be killed by lethal injection to avoid any risk they will be infected and further spread the disease, said Peninsula SPCA director Eugene Falls.

The zoo's kangaroo, donkey, leopards and other caged animals are not thought to be threatened and will not be killed.

Introduction-2

Pathogène obligatoire : complexe tuberculosis

Mycobacteries Complexe Tuberculosis
M. tuberculosis
M.bovis
M. africanum
M.microti
M.pinnipedii
M.caprae
M.canetti
“Dassie bacillus”
M.orygis
M.mungi

Introduction-2

- Pathogène obligatoire : complexe tuberculosis
- Potentiellement pathogène

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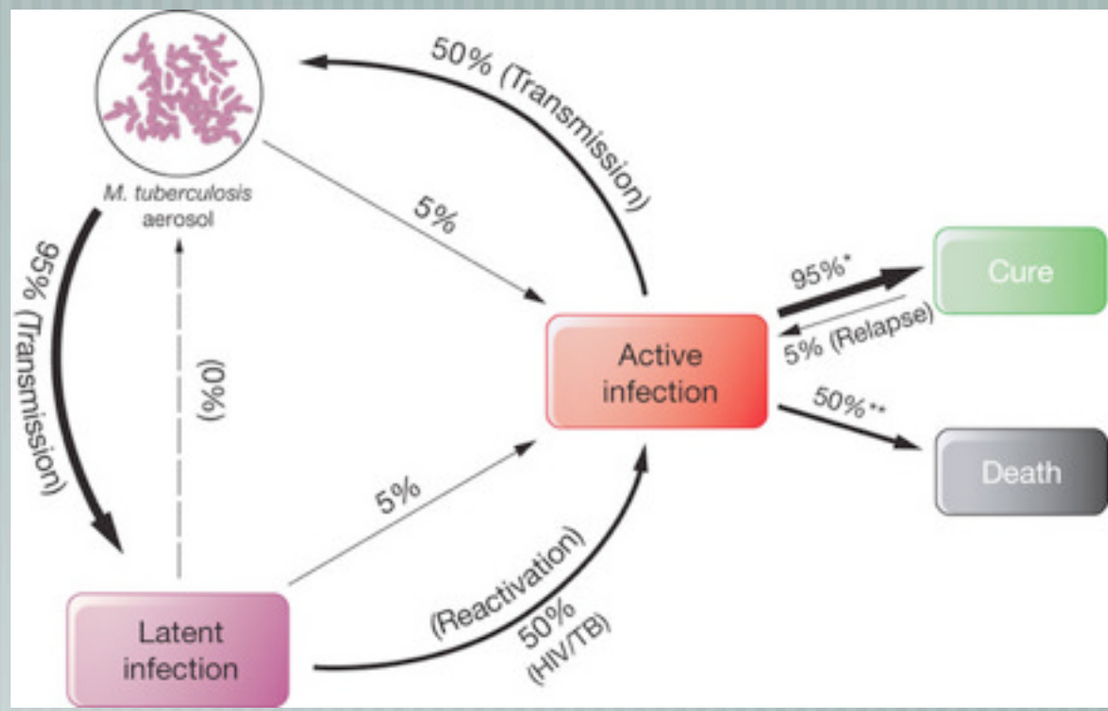
    - Sous espèces avium
  - Mycobactéries environnementales (non tuberculeuses, atypiques)

infections caused by *Mycobacterium kansasii*

Host organism	Infected tissue
Amoeba (*Acanthamoeba castellanii*)[1]	Body
Cockroaches (*Periplaneta americana*)	Body
Chinese soft shell turtle (*Pelodiscus sinensis*)	Lung and carapace
Cardinal tetra (*Paracheirodon axelrodi*)	Tissues with tuberculoid lesions
Siamese fighting fish (*Betta splendens*)	Tissues with tuberculoid lesions
Domestic goat (*Capra hircus*)	Healthy lymph nodes
Squirrel monkey (*Saimiri sciureus sciureus*)	Healthy lymph nodes
Rhesus monkey (*Macaca mulatta*)	Pulmonary lesions
Antelope in zoo	Pulmonary lesions (mixed infection caused by *M. a. avium* and *M. kansasii*)
Llama	Lesioned mesenteric lymph nodes, liver and lungs
Florida manatee (*Trichechus manatus latirostris*)	Tuberculoid nodules in lungs
Dog (*Canis familiaris*)	Pleural fluid from persistent pleural effusion (3-year-old spayed female whippet)
Cattle (*Bos taurus*)	Lesioned lymph nodes
Cattle (*B. taurus*)	Healthy lymph nodes from positive skin-tested animal for bovine tuberculin
Black-tailed deer (*Odocoileus hemionus*)	Multiple granulomas in thoracic cavity

Introduction-2

- Pathogène obligatoire : complexe tuberculosis
- Potentiellement pathogène
  - complexe avium
    - Sous espèces avium
  - Mycobactéries environnementales (non tuberculeuses, atypiques)
- Phénomène de latence



Obligations réglementaires (CEE 92/65)

- But : Tous les ruminants échangés entre les zoos **européens** doivent provenir de troupeaux indemnes de tuberculose

- **«Etablissement agréé»** = aucun cas de **tuberculose bovine** dans les 3 dernières années (= liste A) et pas d'infection au **complexe tuberculosis** chez les Primates/ Felidés/Ruminants en cas de reglementation nationale specifique (liste B)

- Transfert entre 2 etablissements agrées = pas de tests TB !

- M.A.D : tous mammifères, mais *M.tuberculosis* et *M.bovis* seulement

Diagnostic en zoo

Doute → *Certitude* → *Immunologie*

Aspecifique

Examen clinique
Hematologie
Autopsie

Radiographie
Endoscopie
Tomographie

(Colorations)

DIRECT

- Colorations
- **CULTURE**
- Biologie moléculaire (PCR)

Cellulaire

- IDR ou IDC
- LTA ou LPA
- γ-interferon (IGRA)

Humoral

- ELISAs
- Tests Rapides
- Multiplex

Primates Non Humains

Recommendations de l'European Primate Vet, 2009

J Med Primatol doi:10.1111/j.1600-0684.2008.00303.x

ORIGINAL ARTICLE

Guidelines for the prevention and control of tuberculosis in non-human primates: recommendations of the European Primate Veterinary Association Working Group on Tuberculosis

Moshe Bushmitz[1], Alex Lecu[2], Frank Verreck[3], Ellen Preussing[4], Susanne Rensing[5] & Kerstin Mätz-Rensing[6] of the EPV-Tuberculosis Working Group on Non-human Primate Health

1 BFC, Mazor, Israel
2 Parc Zoologique de Paris, Paris, France
3 Biomedical Primate Research Centre, Rijswijk, the Netherlands
4 Novartis Pharma AG, Basel, Switzerland
5 Covance, Münster, Germany
6 German Primate Centre, Göttingen, Germany

Keywords
Diagnosis – handling – non-human primate – recommendation – tuberculosis

Correspondence
Dr Kerstin Mätz-Rensing, Department of Infectious Pathology, German Primate Centre, Kellnerweg 4, D-37077 Göttingen, Germany.
Tel.: +49 551 3851 386;
fax: +49 551 3851 442;
e-mail: kmaetz@gwdg.de

Accepted June 14, 2008.

Abstract

Background Effective tuberculosis (TB) control requires accurate diagnostic methods but the tuberculin skin test has serious limitations. Both false-negative and false-positive reactions are common, resulting in the spread of the infection and devastating TB outbreaks. Results of questionnaire surveys concerning TB testing practices in primate housing facilities showed great differences in testing practices. Although there was some uniformity regarding the sites of application, the amounts of tuberculin used and the time intervals for retesting, a great deal of variety was revealed considering the types of tuberculin preparations, the interpretation of tests and the susceptibility of animals.

Conclusion Here, we summarize the most common practices as regards TB control and prevention for non-human primates, and attempt to establish a uniform guideline based upon our experience with primate husbandry and care programmes as well as recent developments in the literature. The present guideline represents a consensus recommendation intending to harmonize the existing protocols.

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- Faux positifs à l'IDR (*M.avium*, MNT, espèces sensibles)

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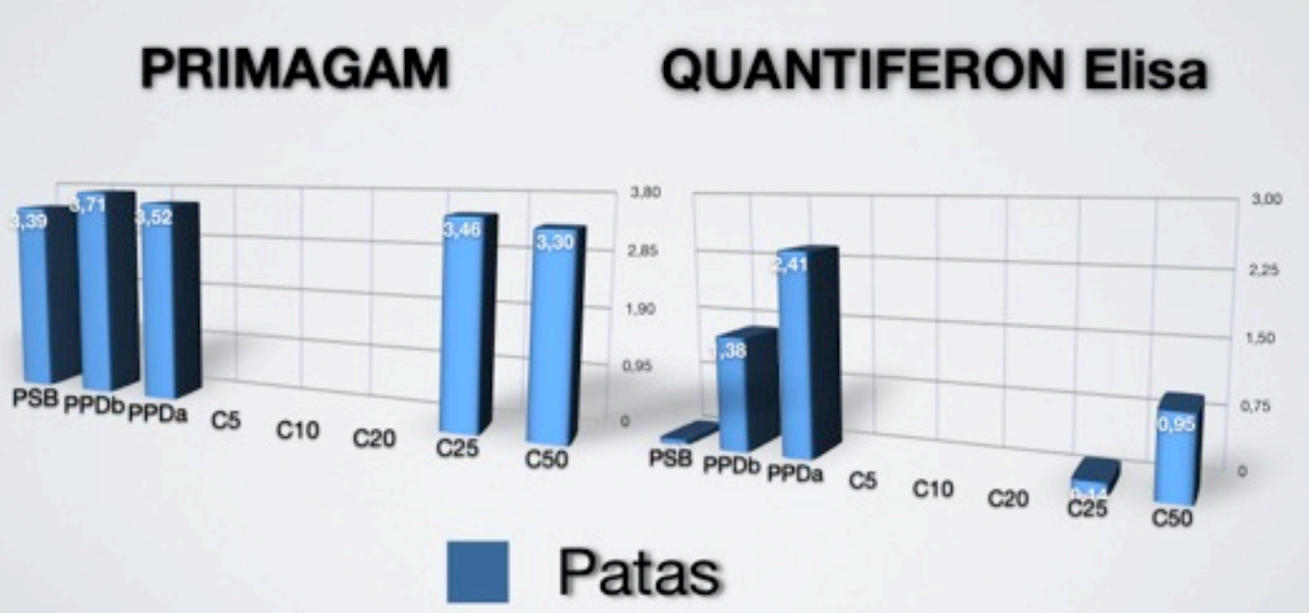
- *M.microti*
- Combinaison sérologie et test au gamma interféron

PRIMAGAM® STIMULATIONS



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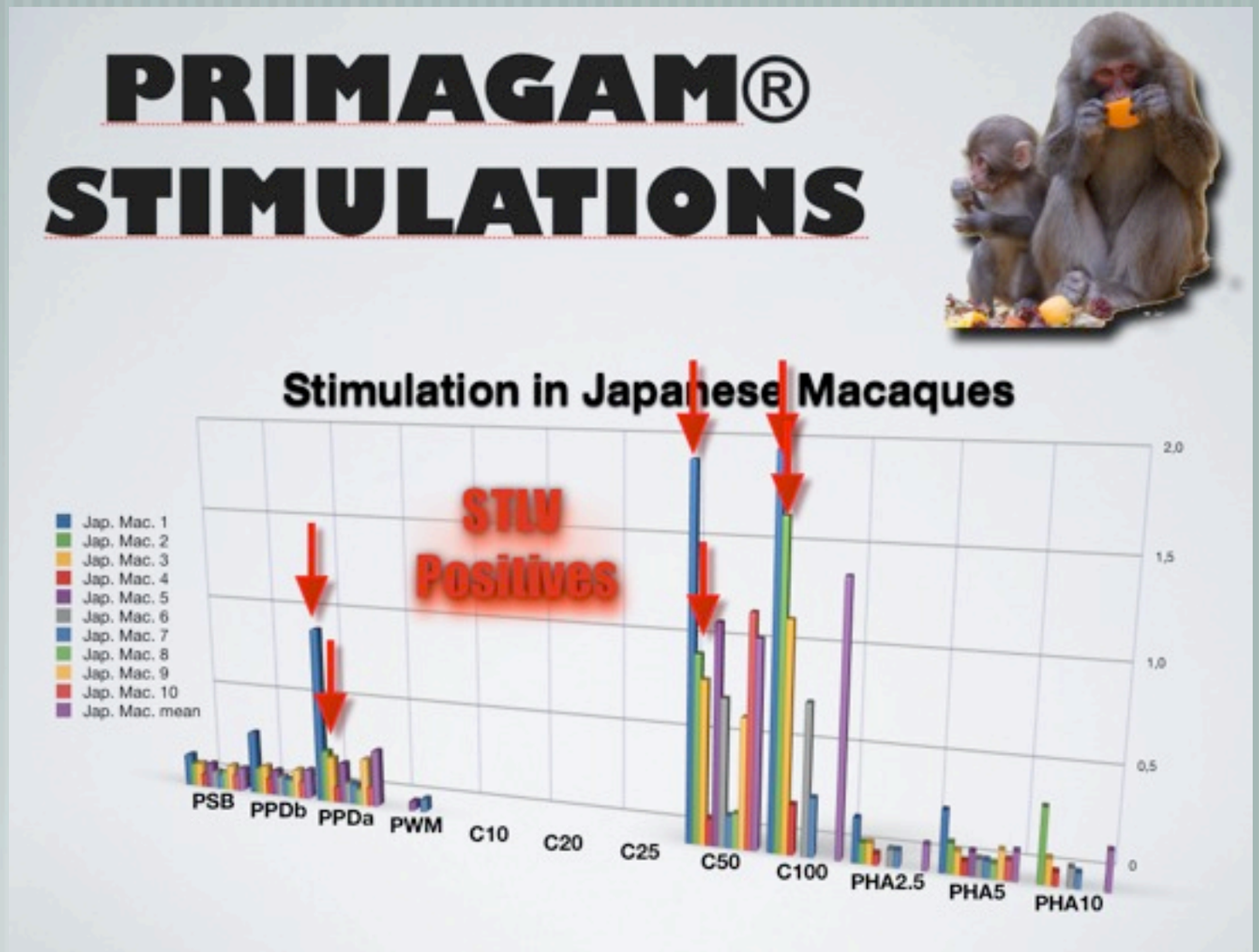

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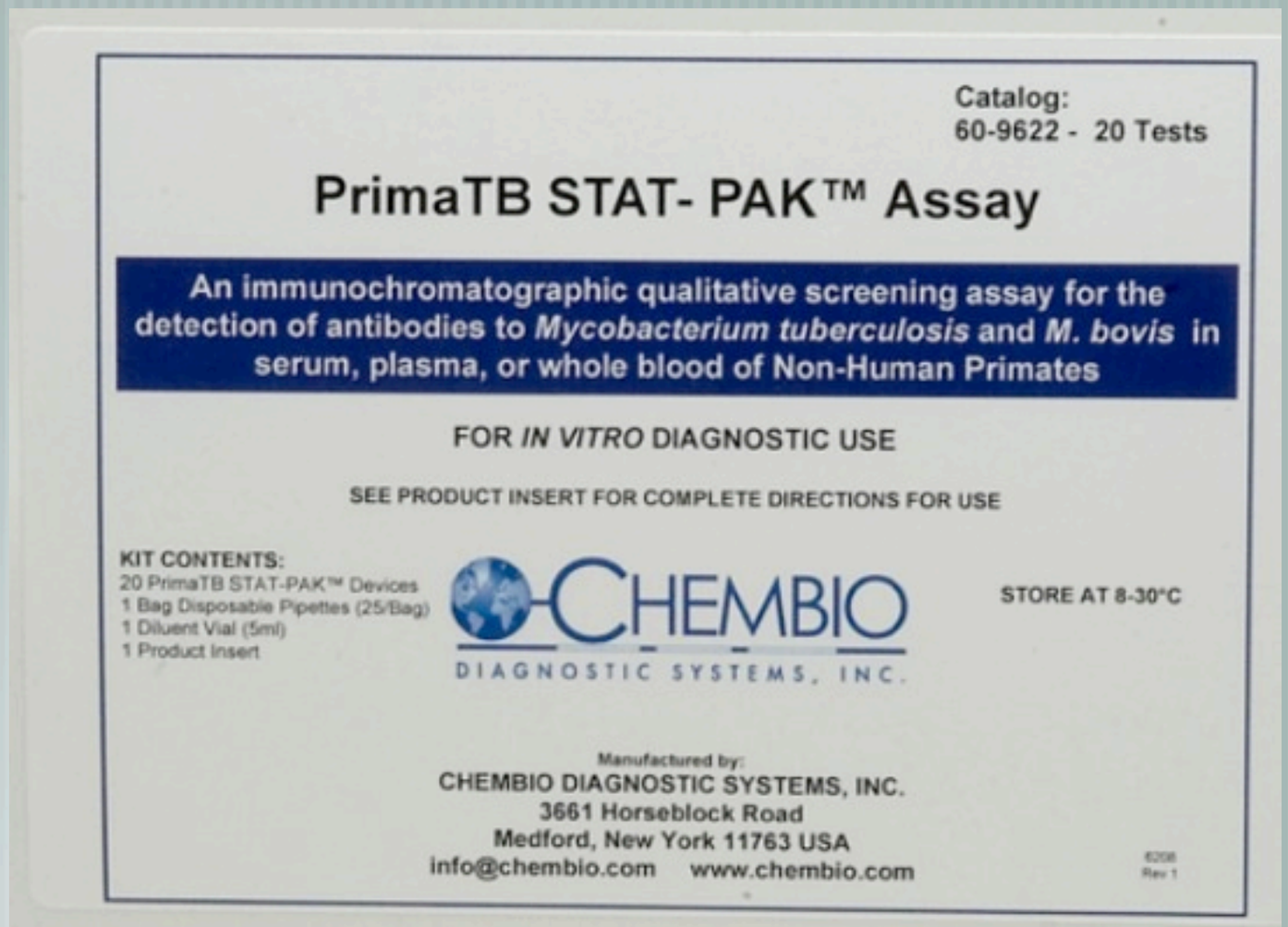
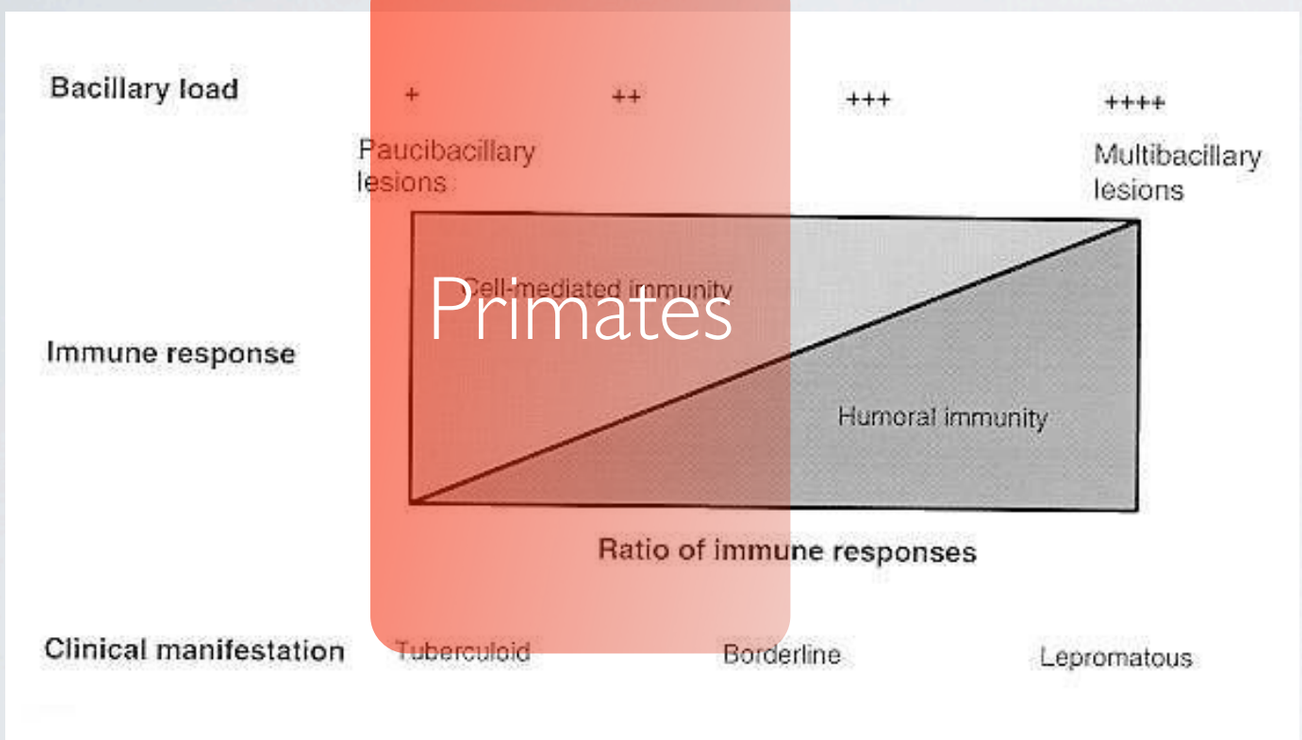


