
Welcome

COMPARISON OF TUBERCULIN SKIN TEST AND LATERAL FLOW RAPID TEST FOR DETECTION OF BOVINE TUBERCULOSIS IN DAIRY CATTLE

مقارنة أختبار التيوبركلين فى الجلد والأختبار السريع للكشف عن السل البقرى فى ماشية الألبان.

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History and background of tuberculosis

I. مرض السل من الامراض الخطيرة منذ بدا الخليقة (حيث تظهر اوراق البردى وجوده منذ الاف السنين)

II. وجود اثار للدرن فى العمود الفقرى لبعض المؤمياوات و منها توت عنخ امون (اشهر ضحاياه) التى تثبت الاورق موته بالدرن

III. ترجع تسميته بالسل الى الطبيب اليونانى القديم (ابقراط)

فى القرن الرابع قبل الميلاد و هو يعنى باليونانية الانحلال و الاضمحلال

اما تسميته بالدرن فهى حديثة لظهوره فى الفحص الباثولوجى على هيئة درنات فى مواضع الاصابة

Tubercular decay has been found in the spines of Egyptian mummy in the British Museum

Remember (very important)

- السل فى اى مكان يعنى السل فى كل مكان
- العدوى الكامنة ------- اصابة فعلية ------ اصابة نشطة
- السل مرض معدى له فترة حضانة من عدة اسابيع الى مدى الحياة
- السل يصيب 1% اصابة فعلية جديدة سنويا من سكان العالم
- السل يمر باربع مراحل
- عدم وجود اصابة سلية ظاهرية او عزل البكتريولوجى لا يعنى عدم وجود العدوى

لماذا الاهتمام بمشكلة الدرن ؟؟

اولا فى الانسان

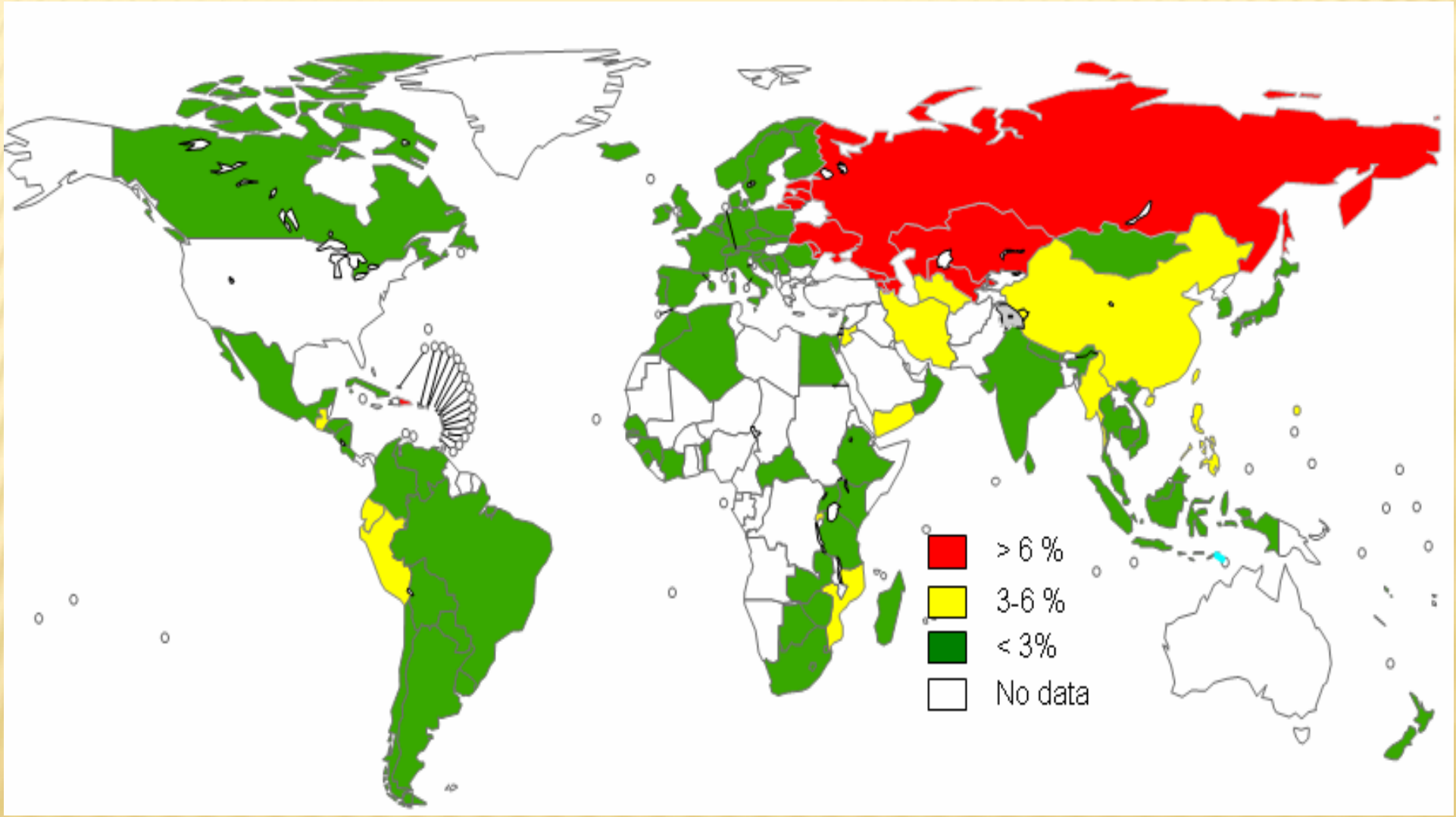
- ثلث سكان العالم مصابون بعدوى العصيات الرئوية (- 2013 (WHO
- يصاب بعدوى الدرن شخص على الاقل كل ثانيه (10الاف شخصا يوميا)
- يتوفى بمرض الدرن شخص كل 4ثوانى
- يؤثر على الشريحة المنتجة فى المجتمع (15-40 سنة)
- اذا لم يعالج المصاب بعدوى الدرن النشط فانه سيعدى 10-15 شخصا اخر فى السنة
- 5% فقط من جملة المصابين بالعدوى يصابون بالعدوى و ذلك عندما تضعف الجهاز المناعى لديهم