TESTS IMMUNOLOGIQUES



Primates

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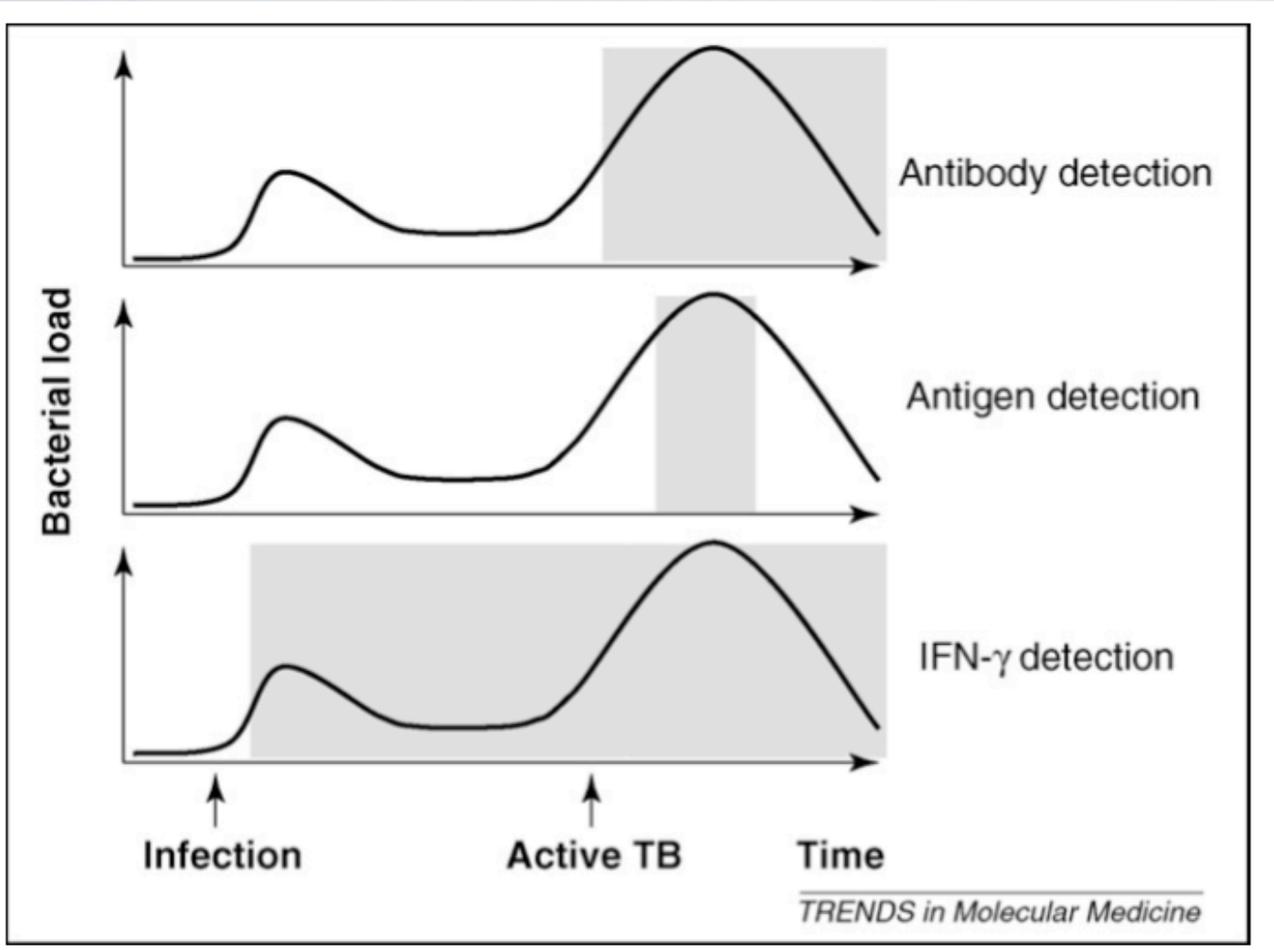

Théorie «Humain»....

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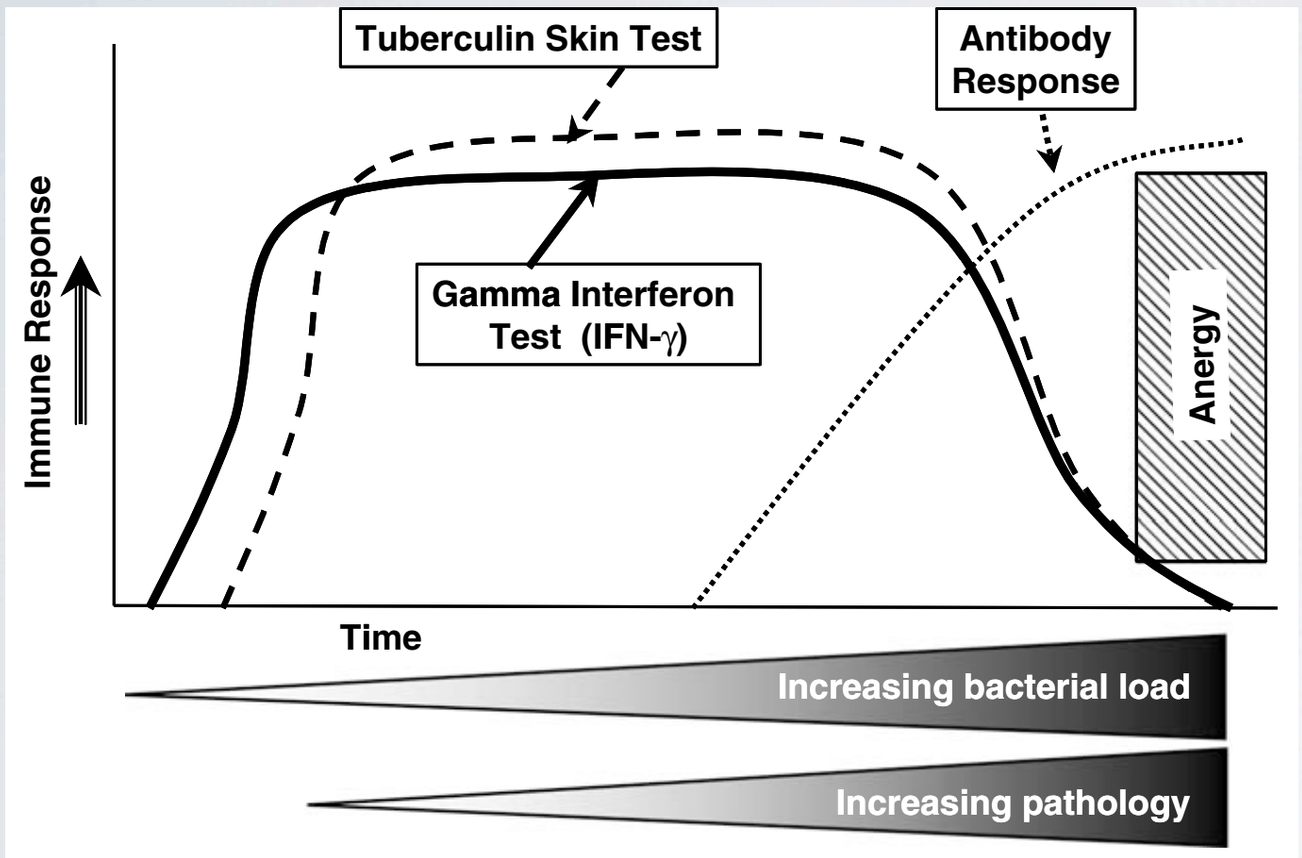


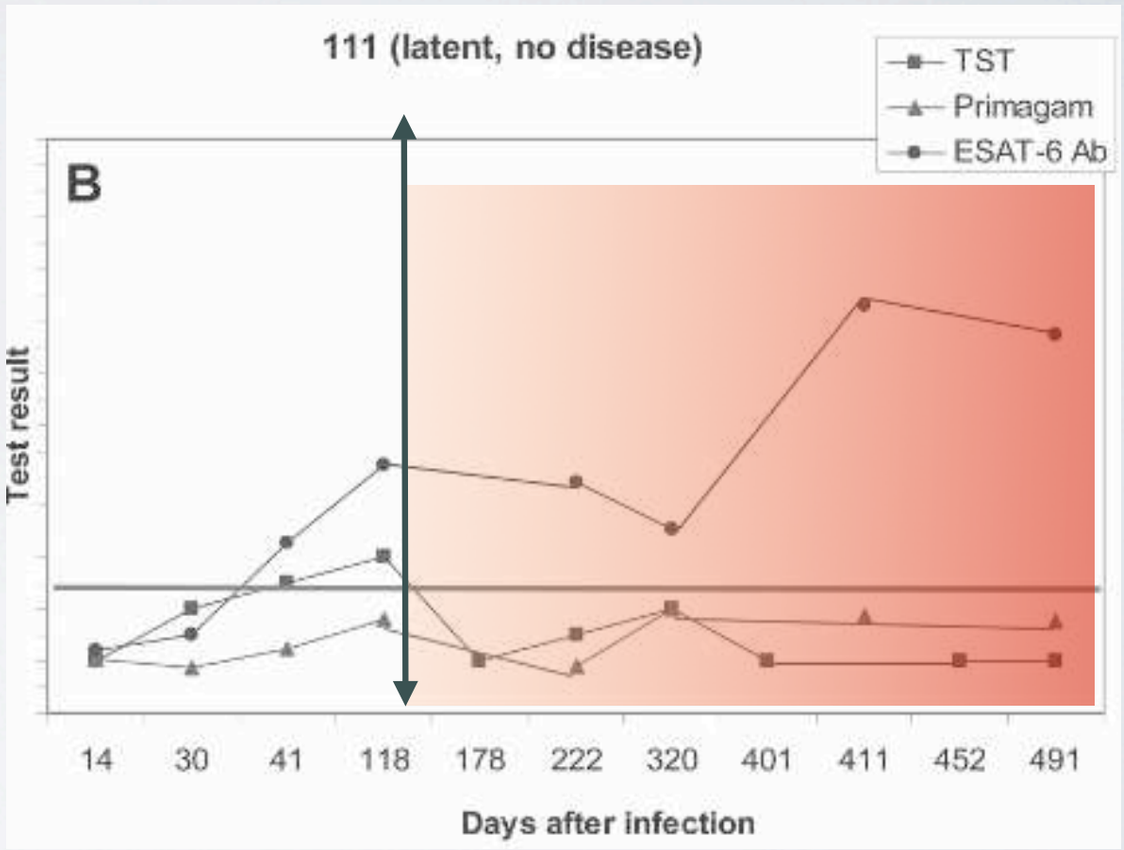
New Approaches to Tuberculosis Surveillance in Nonhuman Primates

ILAR JOURNAL

2008

Journal Vol. 49 (2)

Nicholas W. Lerche, JoAnn L. Yee, Saverio V. Capuano, and Joanne L. Flynn



Théorie «Bovin»....

Théorie «Humain»....

... et pratique primate!

Cellulaire *Immunologie* **Humoral**

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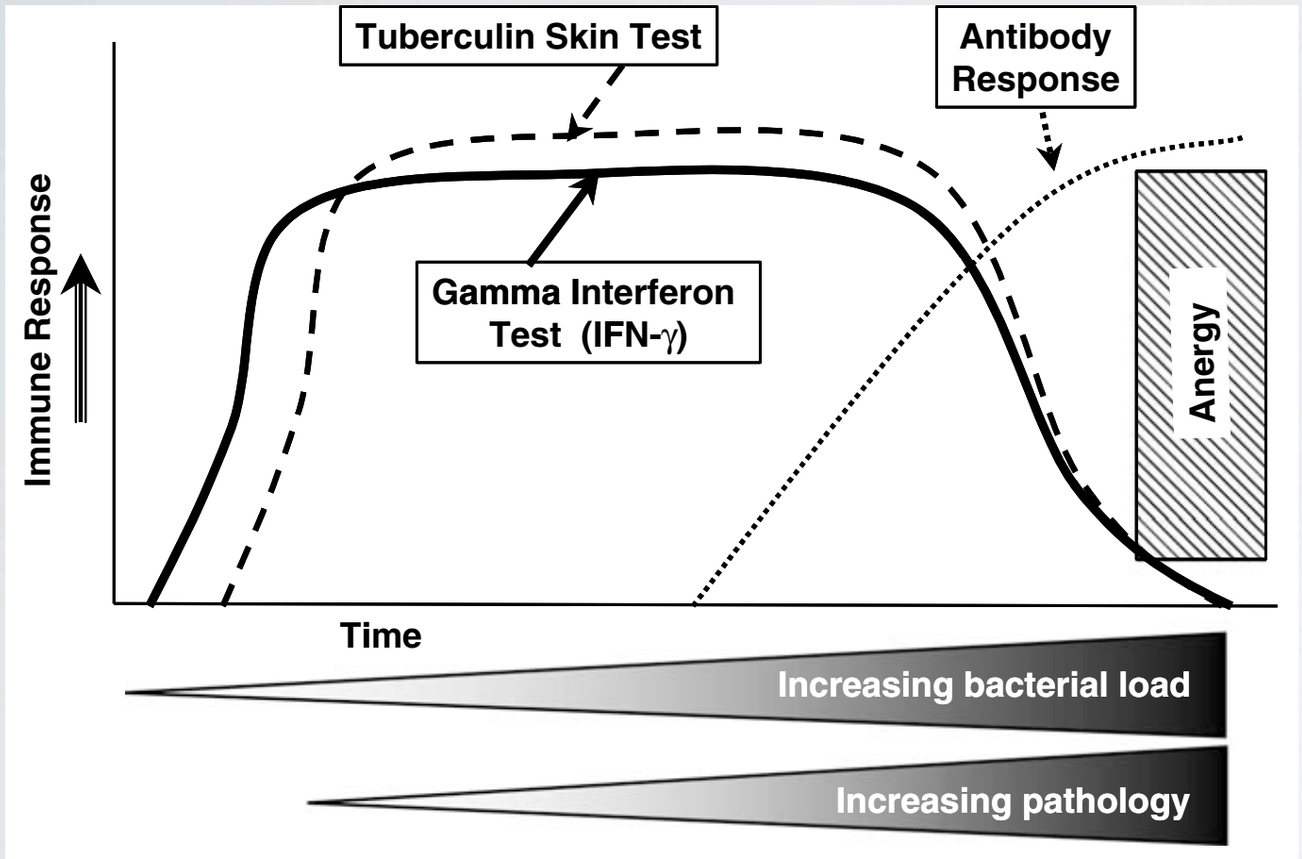

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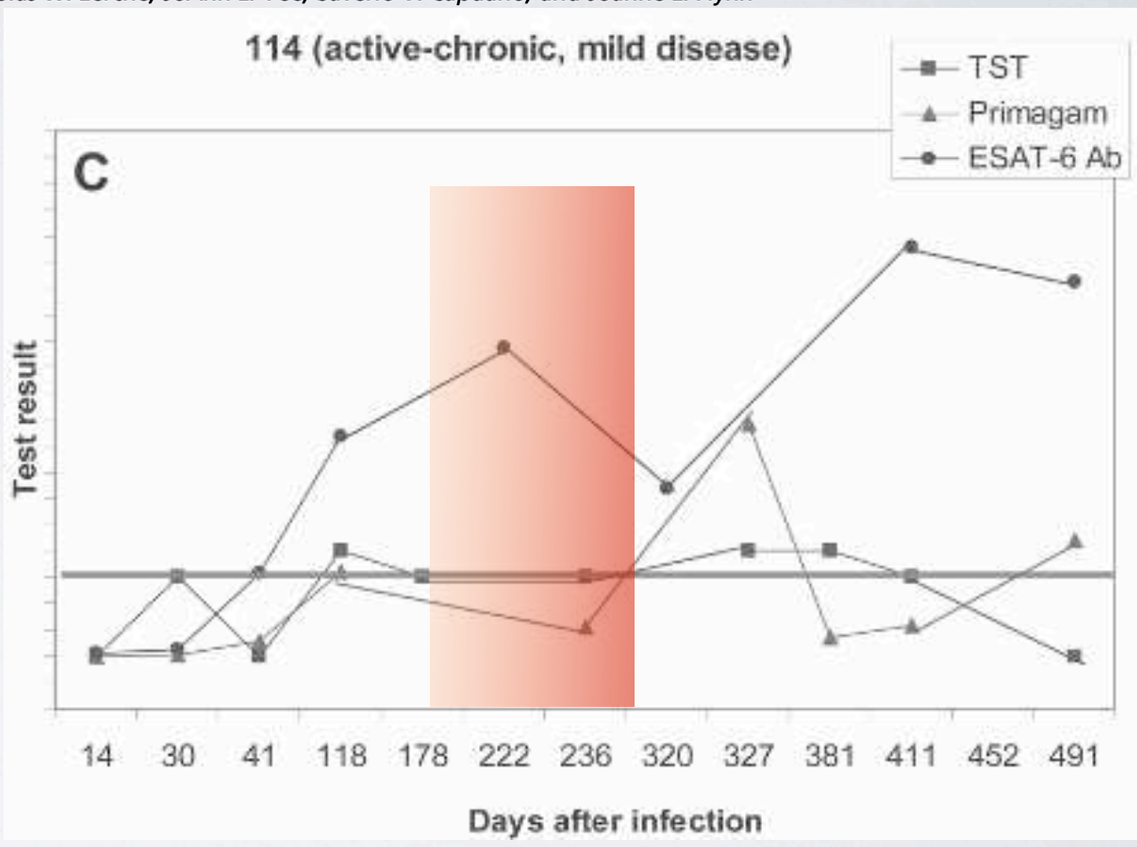

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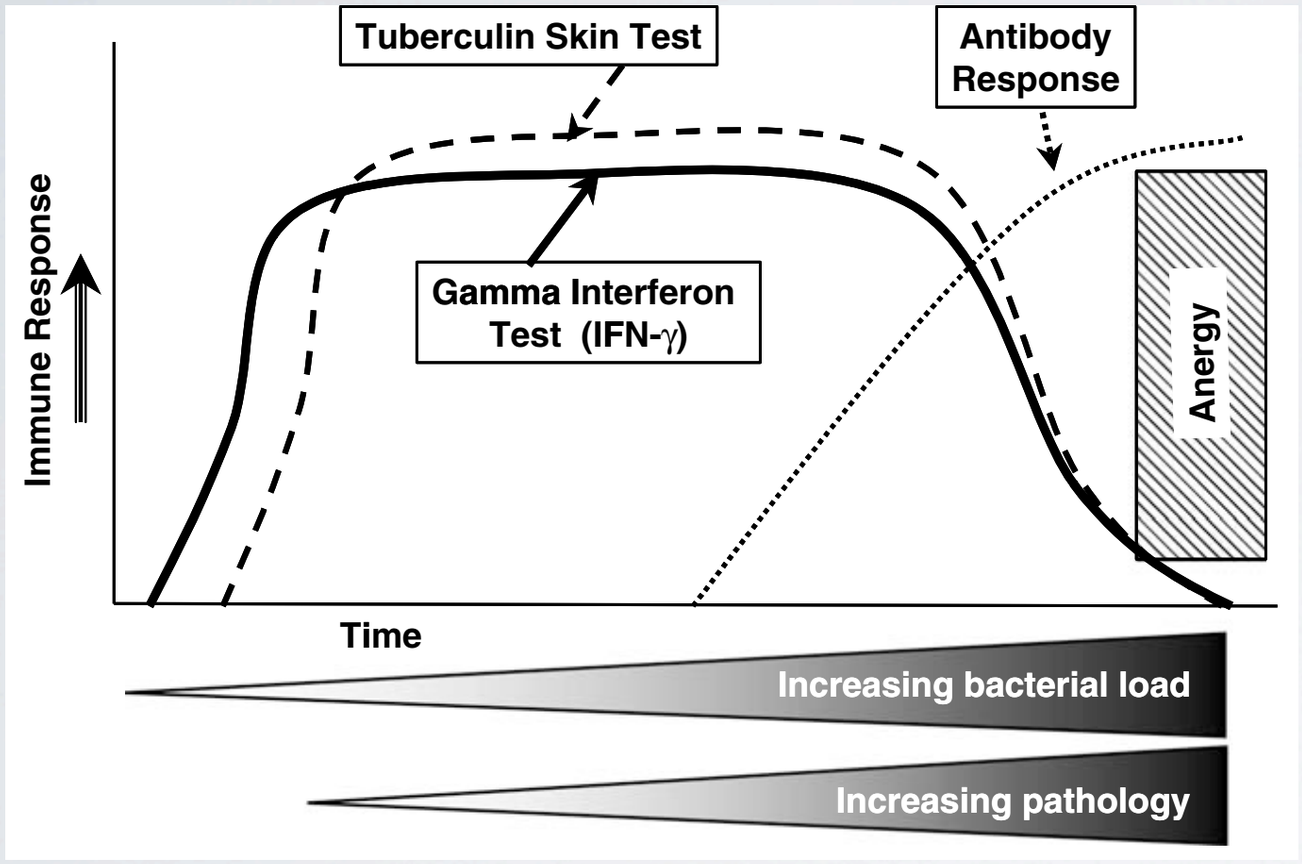

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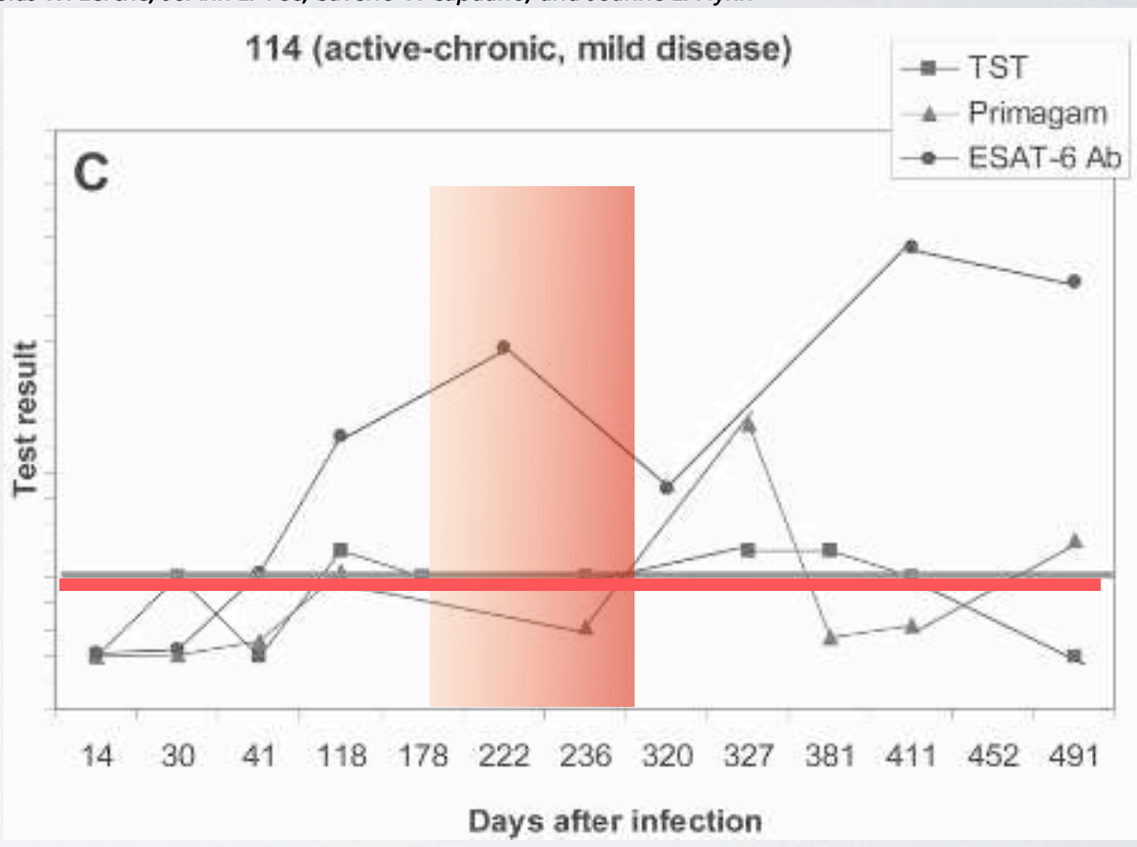

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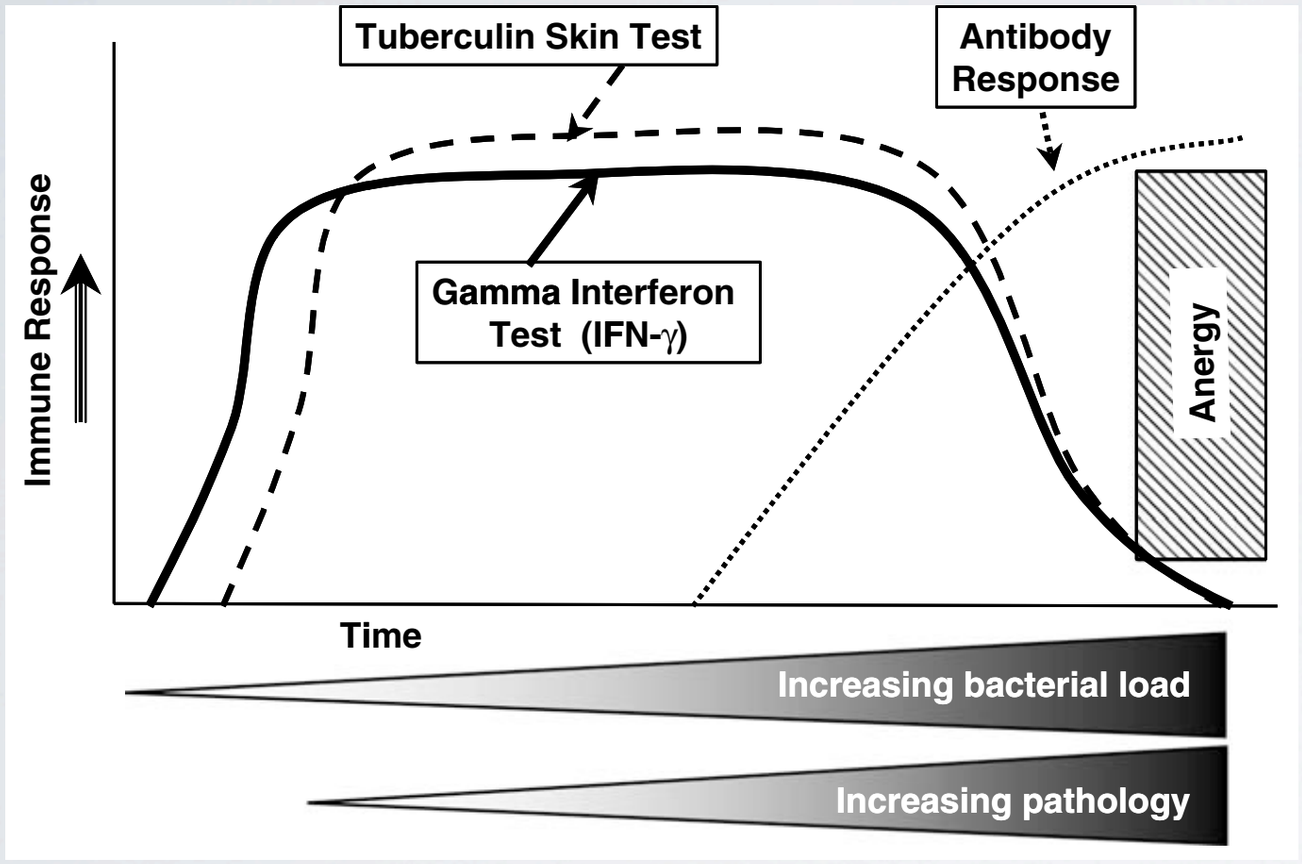

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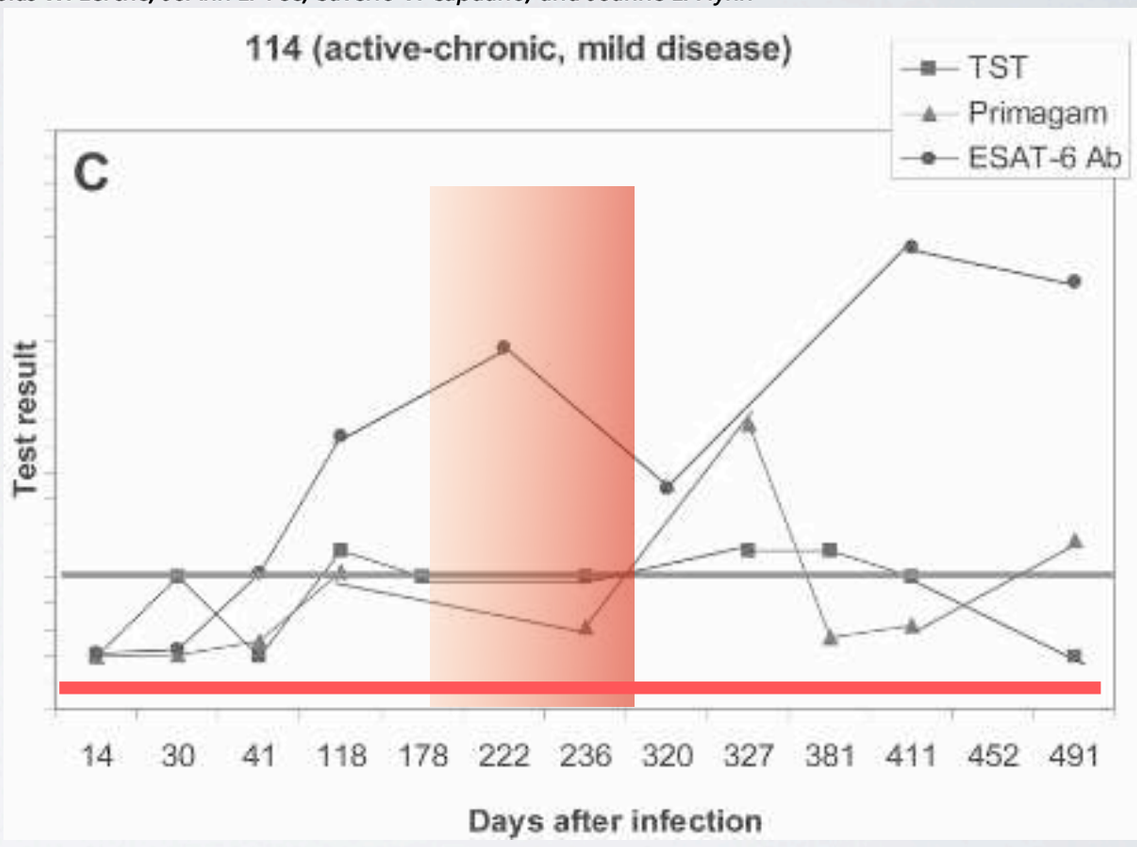

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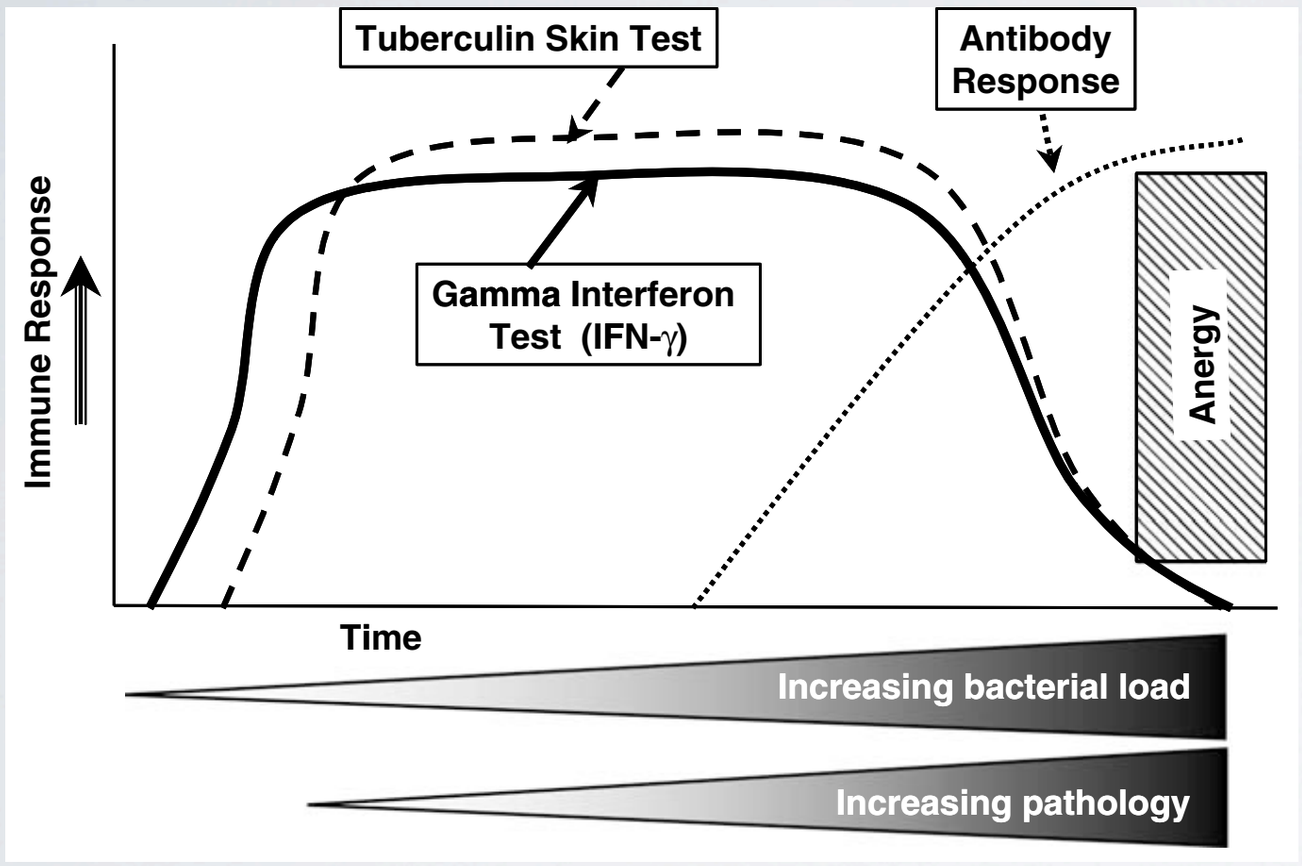

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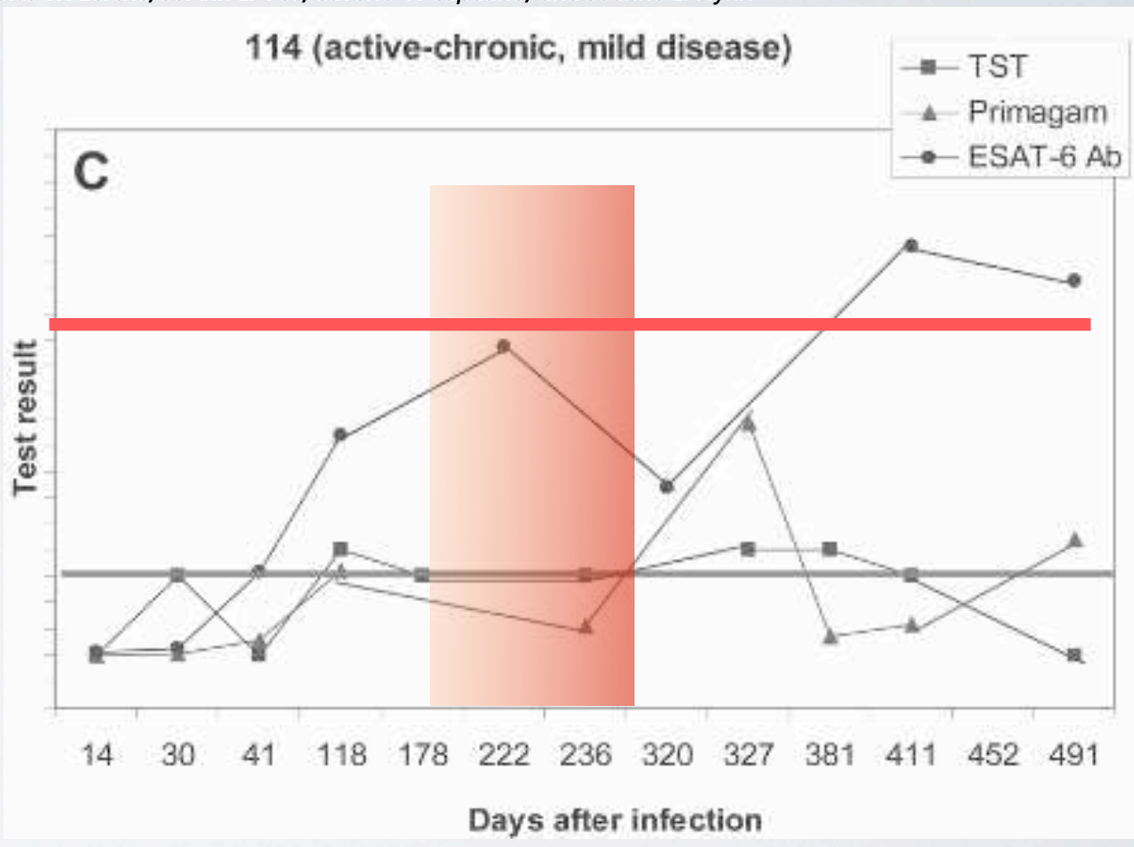