World Health Organization

Global incidence	13.6% 90% children
Annual deaths	2-3 ,000000
Expected 2020	1000,000000 case 35 ,000000 deaths

TB pandemic is growing due to antibiotics-resistant TB strains.

MDR-TB & XDR-TB
THE 2008 REPORT
% of MDR-TB among new TB cases 1994-2007



EXPOSURE, IMMUNE RESPONSE, INFECTION and DISEASE

False-positives? False-negatives?

		CMI diagnosis (SICCT or IFN-γ)	Visible lesions/ *M. bovis* +ve
M. bovis exposure	VERY early infection in latent period	Negative	Negative
	Infection, early stages or disease controlled (?)	Positive	Negative/ Positive
	Infection, progressive disease	Positive/ Negative	Positive
Environmental Mycobacteria	Infection leading to sensitisation	False Positive/ Negative	Negative

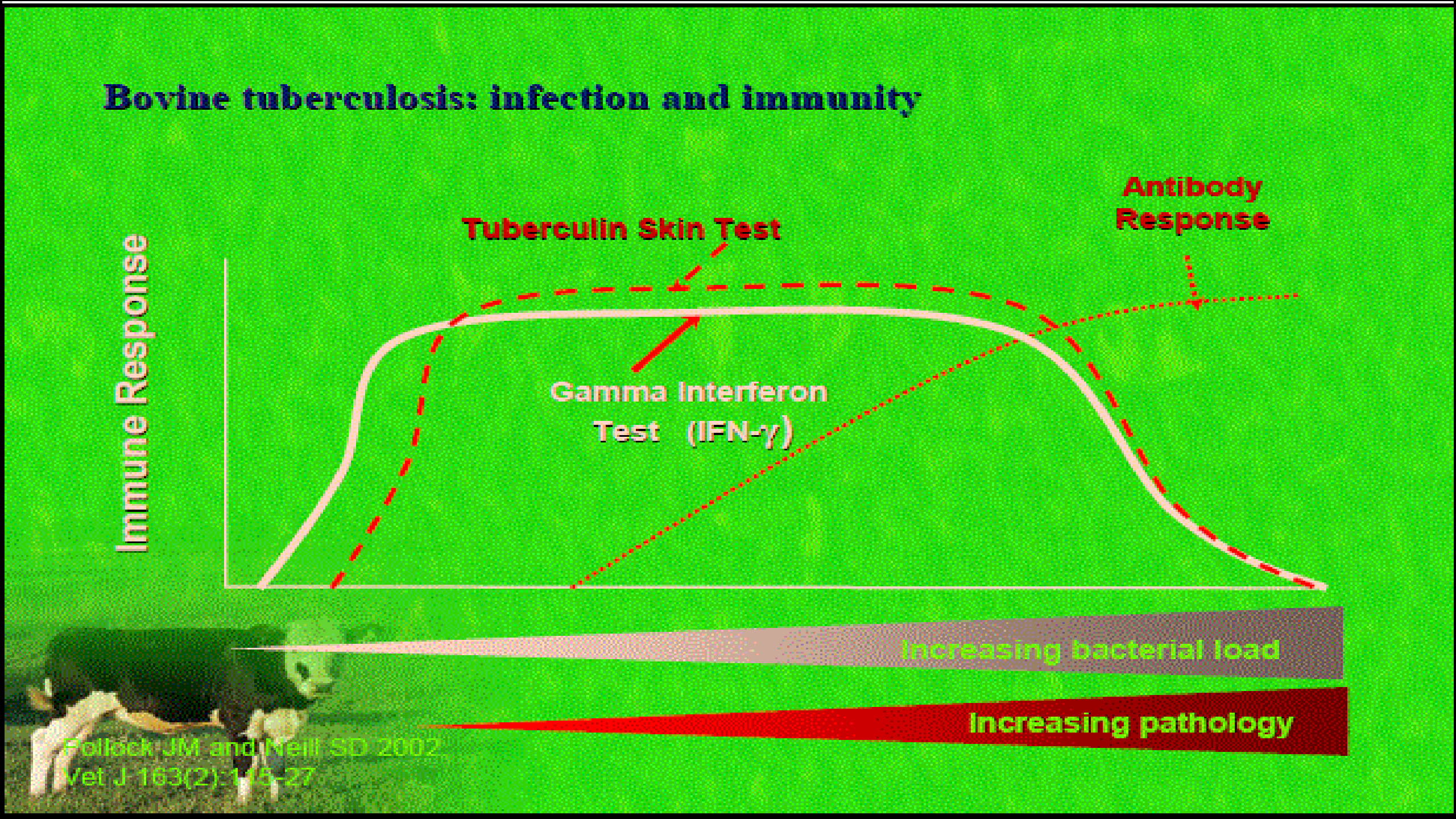