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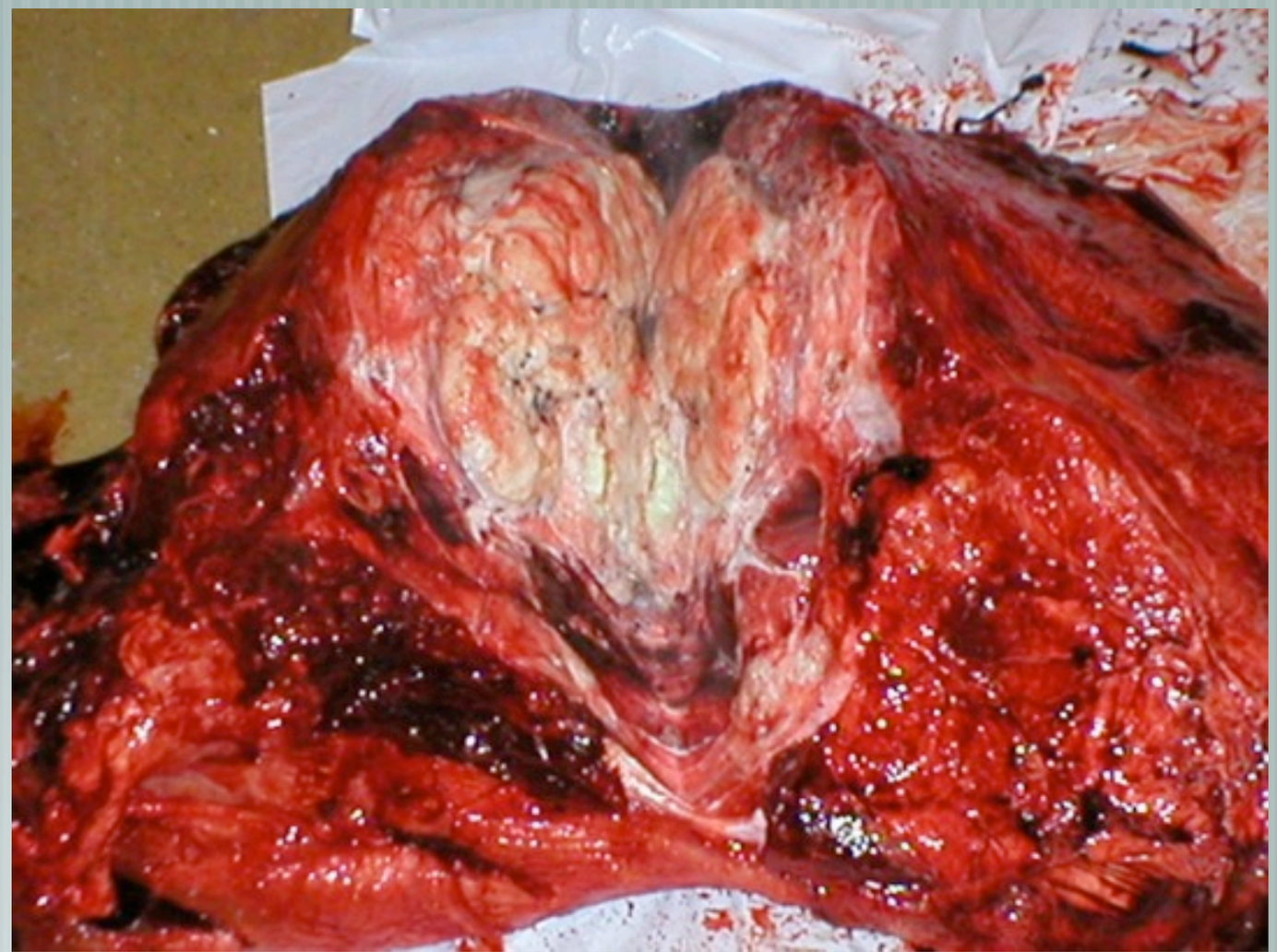
Eléphants

Latence possible



Eléphants

- Latence possible
- «Guidelines» USA et Europe

EEP-recommendations for the prevention of tuberculosis in captive elephants

26 June 2009

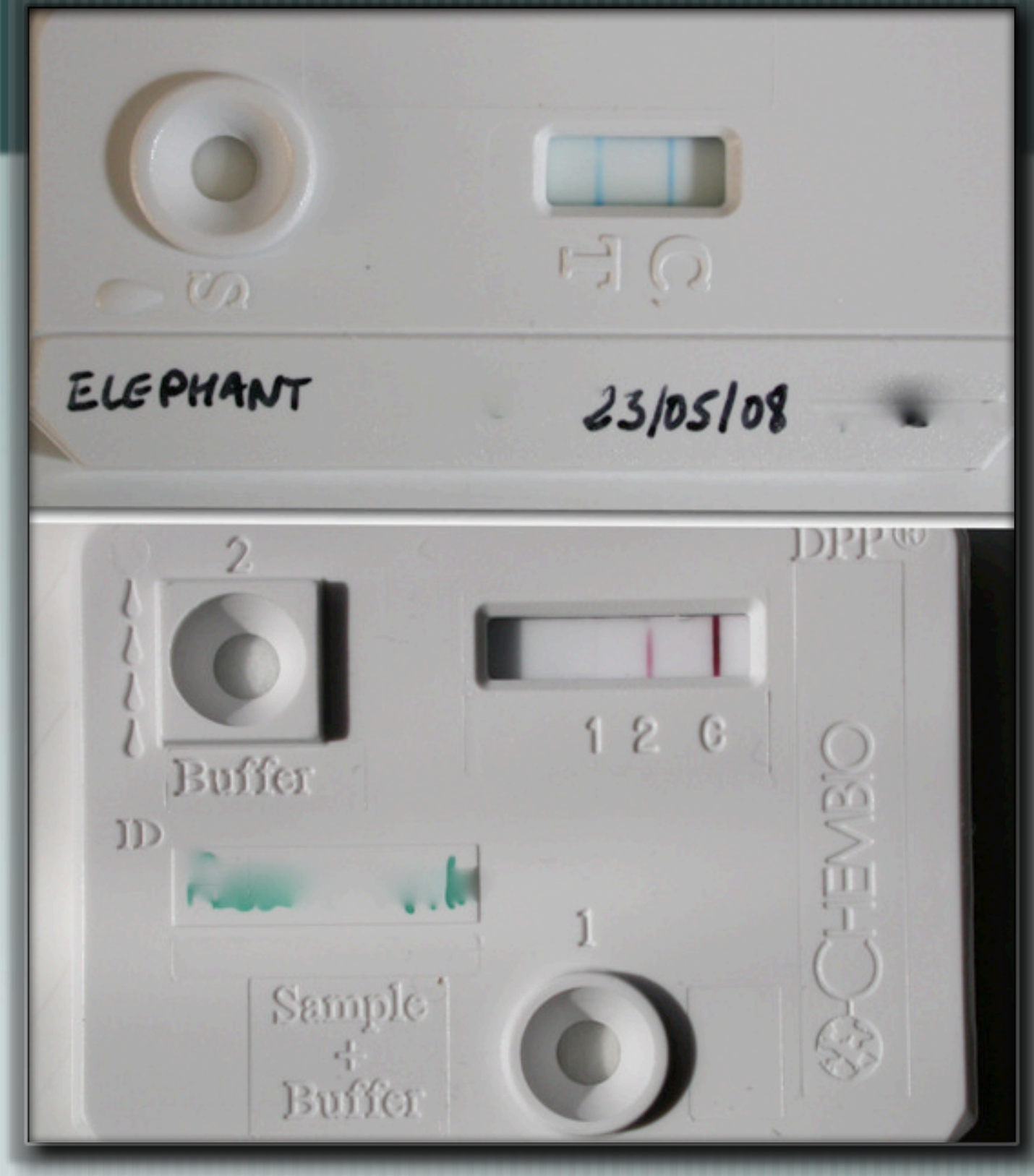
These recommendations for the control of tuberculosis in captive Asian en African elephants are aimed to prevent the spread of Mycobacterium spp. that can cause tuberculosis in mammals. The recommendations are based on the document "TB testing in captive elephants in the EEP, 23 July 2008", (see annex to this document and are reflecting the current possibilities for testing within Europe. The document will be updated when new relevant developments become available.
The interpretation of the available diagnostic tests is under constant evaluation and the panel of experts involved in TB-testing in elephants in recent years will be consulted when questions arise.

Glossery

Antibody test (serum or plasma):	
ELISA:	At present, the Central Veterinary Institute Lelystad is the only institute in Europe running this ELISA on a routine base; antigens used: M.bovis, MPB70 and M.paratuberculosis. Address: Central Veterinary Institute, DSU Edelhertweg 15, 8219 PH Lelystad, the Netherlands
Elephant TB STAT-PAK Assay:	Also known as ERT. Test to be performed by a qualified zoo veterinarian or veterinary institute. The test is available through the following website: www.zootest.com
Culture of suspected material to be sent to:	National Veterinary Laboratory
Tuberculin to be obtained from:	National Veterinary Institute
Trunk wash for culture and/or PCR	See definition in the annex

Eléphants

- Latence possible
- «Guidelines» USA et Europe
- Test sérologiques (STAT PAK® et DPP®) recommandés mais pas de validation hors USA.



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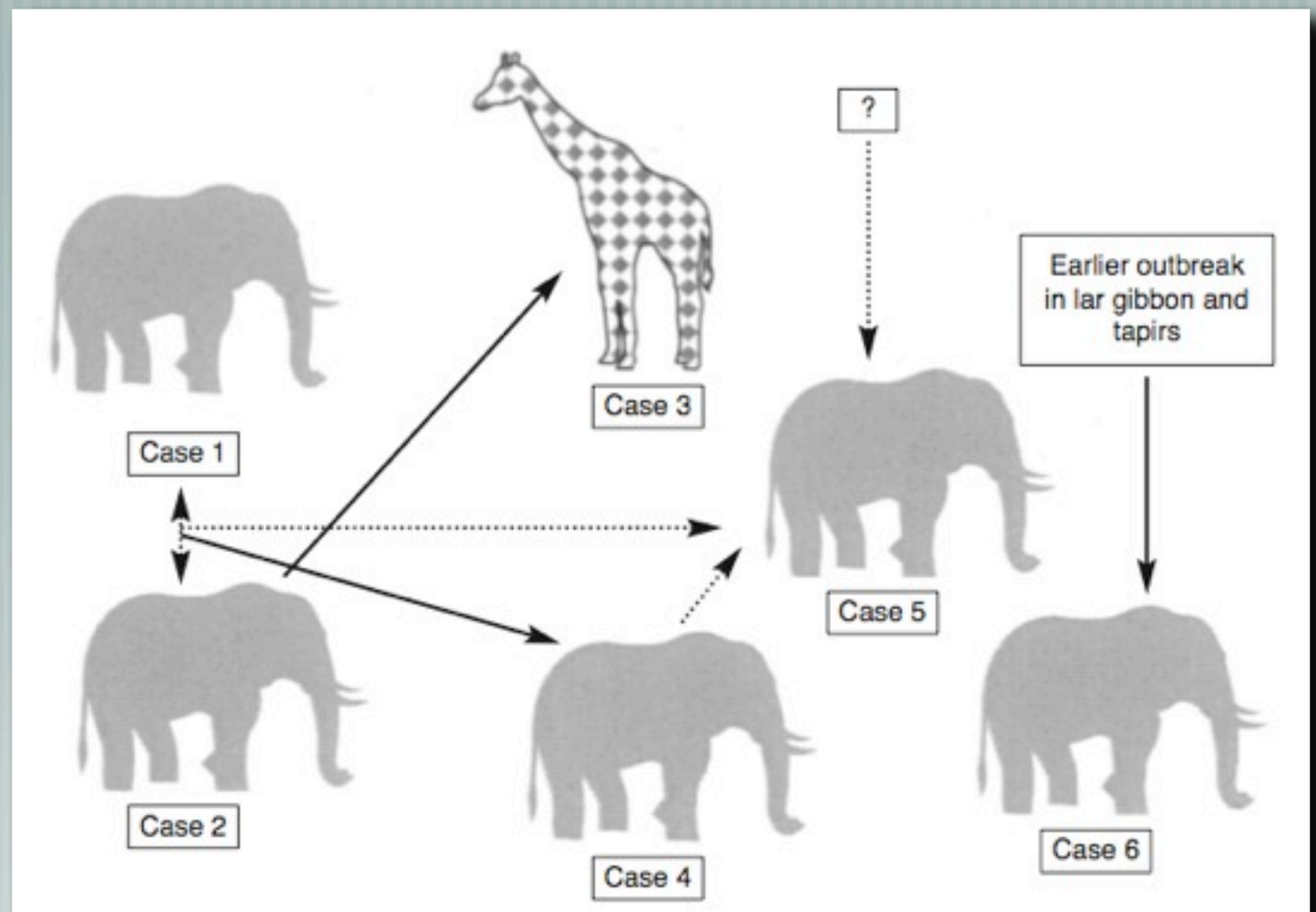

FIG 1: Probable routes by which the infection spread between the animals in the outbreak of tuberculosis. Dotted arrows indicate possible alternative routes of infection; solid arrows indicate probable routes of infection

Eléphants

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- Problématiques du traitement

Journal of Zoo and Wildlife Medicine 42(4): 709–712, 2011

GENITOURINARY AND PULMONARY MULTIDRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN AN ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

Genevieve A. Dumonceaux, D.V.M., Judy St. Leger, D.V.M., Dipl. A.C.V.P., John H. Olsen, D.V.M., Michael S. Burton, V.M.D., David Ashkin, M.D., and Joel N. Maslow, M.D., Ph.D., M.B.A.

Abstract: A female Asian elephant (*Elephas maximus*) developed vaginal and trunk discharge. Cultures were positive for pan-susceptible *Mycobacterium tuberculosis*. Isoniazid and pyrazinamide were given rectally and monitored by serum levels. After being trained at 10 mo to accept oral dosing, treatment was changed and rifampin was added. Oral medications were administered for another 10 mo. A year after completion of therapy, the vaginal discharge increased and cultures yielded *M. tuberculosis*, resistant to isoniazid and rifampin. Treatment with oral ethambutol, pyrazinamide, and enrofloxacin and intramuscular amikacin was initiated. Although follow-up cultures became negative, adverse reactions to medications precluded treatment completion. Due to public health concerns related to multidrug resistant *M. tuberculosis* (MDR-TB), the elephant was euthanized. Postmortem smears from the lung, peribronchial, and abdominal lymph nodes yielded acid-fast bacteria, although cultures were negative. This case highlights important considerations in the treatment of *M. tuberculosis* in animals and the need for a consistent approach to diagnosis, treatment, and follow-up.

Key words: *Elephas maximus*, elephant, tuberculosis, *Mycobacterium tuberculosis*, multidrug resistant tuberculosis.

INTRODUCTION

Tuberculosis (TB) in elephants, although recognized for many years, has posed significant therapeutic, diagnostic, and ethical dilemmas for caretakers, public health agencies, and regulatory bodies alike. In 1996, *Mycobacterium tuberculosis* infection was diagnosed in a herd of captive elephants and subsequently from five additional herds, prompting zoo, circus, and public health bodies to develop a systematic approach to disease.[8] Therapeutic regimens were developed based on those for humans and updated to reflect experience gained.[10] While techniques for diagnosis have progressed with the introduction of serologic testing, advances in treatment have lagged primarily because of the dearth of published data.[3] Major concerns have been the adequacy of treatment, optimal antibiotic combinations, and the possibility that resistant strains would emerge. Protocols for the treatment of tuberculosis in elephants were developed based on human regimens with efficacy determined by serum drug levels and guided by pharmacokinetic studies.[4,5,9,10,11] Because of poor acceptance of oral dosing, rectal regimens were explored and yielded drug levels similar to oral dosing for water-soluble drugs such as isoniazid (INH) and pyrazinamide (PZA).[4,11]

Drug administration issues in elephants can increase the risk for drug resistance, including multidrug resistant TB (MDR-TB). Multidrug resistant tuberculosis is an infection with *M. tuberculosis* that is resistant to isoniazid and rifampin with or without resistance to other TB drugs. Reports of zoonotic spread of *M. tuberculosis* between elephants and humans raises the interspecies spread of MDR-TB as a potential concern.[6] This report describes a case of pulmonary and genitourinary TB in an Asian elephant complicated by recurrent infection with MDR-

From Busch Gardens Tampa Bay, 3605 East Bougainvillea Avenue, Tampa, Florida 33612, USA (Du-

Pinnipèdes ❶ Epidemiologie

- Premiers cas = Allemagne & France

Pinnipèdes ❶ Epidemiologie

- Premiers cas = Allemagne & France
- Foyer majeur aux Pays Bas

INT J TUBERC LUNG DIS 12(12):1469–1473

Transmission of *Mycobacterium pinnipedii* to humans in a zoo with marine mammals

A. Kiers,* A. Klarenbeek,† B. Mendelts,‡ D. Van Soolingen,§ G. Koëter‡

*Department of Tuberculosis Control, GGD Fryslân, Leeuwarden, †Zoo Emmen, Emmen, ‡Department of Tuberculosis Control, GGD Groningen, Groningen, §National Mycobacteria Reference Laboratory, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands

SUMMARY

OBJECTIVES: An outbreak of tuberculosis (TB) in sea lions occurred recently in a zoo in the Netherlands. The disease was detected in a captive colony consisting of 29 animals kept in an open air basin with an indoor night house. Approximately 25 animal keepers were in close contact with the animals.

METHODS: The sea lions were investigated using the tuberculin skin test (TST) with avian and bovine purified protein derivative (PPD) and, in case of positivity, necropsied. A survey was conducted among the animal keepers including TSTs with *Mycobacterium tuberculosis* complex PPD tuberculin, a chest X-ray and an interferon-gamma release assay (IGRA).

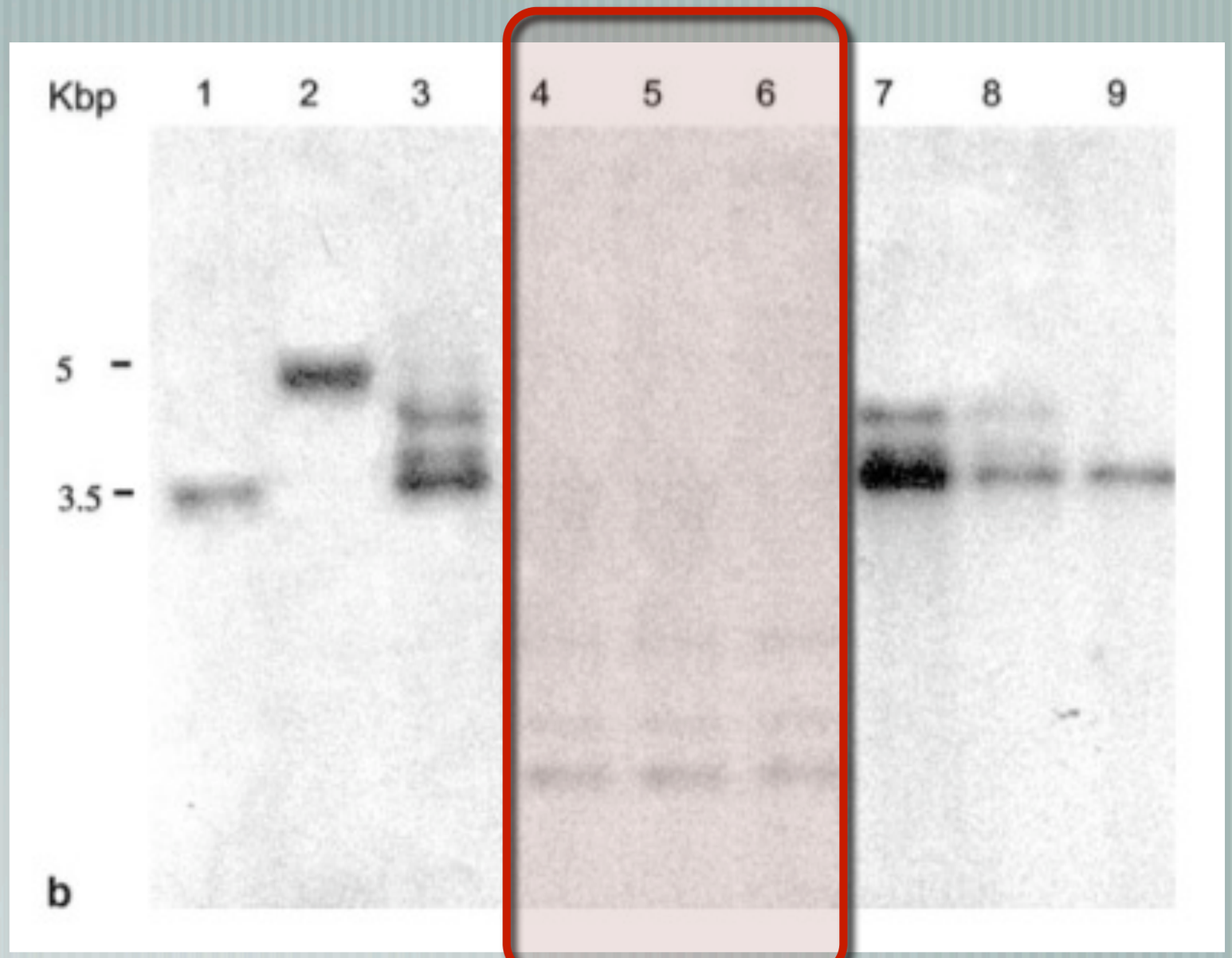
RESULTS: Necropsy was positive for TB in 13 of the 29 sea lions. Three cases of pulmonary involvement were found. Only one of these was infectious and it was therefore regarded as the source case. The causative mycobacterium was identified as *M. pinnipedii*. Six of the 25 animal keepers were TST-positive; in five of these, infection was confirmed by a positive IGRA.

CONCLUSION: Transmission of *M. pinnipedii* infection from sea lions to humans was established by TST. IGRA results largely agreed with the TST results. Nebulisation when cleaning the sea lions' enclosure was most likely the main cause of transmission to humans.

KEY WORDS: tuberculosis; *Mycobacterium pinnipedii*; transmission; latent tuberculosis infection; interferon-gamma assay

Pinnipèdes ❶ Epidemiologie

- Premiers cas = Allemagne & France
- Foyer majeur aux Pays Bas
- *M.pinnipedii*



Pinnipèdes ❶ Epidemiologie

- Premiers cas = Allemagne & France
- Foyer majeur aux Pays Bas
- *M.pinnipedii*
- Concerne surtout *O.byronia*
- Cas index : animaux importés
- Chili >> Uruguay >> Pérou

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- Premiers cas = Allemagne & France
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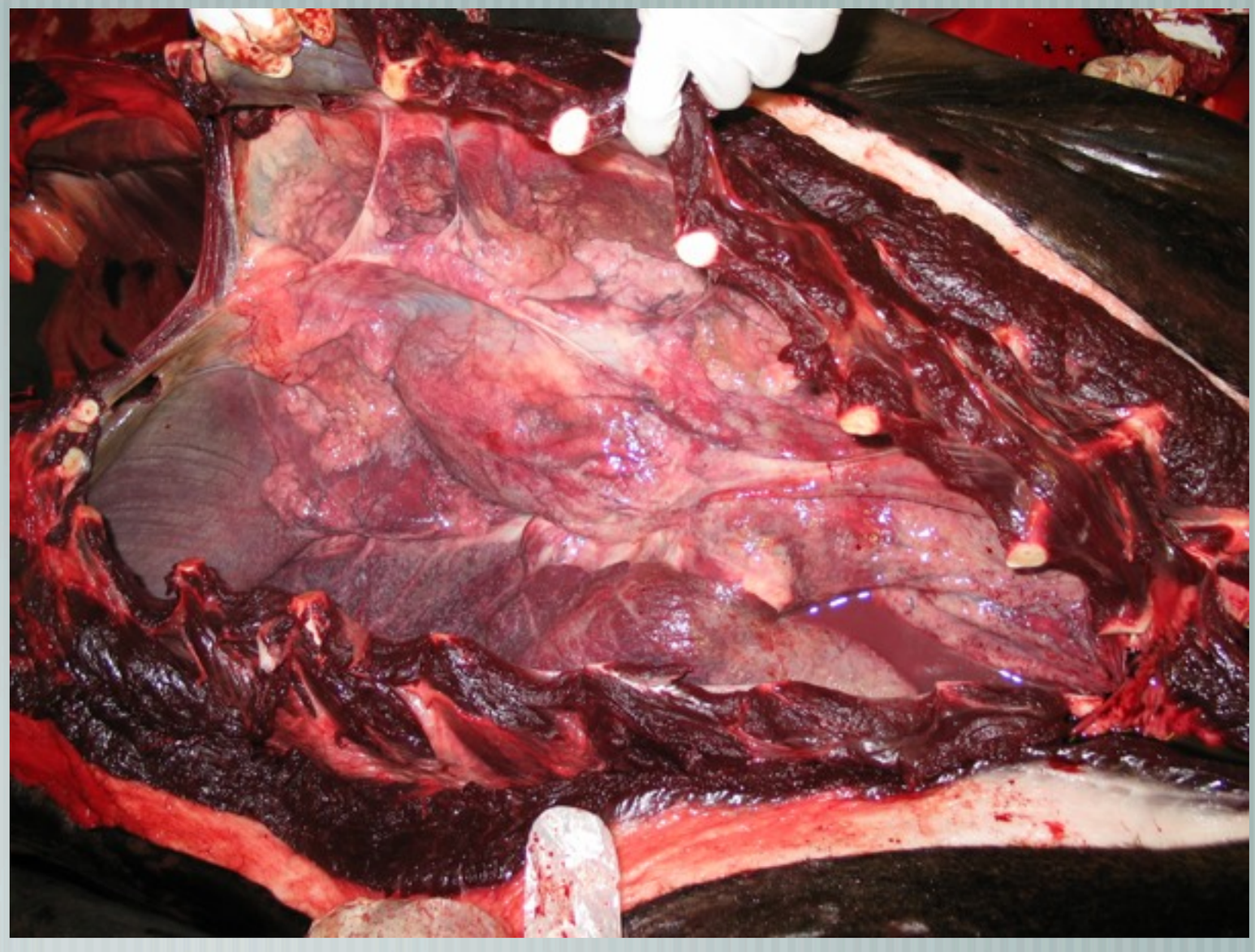
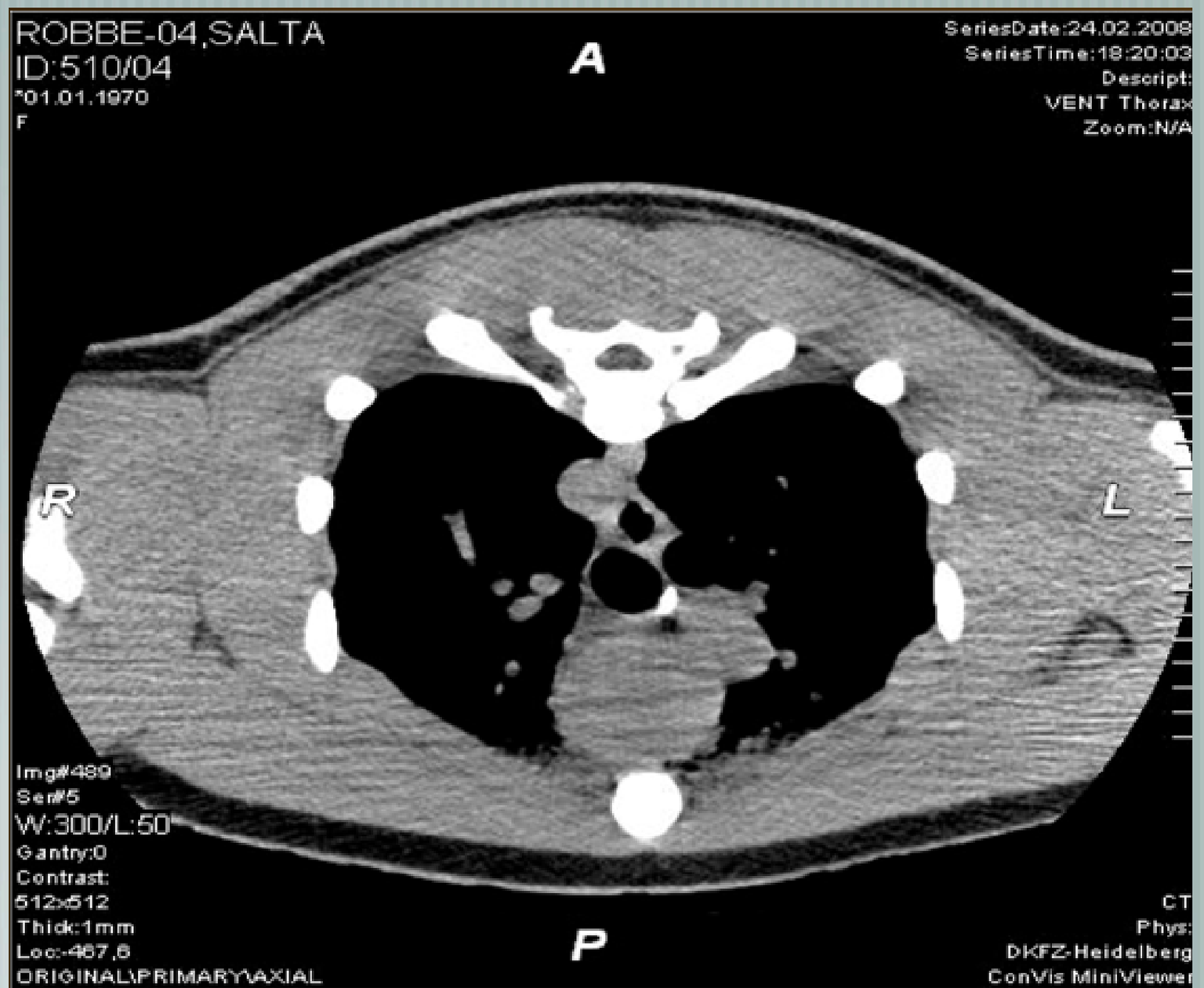