Latency

N V L

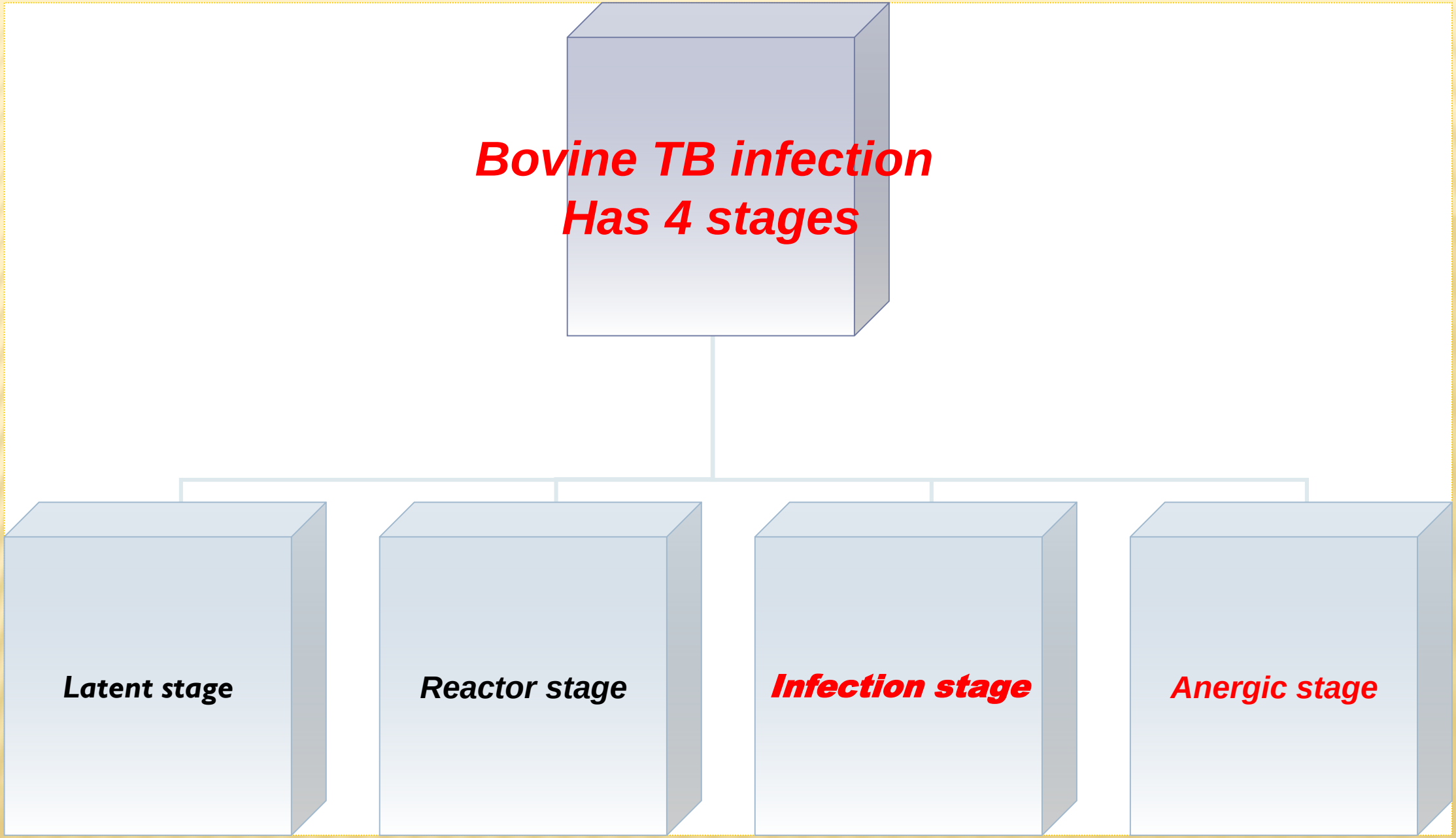
Anergy

(inspired by Dannenberg, e.g. Tuberculosis, 2000)

Bovine tuberculosis: infection and immunity



Pollock JM and Neill SD 2002 Vet J 163(2): 115-27


Bovine TB infection
Has 4 stages
Latent stage
Reactor stage
Infection stage
Anergic stage

1. Latent stage

1. “Of length in order of a few weeks”

2. The animal is infected but neither infect other animal nor test positive for TB

2. The reactor stage

- The animal tests positive but is not infectious

3. The infectious stage

- The disease which follows is typically identifiable by the appearance of open lesions (and is thus assumed to be identifiable by abbattoir inspection) ,

- through there is evidence of infectiousness without visible lesion.