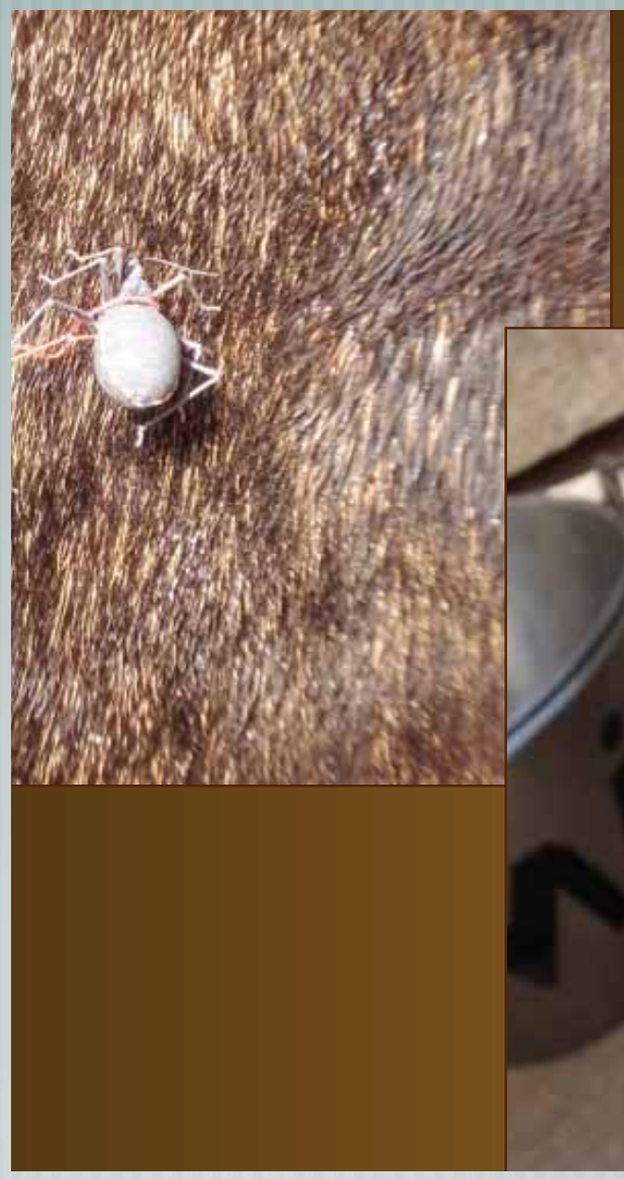
Pinnipèdes ❷ Diagnostic

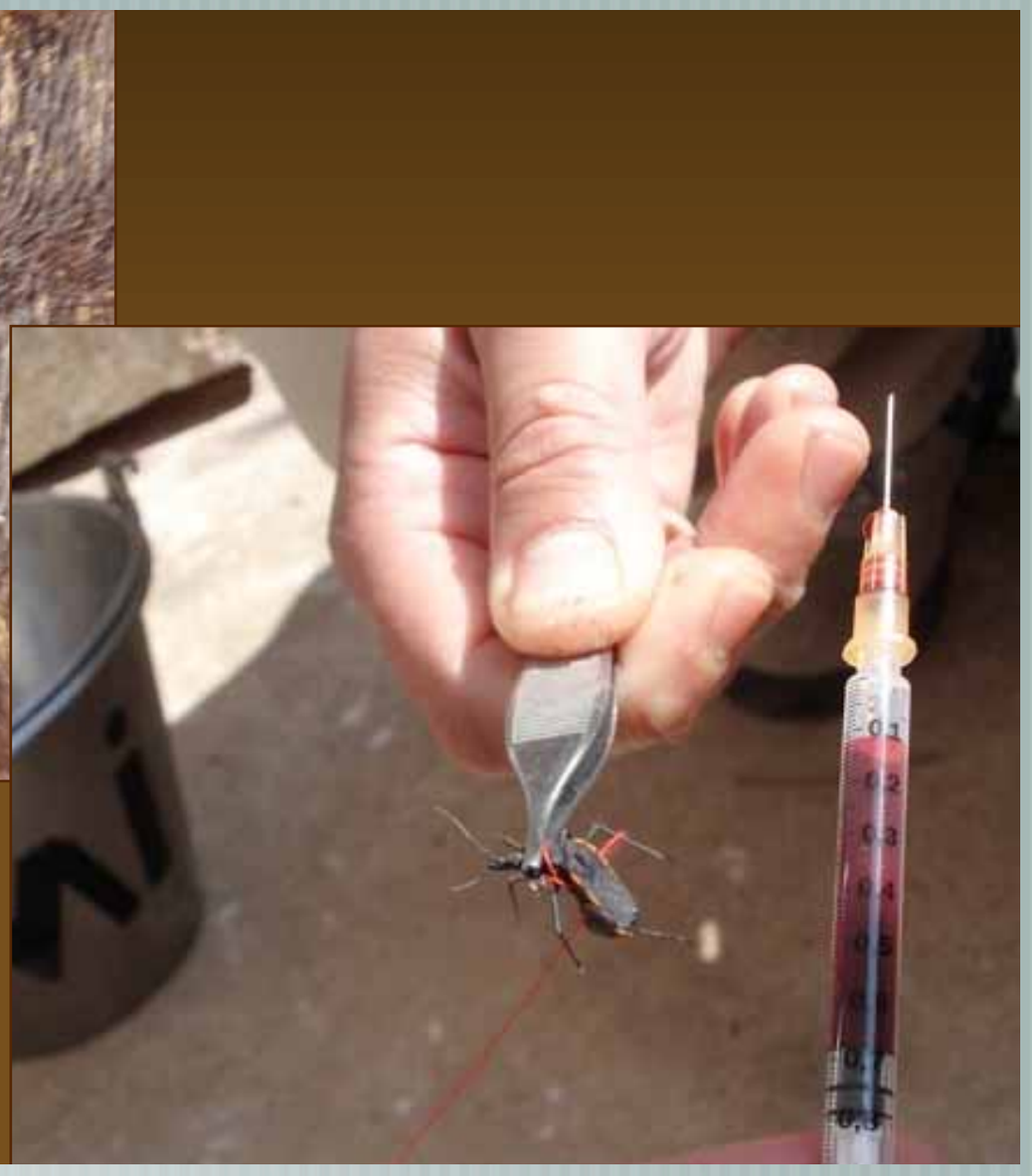
Imagerie : scanner



Pinnipèdes ❷ Diagnostic

- Imagerie : scanner
- Serologie



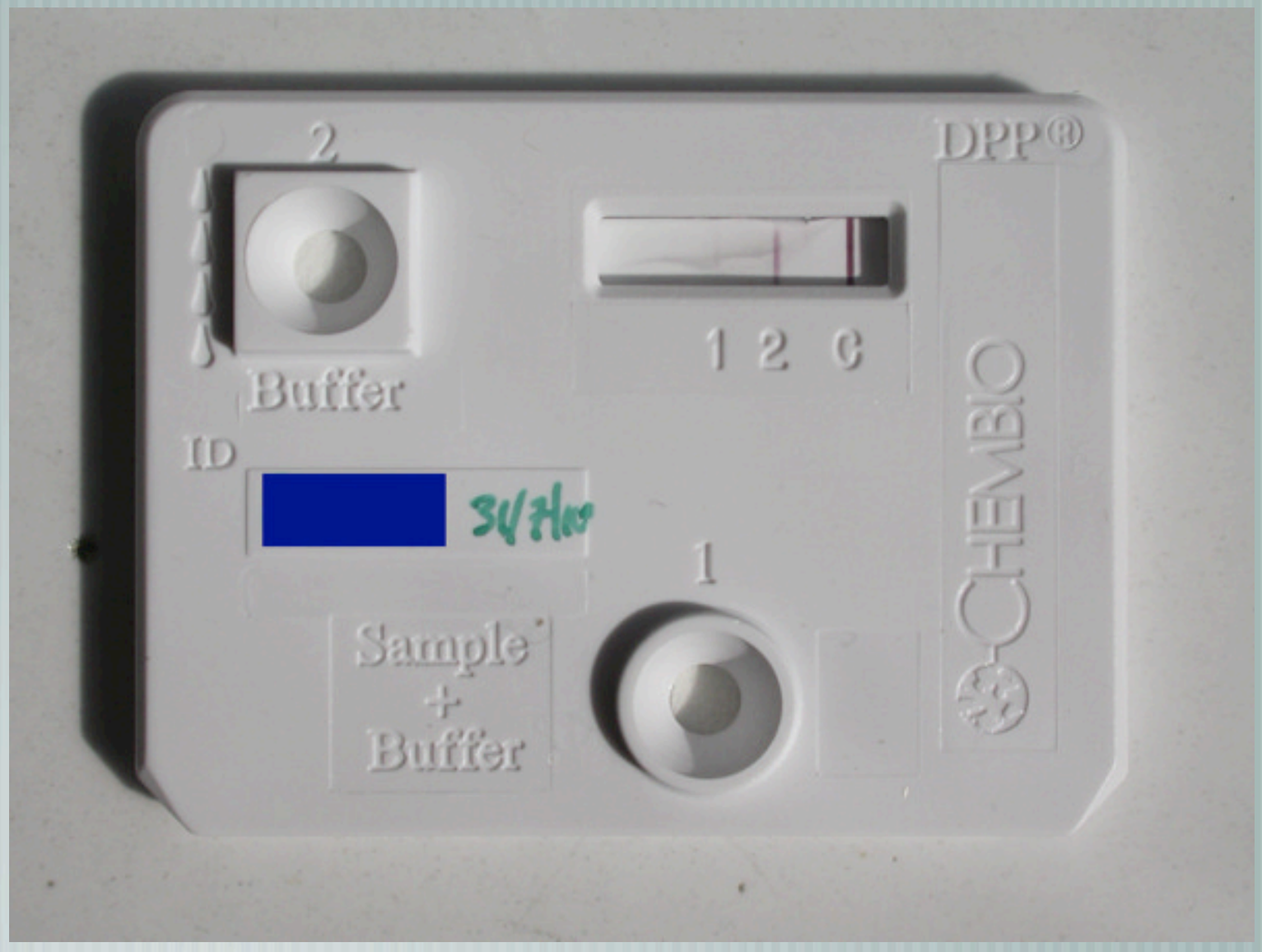


Pinnipèdes ❷ Diagnostic

- Imagerie : scanner
- Serologie
- Elephant STATPAK®, DPP®

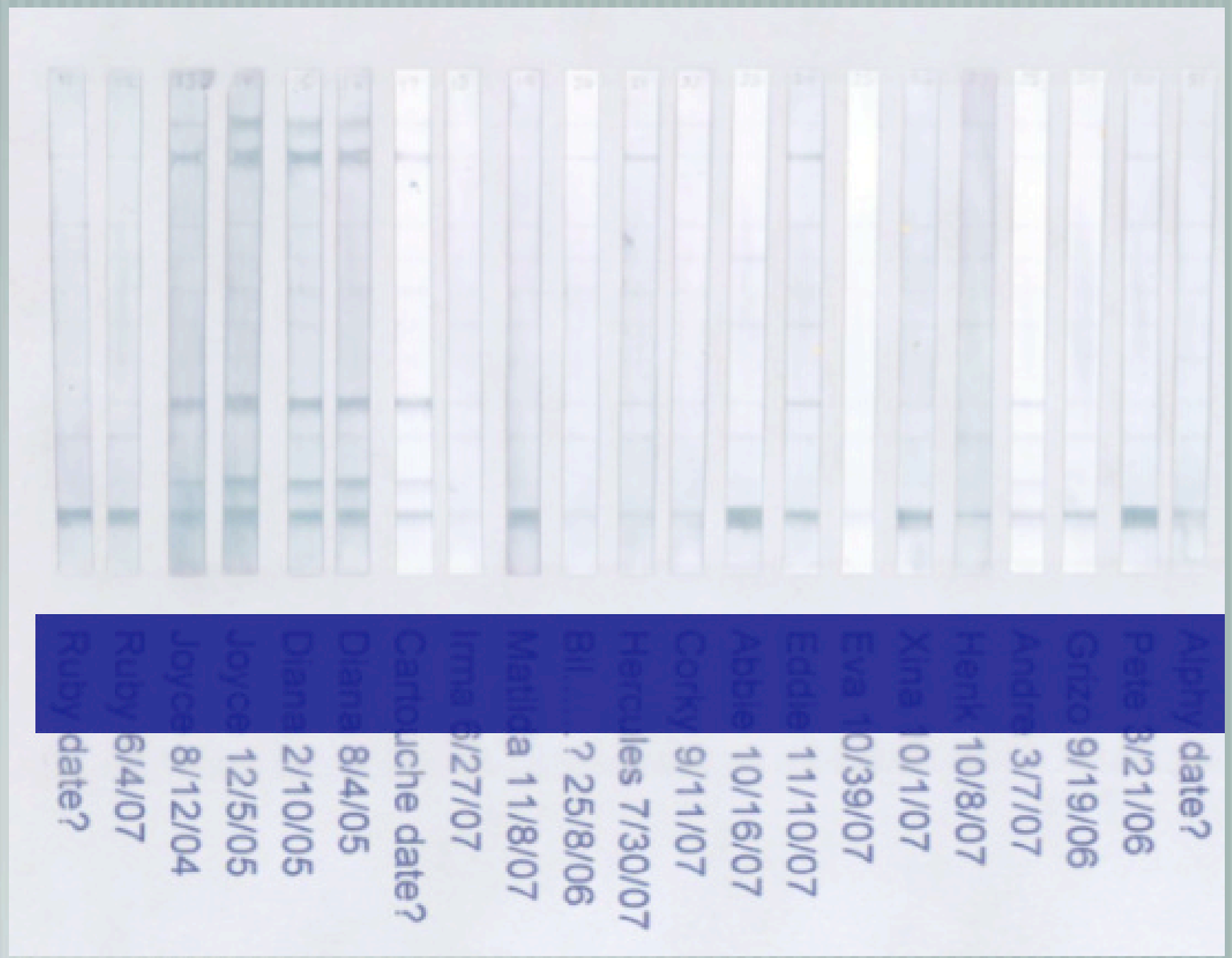
Pinnipèdes ❷ Diagnostic

- Imagerie : scanner
- Serologie
- Elephant STATPAK®, DPP®



Pinnipèdes ❷ Diagnostic

- Imagerie : scanner
- Serologie
- Elephant STATPAK®, DPP®



Pinnipèdes ❷ Diagnostic

- Imagerie : scanner
- Serologie
- Elephant STATPAK®, DPP®
- Détection directe

Pinnipèdes ❷ Diagnostic

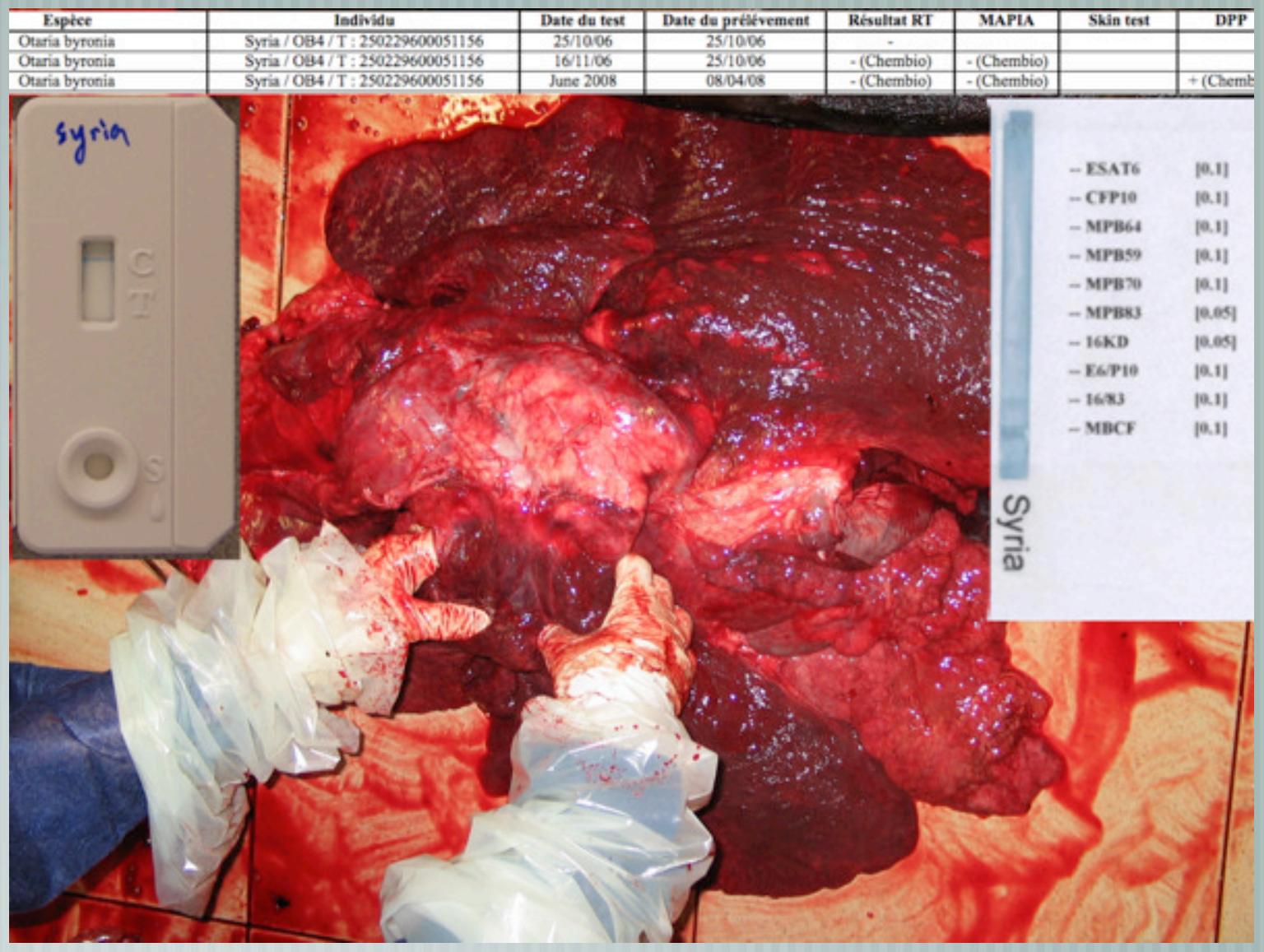
- Imagerie : scanner
- Serologie
- Elephant STATPAK®, DPP®
- Détection directe
- Faux positifs

Sealions Booster Study Results Part 1

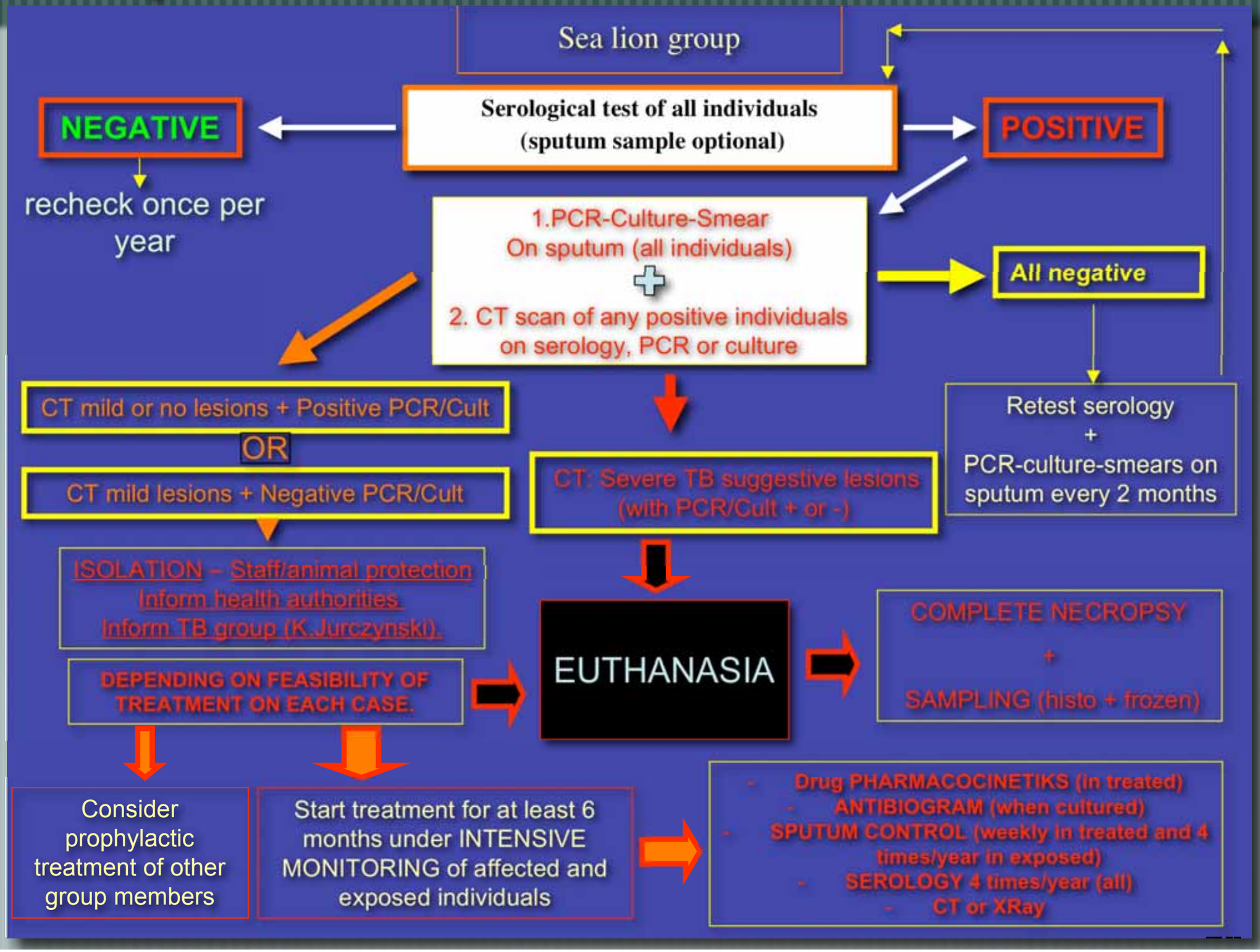
Animal	1st Blood sample			Skin Test		2nd blood sample		
	Date J0	results		Date	results	Date	results	
		DPP	RT				DPP	RT
Fridolin	11/10/09	-	-	16/11/09	-	07/12/09 =J+21	1 :+++ 2: +	-
Speedy	29/10/09	1 : - 2: +	-	16/11/09	+++	03/12/09 =J+17	1: +++ 2: +	+/-
Algon	4/11/09	-	-	16/11/09	+	21/12/09 =J+35	1:+++ 2: +	+
Xino	04/11/09	-	-	16/11/09	+/-	03/12/09 =J+17	1: + 2 :-	-
Manolito	23/10/09	1: - 2: +	-	16/11/09	+++	07/12/09 =J+21	1:++ 2: -	+/-

Pinnipèdes ❷ Diagnostic

- Imagerie : scanner
- Serologie
- Elephant STATPAK®, DPP®
- Détection directe
- Faux positifs
- Faux négatifs

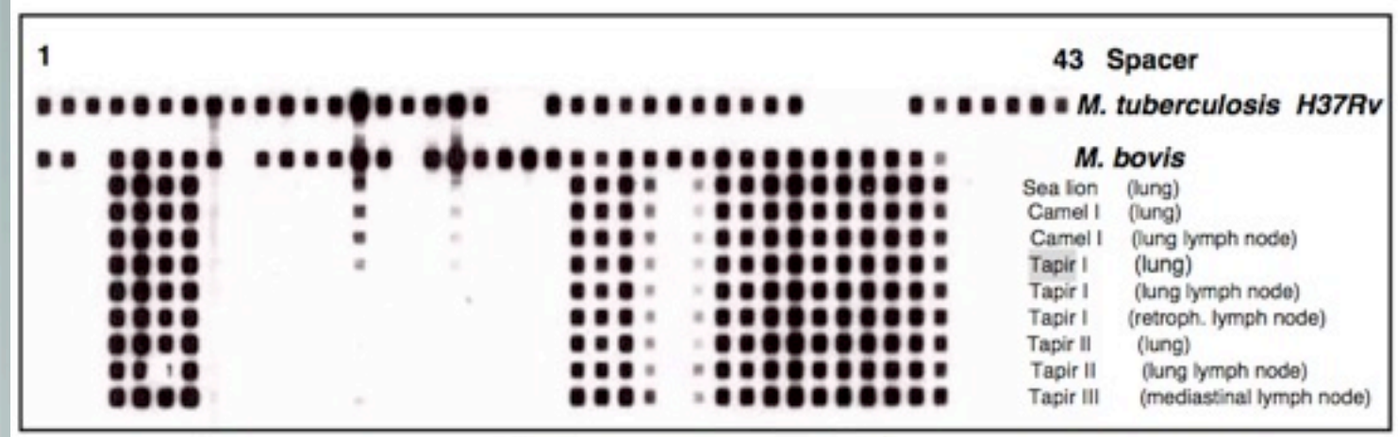


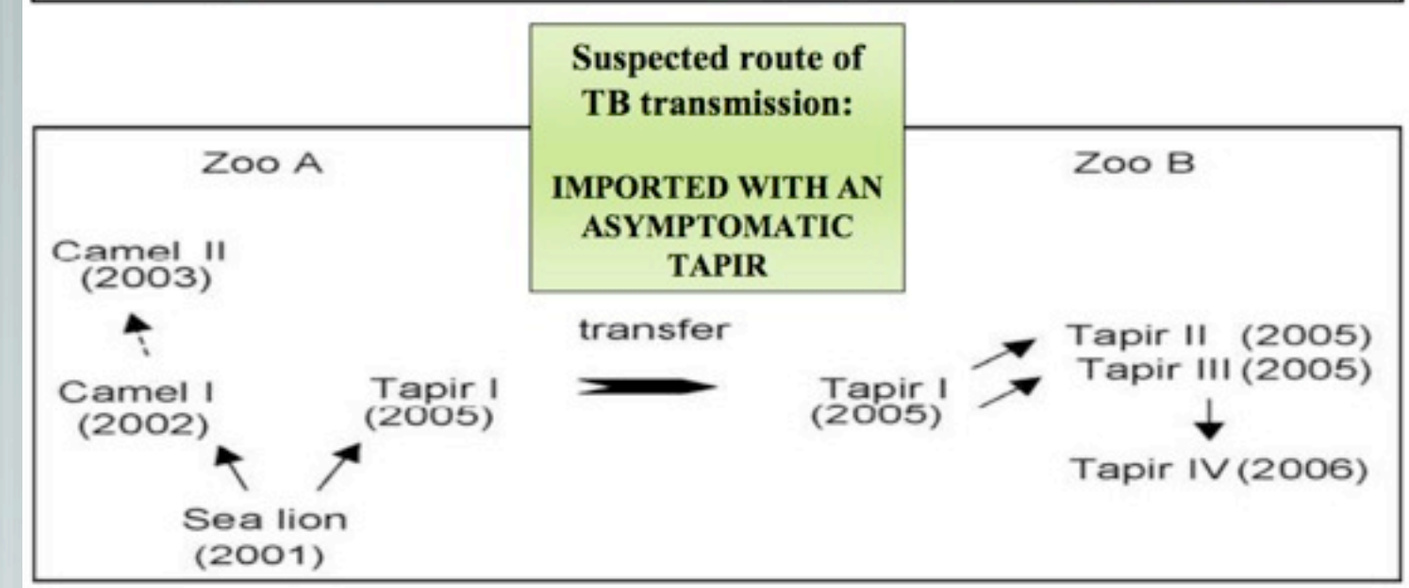
Pinnipèdes ❸: conduite à tenir



Tapirs ❶

M.bovis, M.tuberculosis + transmission de *M.pinnipedii* depuis otaries





Tapirs ❶

- *M.bovis, M.tuberculosis* + transmission de *M.pinnipedii* depuis otaries
- Serologie, IDR

Tapirs ❶

- *M.bovis, M.tuberculosis* + transmission de *M.pinnipedii* depuis otaries
- Serologie, IDR
- exposition de l'espèce (biologie) ⇨ impact sur la specificité des tests?

Date	Sample	Test	Hutan
June 2008	Sputum from floor	Microscopy	Scanty AFB
		Culture	-ve
		MTBC PCR	NA
July 2008	Bronchiolar lavage	Microscopy	-ve for AFB
		Culture	*M. chelonae*
		MTBC PCR	-ve
Nov 2008	Bronchiolar lavage	Microscopy	-ve for AFB
		Culture	*M. avium*
		MTBC PCR	-ve
May 2009	Bronchiolar lavage	Microscopy	-ve for AFB
		Culture	-ve
		MTBC PCR	-ve
January2010	Blood clot (epistaxis)	Microscopy	-ve for AFB
		Culture	NA
		MTBC PCR	NA

Tapirs ❷: Conduite à tenir

location	sex	house name	Int #						tb status				
Amsterdam	M	DICK	466	Mulhouse 91	Amsterdam 93				POS	1			
	F	AYA	656	Belfast 02	Amsterdam 04				POS	1			
	M	TIOMAN	919	Amsterdam 08					NEG TO FOLLOW		1		
Antwerp	M	NIKO	498	Berlin 96	Antwerp 99	Rotterdam 06	Antwerp 07	POS 07 *M. avium*	NEG TO FOLLOW			1	
	F	SINGORA	472	Rotterdam 93	Antwerp 95			POS 09 *M. avium*	NEG TO FOLLOW			1	
Belfast	M	ELMER	414	Milwaukee 90	Minnesota 91	Mulhouse 92	Belfast 95		NEG		1		
	F	GLADYS	434	Toronto 92	Belfast 94				NEG		1		
	F	HARAPAN		Belfast 09					not tested yet (young)				1
Bekesbrne (Howletts)	M	HUTA	839	Jakarta 78	Bekesbrne 92				???				1
	F	RINA	841	Jakarta 81	Bekesbrne 92				???				1
	F	SUSAN	842	Jakarta 83	Bekesbrne 92				???				1
Copenhage	M	GIRANG	470	Dortmund 93	Copenhage 94				NEG		1		
	F	NURR	643	Singapore 00	Copenhage 06				NEG		1		
Dortmund	M	JINAK	783	Dortmund 00					NEG		1		
	F	ARIA	490	Oklahoma 96	Munich 97	Dortmund 00		POS 08 *M. chelonae*	NEG TO FOLLOW			1	
	M	MOWGLI		Dortmund 09					not tested yet (young)				1
Dresden	F	TKN 2	156	Wulff ~72	Dresden 72				???				1
Edinburgh	M	KAKA	786	Dortmund 03	Edinburgh 04				NEG (RT 4/3/09)		1		
	F	SAYANG	741	London 03	Edinburgh 04				NEG (RT 26/1/09)		1		
Fuengirola	F	ESPERANZA	620	Melaka 00 (wb)	Fuengirola 04				DOUBT POS			1	
	F	DEWI	507	Singapore 98	Fuengirola 03				DOUBT POS			1	
Lisieux	M	BENGTIE	824	Copenhage 07	Lisieux				NEG		1		
	F	INDIRA	815	Antwerp 07	Lisieux 09			POS 09 *M. avium*	NEG TO FOLLOW			1	
London	M	HUTAN	485	Dortmund 95	London 97			POS 09 *M. avium* & *M. chelonae (culture)*	POS	1			
	F	GERTRUDE	748	Belfast 04	London 05				NEG (skin test & RT January 2009)		1		
	M	HUNTER	900	London 08					NEG (skin test & RT January 2009)		1		
Madrid	M	PETER	422	Sandiego 91	Heidelberg 93	Madrid 00			NEG		1		
	F	TAPM3	488	Nurnberg 96	Madrid 98				NEG		1		
Munich	M	NEMO	475	Nurnberg 94	Munich 95				???				1
	F	CORA	568	Pretoria 89	Munich 91				???				1
Nurnberg	M	HENK	501	Amsterdam 97	Nurnberg 98			test on serum 2005	NEG ??? TO RETEST		1		
	F	INDAH	508	Dortmund 98	Nurnberg 98				NEG		1		
	M	BANJU	788	Nurnberg 05	Fuengirola 08	Nurnberg 08			POS	1			
	M	PI	825	Belfast 07	Nurnberg 09				NEG, skin test June 2009, blood stored		1		
	F	1/09		Nurnberg 09					not tested yet (young)				1
Overloon	F	TUMPAT II	814	Amsterdam 07	Overloon 07				NEG TO FOLLOW		1		
	M	TOBI	204	Amsterdam 75	Munich 76	Aalborg 96	Overloon 04		NEG		1		
	M	LOMBOK	799	Dortmund 06	Overloon 08				NEG		1		
Port Lympne	M	DAENG	460	Singapore 92	Lympne 97				NEG		1		
	M	KINGUT	583	Jakarta ? (wb)	Lympne 03	Bekesbrne 03	Sandwich 07		NEG		1		
	F	LIDAENG	637	Lympne 00					NEG		1		
	F	MULACCA	655	Lympne 02	Bekesbrne 06	Sandwich 07			NEG		1		
	F	TENGUI	792	Lympne 06					NEG		1		
	F	ISTANA	497	San Diego 96	Marwell 98	Lympne 04			NEG		1		
Pretoria	M	CAESAR	293	Dortmund 82	Pretoria 83				???				1
	F	CHIVAZ	502	Pretoria 97					???				1
Rotterdam	M	JAMES / RAI	478	Singapore 95	Marwell 97	Rotterdam 02	Antwerp 06	Rotterdam 07	NEG		1		
	F	GANESHA	747	Antwerp 05	Rotterdam 07				NEG		1		
Sandwich	M	VASAN		Edinburgh 07	Sandwich 08				NEG		1		
	F	INDAH		Edinburgh 08	Sandwich 09				NEG (RT 6/5/09)		1		
Stuttgart	M	THAI	555	Bangkok 89	Stuttgart 89	Mulhouse 02	Stuttgart 03		not planned to be tested, no breeding				1
Twycross	F	MADGE	654	Nurnberg 02	Twycross 03				POS	1			
	M	COLIN	634	Amsterdam 99	Heidelberg 00	Twycross 07			POS	1			
21 participants										8	29	6	12

Tapirs ❷: Conduite à tenir

Flow-Chart Tuberculosis in Malayan Tapirs

Clinically healthy?

No → General health check (Blood-test, faeces, x-ray, ultrasound ...)

Yes → Comparative skin-test + Rapid-Test + ELISA (negative = MBP70<5)

All tests negative → **NEGATIVE**

One or more tests positive → **DOUBTFUL**

Culture (positive) / AF-Stain (positive) → PCR (positive)

negative → Institutional cycle: Two negative tests after 6 and 12 months (skin-test + RT + ELISA)

POSITIVE → Institution has to decide about euthanasia or treatment

Gestion de 3 populations

Autres espèces

Daman & Dassie bacillus

Autres espèces

- Daman & Dassie bacillus
- Camelidés & *M.microti*
- Ongulés & *M.bovis* :
- Mycobactéries environnementales

Carnivores sauvages..

Félidés

Carnivores sauvages..

Félidés

DISPATCHES

Pulmonary Tuberculosis due to *Mycobacterium bovis* subsp. *caprae* in Captive Siberian Tiger

Ákos Lantos,* Stefan Niemann,† László Mezõsi,‡ Endre Sós,‡ Károly Erdélyi,§ Sándor Dávid,¶ Linda M. Parsons,# Tanja Kubica,† Sabine Rüsch-Gerdes,† and Ákos Somosköví***

We report the first case of pulmonary tuberculosis caused by *Mycobacterium bovis* subsp. *caprae* in a captive Siberian tiger, an endangered feline. The pathogen was isolated from a tracheal aspirate obtained by bronchoscopy. This procedure provided a reliable in vivo diagnostic method in conjunction with conventional and molecular tests for the detection of mycobacteria.

Mycobacterium bovis, a member of the *M. tuberculosis* complex (MTBC), can cause tuberculosis in a wide range of domestic and wild animals and also in humans (1,2). Routine differentiation of *M. bovis* is based on a number of phenotypic characteristics and biochemical tests (2). *M. bovis* shows dysgonic growth on Löwenstein-Jensen (LJ) medium and has been described as negative for nitrate reduction and niacin accumulation (2). As a further criterion for the differentiation of *M. bovis*, intrinsic resistance to pyrazinamide (PZA) has been described (2). However, more recently, PZA-susceptible strains of *M. bovis* were found in Spain and Germany; these strains were also characterized by specific molecular techniques (3–5). As a consequence, *M. bovis* was split into two subspecies: *M. bovis* subsp. *bovis*, which showed resistance to PZA, and *M. bovis* subsp. *caprae*, which was sensitive to PZA (6,7). *M. bovis* subsp. *caprae* was initially isolated from sheep and goats in Spain (3,4,7); however, further studies confirm its infectivity in humans, cattle, and red deer (6,8). We report the unusual case of a *M. bovis* subsp. *caprae* infection in a captive Siberian tiger.

*Semmelweis University, Budapest, Hungary; †National Reference Center for Mycobacteria, Borstel, Germany; ‡Budapest Zoological and Botanical Garden, Budapest, Hungary; §Central Veterinary Institute, Budapest, Hungary; ¶Korányi National Institute for Tuberculosis and Respiratory Medicine, Budapest, Hungary; #New York State Department of Health, Albany, New York, USA; and **University at Albany, Albany, New York, USA

Case Report

An 8-year-old male Siberian tiger at the Budapest Zoological and Botanical Garden had episodes of coughing in October 2001. Because the coughing did not stop in 6 to 7 days, an expectorant (Bisolvon; Boehringer Ingelheim Vetmed Gmbh., Ingelheim am Rhein, Germany) was given for 10 days. His condition showed a temporary improvement; however, after a few weeks, the animal started coughing again, and his appetite decreased. Amoxicillin plus clavulanic acid (Amoksiklav; Lek Animal Health, Ljubljana, Slovenia) and ketoprophen (Ketofen, Merial, Lyon, France) therapy was given for 7 days. The tiger's condition did not show any notable improvement. In addition, in May 2002, the animal's respiratory rate became elevated, he became dyspneic and emaciated, and his daily activity substantially decreased. Further antibacterial treatment was administered (cefatroxil, Cefa-cure; Intervet, Boxmeer, the Netherlands) during that month without clinical effect. At that point, the animal was anesthetized, and tracheoscopy was performed with a flexible 56-cm bronchoscope (Olympus B3R; Tokyo, Japan (Figure 1). The examination found a large amount of purulent mucus in the trachea. Therefore, several tracheal washings were taken for microbiologic tests by using a commercially available tracheal suction set (Medinorm Medizintechnik GmbH, Quierschied, Germany (Figure 1). A chest radiograph showed a severe and extensive bronchointerstitial pattern with cavernous lesions in both lungs.

Nine days after the specimens were taken, cultures for mycobacteria showed growth in the broth-based MGIT 960 system (Becton-Dickinson Microbiology Systems, Sparks, MD). The acid-fast organism that was isolated was identified as MTBC by the AccuProbe TB assay (Gen-Probe Inc., San Diego, CA).

Since the tiger had stopped eating and his condition had dramatically deteriorated, the animal was euthanized and

Figure 1. Obtaining a tracheal washing of the Siberian tiger by bronchoscopy.

Carnivores sauvages..

- Félidés
- Herpestidés

Carnivores sauvages..

- Félidés
- Herpestidés
- Mustelidés
- Procyonidés

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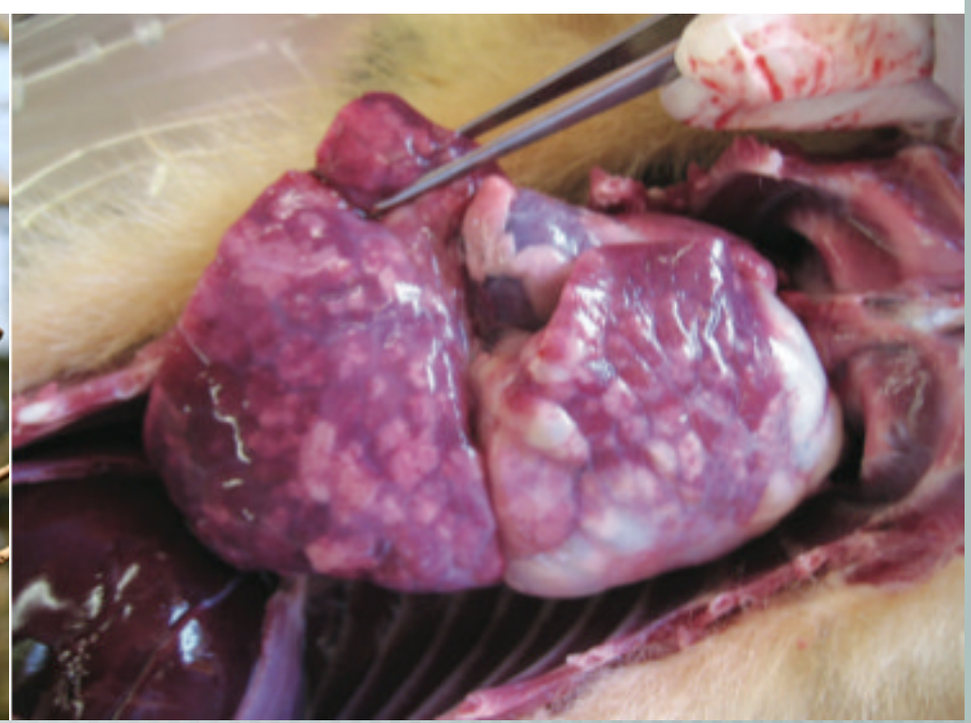
AN OUTBREAK OF TUBERCULOSIS BY *MYCOBACTERIUM BOVIS* IN COATIS (*NASUA NASUA*)

Patrícia Sayuri Murakami, D.V.M., M.Sc., Fernanda Monego, D.V.M., M.Sc., John L. Ho, M.D., Andrea Gibson, B.Sc., Ph.D., Ricardo Guilherme D'Otaviano de Castro Vilani, D.V.M., Ph.D., Grazielle Cristina Garcia Soresini, D.V.M., Sonia Regina Brockelt, Pharmac. Bioch., Sonia Maria Biesdorf, D.V.M., M.Sc., Renata Benício Neves Fuverki, D.V.M., Sueli Massumi Nakatani, Pharmac. Bioch., Ph.D., Irina Nastassja Riediger, Pharmac. Bioch., M.Sc., Ana Laura Grazziotin, D.V.M., Andrea Pires do Santos, D.V.M., Ph.D., Ivan Roque de Barros Filho, D.V.M., Ph.D., and Alexander Welker Biondo, D.V.M., Ph.D.



Discussion -1

- Différences majeures entre :
  - Les législations nationales
  - Les outils diagnostiques disponibles :tuberculine, capacité des laboratoires

Rv number	Molecular mass	Functional group[1]	Protein class[1]	PPDbov BR	PPDbov UK	PPDavi BR	PPDavi UK	Protein identity
Rv0014c	66509	9	TMHMM	–	–	x	x	Involved in signal transduction
Rv0054	17400	2	C	x	–	x	x	Single strand binding protein
Rv0125	37326	7	SIGNALP	–	x	x	–	Probable serine protease
Rv0129c	36791	1	TATP	–	x	x	x	Antigen 85C, mycolytransferase
Rv0187	23079	7	C	–	–	x	–	Hypothetical protein
Rv0192	38903	10	C	–	–	x	x	Hypothetical protein
Rv0216	35994	7	C	x	x	x	x	Conserved hypothetical protein
Rv0237	39518	3	C	–	–	–	x	Conserved lipoprotein
Rv0242c	46916	1	C	x	x	–	–	3-Oxoacyl-[ACP] reductase
Rv0243	46307	1	C	–	x	–	–	Acetyl-CoA C-acetyltransferase
Rv0248c	71092	7	C	x	x	–	–	Probable flavoprotein subunit of Rv0247c
Rv0270	59856	1	C	–	x	–	–	Acyl-CoA synthase
Rv0281	33092	10	C	–	–	x	–	Conserved hypothetical protein
Rv0333	13066	6	C	–	x	–	–	Hypothetical protein
Rv0350	66659	0	C	x	x	x	x	70 kD heat shock protein
Rv0351	21294	0	C	–	x	–	x	Stimulates DnaK ATPase activity
Rv0393	45723	5	C	–	–	x	–	Hypothetical protein
Rv0407	36956	7	C	–	–	x	–	F420-dependent glucose-6-phosphate ehydrogenase
Rv0431	16855	3	C	–	x	–	–	Tuberculin related peptide (AT103)
Rv0440	56561	0	C	x	x	x	x	60 kD chaperonin 2
Rv0455c	19766	10	SIGNALP	–	–	x	x	Hypothetical protein
Rv0462	49437	7	C	–	x	–	–	Probable dihydrolipoamide dehydrogenase
Rv0467	47228	7	C	x	x	–	–	Isocitrate lyase
Rv0468	30766	1	C	x	–	–	–	3-Hydroxyacyl-CoA dehydrogenase
Rv0469	33095	1	C	x	–	–	–	Possible mycolic acid synthase UMAA
Rv0475	21391	3	C	–	x	–	–	Possible exported protein
Rv0503c	37359	1	C	–	x	–	–	Cyclopropane mycolic acid synthase 2
Rv0543c	11279	10	C	–	–	–	x	Conserved hypothetical protein
Rv0559	12116	3	SIGNALP	–	–	–	x	Conserved hypothetical protein
Rv0569	14372	10	C	–	x	–	–	Conserved hypothetical protein
Rv0577	27251	10	C	–	x	–	–	Conserved hypothetical protein
Rv0583c	23725	3	SIGNALP	–	x	–	–	Equivalent to MKU20446_1 MK35
Rv0632c	24454	1	C	x	x	–	–	Enoyl-CoA hydratase/isomerase superfamily
Rv0639	25431	2	C	–	x	–	–	Transcription antitermination protein
Rv0652	13432	2	C	x	x	x	x	50S ribosomal protein L7/L12
Rv0680c	13166	3	TMHMM	–	–	–	x	Conserved hypothetical protein
Rv0685	43566	2	SIGNALP	x	x	x	x	Elongation factor EF-Tu
Rv0703	10951	2	C	–	x	–	–	50S ribosomal protein L23
Rv0707	29928	2	C	–	x	–	–	30S ribosomal protein S3
Rv0719	19377	2	C	–	x	–	–	Probable 50S ribosomal protein L6 RPLF
Rv0733	20113	7	C	–	x	–	–	Probable adenylate kinase
Rv0761c	36283	7	C	–	x	–	–	Zinc-containing alcohol dehydrogenase
Rv0786c	13917	10	C	–	–	x	–	Conserved hypothetical protein
Rv0787A	8589	10	C	–	–	–	x	Conserved hypothetical protein
Rv0793	11191	10	C	–	–	–	x	Conserved hypothetical protein
Rv0801	12618	10	C	–	x	–	–	Hypothetical protein
Rv0831c	33921	10	C	x	x	–	–	Conserved hypothetical protein
Rv0884c	40266	7	C	x	–	–	–	Phosphoserine aminotransferase
Rv0896	48032	7	C	x	x	–	–	Citrate synthase 1
Rv0905	26127	1	C	–	x	–	–	Enoyl-CoA hydratase/isomerase superfamily
Rv0932c	38110	3	SIGNALP	–	x	–	–	PstS component of phosphate uptake
Rv0983	46452	7	TMHMM	–	–	x	x	Conserved hypothetical protein
Rv0984	18618	7	C	x	–	–	–	Molybdenum cofactor biosynthesis, protein B
Rv1070c	27370	1	C	x	–	–	–	Enoyl-CoA hydratase/isomerase superfamily
Rv1080c	17844	2	SECRETOMEP	x	–	–	–	Transcription elongation factor G
Rv1093	45058	7	C	x	x	–	–	Serine hydroxymethyltransferase
Rv1157c	36448	10	SIGNALP	–	–	–	x	Conserved hypothetical protein
Rv1133c	81816	7	C	x	x	–	–	5-Methyltetrahydropteroyltriglutamate-homocystein
Rv1174c	10988	3	SIGNALP	–	x	x	x	Hypothetical protein
Rv1177	11807	7	C	–	–	x	x	Probable ferredoxin
Rv1181	168084	1	C	–	x	–	–	Probable polyketide beta-ketoacyl synthase PKS4
Rv1198	9979	3	C	–	x	–	–	ESAT-6 like protein 1
Rv1270c	24973	3	SIGNALP	–	x	–	–	Lipoprotein
Rv1291c	11025	3	SIGNALP	–	–	–	x	Conserved hypothetical protein
Rv1352	12955	10	SIGNALP	–	x	–	–	Conserved hypothetical protein
Rv1388	12097	2	C	–	x	–	x	Integration host factor
Rv1392	43447	7	C	x	–	–	–	S-adenosylmethionine synthase
Rv1411c	35588	3	SIGNALP	–	x	x	x	Lipoprotein
Rv1436	36105	7	C	x	x	x	x	Glyceraldehyde 3-phosphate dehydrogenase
Rv1445	25772	7	C	–	–	x	–	Probable 6-Phosphogluconolactonase DEVB
Rv1448c	40723	7	C	x	x	x	–	Transaldolase
Rv1471	13308	7	C	–	–	x	–	TrxB protein

Discussion -1

- Différences majeures entre :
  - Les législations nationales
  - Les outils diagnostiques disponibles :tuberculine, capacité des laboratoires

Table 1 (*continued*)

Rv number	Molecular mass	Functional group[§]	Protein class[†]	PPDbov BR	PPDbov UK	PPDavi BR	PPDavi UK	Protein identity
Rv1475c	102728	7	C	x	x	x	–	Aconitate hydratase
Rv1608	16894	0	C	–	–	–	x	Probable peroxidoxin BcpB
Rv1630	53199	2	C	–	x	–	–	30S ribosomal protein S1
Rv1661	220844	1	C	–	–	–	x	Probable polyketide synthase pks7
Rv1662	167095	1	C	–	–	–	x	Probable polyketide synthase pks8
Rv1637	27936	10	C	–	–	x	–	Conserved hypothetical protein
Rv1687c	27940	3	C	–	–	–	x	Probable ABC-transporter
Rv1758	17868	3	C	–	–	–	x	Probable cutinase cut1
Rv1781	79744	7	C	–	–	–	x	Probable 4-Alpha-Glucanotransferase Malq
Rv1789	38588	6	C	–	–	x	x	PPE Family protein
Rv1793	9993	3	C	–	x	x	x	ESAT-6 like protein 5
Rv1796	60275	7	TMHMM	–	–	–	x	Conserved hypothetical protein
Rv1802	46021	6	C	–	–	x	–	PPE Family protein
Rv1808	39899	6	C	–	–	x	–	PPE Family protein
Rv1826	14229	7	C	–	x	–	–	Glycine cleavage system H protein
Rv1827	17240	10	C	x	x	x	–	Conserved hypothetical protein
Rv1837c	80450	7	C	x	x	x	–	Malate synthase
Rv1855c	33256	7	C	–	x	x	–	Probable monooxygenase
Rv1860	28746	3	SIGNALP	–	x	x	x	Precursor of Apa (45/47 kD secreted protein)
Rv1876	18443	7	C	–	x	x	x	Bacterioferritin
Rv1886c	30814	1	TATP	–	x	x	x	Antigen 85B, mycolyltransferase
Rv1893	7463	10	C	–	x	x	x	Hypothetical protein
Rv1915	40489	7	C	–	x	–	–	Probable isocitrate lyase aceAa
Rv1916	85437	7	C	x	x	–	–	Isocitrate lyase, [beta] module
Rv1926c	16504	3	SIGNALP	x	x	–	–	Hypothetical protein
Rv1932	17057	0	C	–	x	x	x	Thiol peroxidise
Rv1980c	25081	3	SIGNALP	x	x	x	x	Secreted immunogenic protein Mpb64/Mpt64
Rv1984c	24210	3	SIGNALP	–	x	–	–	Probable secreted protein
Rv2031c	16086	0	C	x	x	–	–	14 kD antigen, heat shock protein Hsp20 family
Rv2140c	18622	10	C	–	x	–	–	Conserved hypothetical protein
Rv2145c	28260	3	C	x	x	x	x	Antigen 84 (aka wag31)
Rv2162c	44478	6	SIGNALP	–	–	–	x	PE-PGRS Family protein
Rv2198c	30954	3	TMHMM	–	–	–	x	Probable conservedmemebrane protein MMPS3
Rv2215	57110	7	C	–	x	–	–	Dihydrolipoamide succinyl transferase
Rv2220	53707	7	C	–	x	–	–	Glutamine synthase class I
Rv2241	100481	7	C	–	x	–	–	Pyruvate dehydrogenase E1 component
Rv2244	12516	1	C	x	x	x	x	Acyl carrier protein (meromycolate extension)
Rv2246	44421	1	C	–	x	–	–	[Beta]-ketoacyl-ACP synthase (meromycolate
Rv2347c	10977	3	C	–	–	–	x	Putative ESAT-6 Like protein ESXP
Rv2376c	16653	3	SIGNALP	–	x	–	–	Conserved hypothetical protein
Rv2428	21566	0	C	–	–	–	x	Alkyl hydroperoxide reductase C protein
Rv2467	94683	7	C	–	–	x	–	Probable aminopeptidase
Rv2468	17288	10	C	–	–	x	x	Conserved hypothetical protein
Rv2557	24679	10	C	–	x	–	–	Conserved hypothetical protein
Rv2593c	20177	2	C	–	x	–	–	Holliday junction binding protein, DNA helicase
Rv2623	31747	3	C	–	x	–	–	Conserved hypothetical protein
Rv2626c	15679	10	C	x	x	–	–	Conserved hypothetical protein
Rv2744c	29257	10	C	–	–	x	x	Conserved 35 kDa alanine rich protein
Rv2779c	19871	9	C	–	x	–	–	Possible transcriptional regulatory protein
Rv2787	63848	10	C	–	x	–	–	Conserved hypothetical alanine protein
Rv2847c	41938	7	C	–	–	–	x	Conserved hypothetical protein
Rv2873	24412	3	SIGNALP	x	x	–	–	Surface lipoprotein Mpt83
Rv2875	8674	3	SIGNALP	x	x	–	–	Major secreted immunogenic protein Mpt70
Rv2878c	18959	3	SIGNALP	–	x	–	–	Secreted protein Mpt53
Rv2882c	20815	2	C	–	–	–	x	Ribosome recycling factor
Rv2889c	28851	2	C	–	x	–	–	Elongation factor EF-Ts
Rv2919c	12220	9	C	–	–	–	x	Nitrogen regulatory protein
Rv2940c	24296	3	SIGNALP	x	x	–	–	Lipoprotein
Rv2945c	36626	7	C	–	x	–	–	Ketol-acid reductoisomerase
Rv3001c	31700	7	C	x	x	–	–	Electron transfer flavoprotein [alpha] subunit
Rv3028c	24590	3	SIGNALP	x	x	x	–	Probable secreted protein
Rv3036c	24406		SIGNALP	–	–	x	x	Probable conserved secreted protein TB22.2
Rv3045	37508	7	C	x	–	–	–	Alcohol dehydrogenase
Rv3046	13350	10	C	–	–	x	x	Conserved hypothetical protein
Rv3048c	37025	2	C	–	x	–	–	Ribonucleoside-diphosphate small subunit
Rv3196A	7252	16	C	–	x	–	–	Hypothetical protein
Rv3248c	54343	7	C	x	x	x	–	Adenosylhomocysteinase
Rv3285	63750	1	C	–	–	x	–	Probable bifunctional protein acetyl
Rv3354	13065	10	SIGNALP	–	x	–	–	Conserved hypothetical protein
Rv3368c	23732	7	C	–	–	x	–	Probable oxidoreductase
Rv3392c	32461	1	C	x	–	–	–	Cyclopropane Fatty acid synthase
Rv3417c	55858	0	C	x	x	–	–	60 kD chaperonin 1
Rv3418c	10798	0	C	x	x	–	x	10 kD chaperone

(*continued on next page*)

Discussion -1

- Différences majeures entre :
  - Les législations nationales
  - Les outils diagnostiques disponibles :tuberculine, capacité des laboratoires
  - gestion et prévalence TB humaine
- Persistance des réservoirs sauvages en interface

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  - gestion et prévalence TB humaine
- Persistance des réservoirs sauvages en interface
- Impact des importations du milieu sauvage à grande longévité (éléphants, otaries)

Discussion (2)

- TB WG :
  - Suivre le circuit épidemiologique des souches circulantes

Discussion (2)

TB WG :

- Suivre le circuit épidemiologique des souches circulantes
- Mettre à jour les recommandations et les connaissances sur l'efficacité des outils diagostiques

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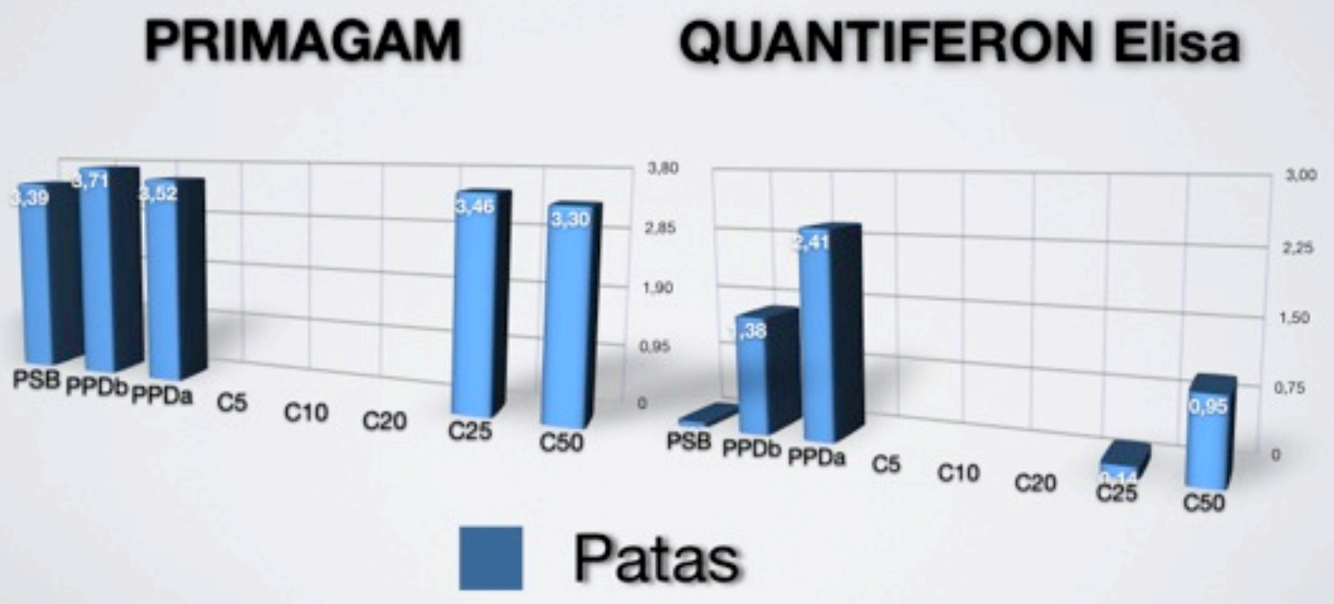

Discussion (2)

TB WG :

- Suivre le circuit épidemiologique des souches circulantes
- Mettre à jour les recommandations et les connaissances sur l'efficacité des outils diagostiques

Evaluation of DNA Extraction Techniques for Detecting *Mycobacterium tuberculosis* Complex Organisms in Asian Elephant Trunk Wash Samples ▽

Meagan K. Kay[1], Lyndsey Linke[2], Joni Triantis[2], M. D. Salman[2*] and R. Scott Larsen[1]

School of Veterinary Medicine, University of California, Davis, California,[1] Animal Population Health Institute, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado[2]

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Rapid and sensitive diagnostic assays for the detection of tuberculous mycobacteria in elephants are lacking. DNA extraction with PCR analysis is useful for tuberculosis screening in many species but has not been validated on elephant trunk wash samples. We estimated the analytical sensitivity and specificity of three DNA extraction methods to detect *Mycobacterium tuberculosis* complex organisms in trunk wash specimens. A ZR soil microbe DNA kit (ZR) and a traditional salt and ethanol precipitation (TSEP) approach were evaluated under three different treatment conditions: heat treatment, phenol treatment, and contamination with *Mycobacterium avium*. A third approach, using a column filtration method, was evaluated for samples contaminated with soil. Trunk wash

Conclusion

- Le diagnostic de la tuberculose est une analyse des risques : le clinicien/laboratoire pose son propre seuil limite !
- Limites de performances des tests

TEST Se=95% Sp= 95% Prévalence 5%	Malade	Non malade	Valeurs Prédictives du test
	5	**95**	
Test +	4,75	4,75	VPP=4,75/(4,75+4,75)=**0.50**
Test -	0,25	90,25	VPN=90,25/(90,25+0,25)=**0.99**

Conclusion

Le diagnostic de la t
le clinicien/laborato

Limites de performa

TEST Se=95% Sp= 95% Prévalence 5%	Malade	
	5	
Test +	4,75	
Test -	0,25	

Species	Test	Se[a,b] of test	Sp[a] of test	Number of animals tested	Infecti (N)/ex (E)
Badger (*Meles meles*)	IFN-γ assay	80.9%	93.6%	235	N
	Brock ELISA	48.9%	93.6%	235	N
	RT qPCR	70.6%	90.7%	247	
	Rapid test	50.7%	93.1%	1532	N
	Brock	49.2%	93.1%	1464	N
	STAT-PAK[c]				
	Brock ELISA	68%	NE	128	N
	MAPIA	48.7%	88.0%	178	N
	Rapid test	52.6%	95.0%	178	N
	Brock ELISA	47.4%	89.0%	178	N
Possum (*Trichosurus Vulpecula*)	Rapid test	44.7%	85.7	129	N
Fallow deer (*Dama dama*)	CervidTB STAT-PAK	80.1	NE	134	N
	CervidTB STAT-PAK	91%	91%	139	N
	Dual path platform VetTB test	91%	99%	139	N
Red deer (*Cervus elaphus elaphus*)	RT qPCR	78.6%	97.5%	15	E
	IFN-γ ELISA (Cervigam)	70%	100%	15	E
	LPA	65.7%	92.5%	15	E
	CervidTB STAT-PAK	86.5%	83.8%	157	N + E
	DPP VetTB test	84.6%	91.4%	157	N + E

Conclusion

- Rupture entre les moyens et les objectifs
  - Manque de cadre réglementaire sur les espèces sauvages et les carnivores domestiques
    - coïncidant avec la réalité des possibilités diagnostiques (test IDR,...)
    - la finalité doit elle être la même que pour les animaux de rente?
  - Quelle prévalence dans nos populations captives ?

Conclusion

- De l'animal à l'homme ?
  - quelques cas mais surtout risque professionnel

- De l'homme à l'animal
  - probable origine dans les P.E.V.D
- **<u>De l'animal à l'animal :</u>**
  - Bien plus réel (publications) et plus préoccupant !

Conclusion

Epidemiol. Infect., Page 1 of 10. doi:10.1017/S095026881300068X

Transmission of *Mycobacterium tuberculosis* from an Asian elephant (*Elephas maximus*) to a chimpanzee (*Pan troglodytes*) and humans in an Australian zoo

N. STEPHENS[1,2]*, L. VOGELNEST[3], C. LOWBRIDGE[1], A. CHRISTENSEN[1], G. B. MARKS[4], V. SINTCHENKO[5,6] AND J. McANULTY[1]

[1] *Health Protection, Ministry of Health, New South Wales, Australia*
[2] *Health Protection Branch, Department of Health, Victoria, Australia*
[3] *Taronga Zoo, Taronga Conservation Society Australia, New South Wales, Australia*
[4] *Woolcock Institute of Medical Research, New South Wales, Australia*
[5] *Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Sydney New South Wales, Australia*
[6] *Sydney Medical School, The University of Sydney, New South Wales, Australia*

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