4. Anergic stage

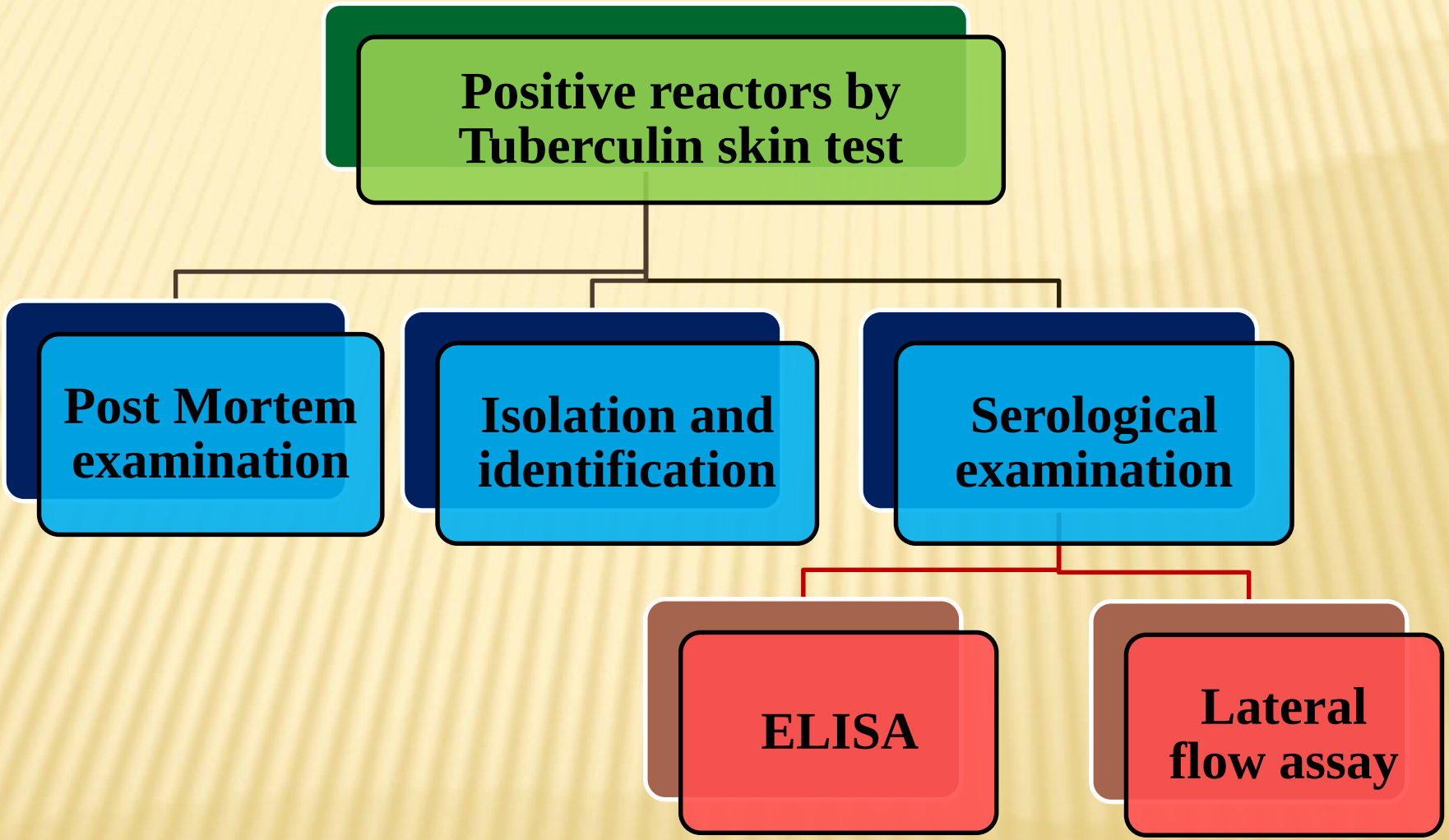
- Eventually, an anergic stage is reached where

  highly infectious animals no longer reactor to tuberculin-based TB test.

THE AIM OF THE WORK

1. **Determination of the Prevalence of Tuberculosis in dairy cattle farms in some Egyptian Governorates.**
2. **Evaluation of ELISA for serodiagnosis of Bovine Tuberculosis.**
3. **Evaluation of recent lateral flow test as a rapid field test for diagnosis of Tuberculosis.**

Study design



Material and Methods

MATERIAL AND METHODS:

Materials:

- **Animals:**
  - **A total of** (1900) **cross-breed dairy cattle were tested by SID cervical tuberculin test**OIE (2009)**.**
  - **Serum samples were collected from the positive reactors for serological testing using ELISA and lateral flow immunochromatographic assay.**
  - **The positive reactor animals were slaughtered for post mortem and bacteriological examination.**

• Tuberculin test:

- **Bovine tuberculin:**
  Purified protein derivatives (PPD) produced in bacterial diagnostic product department in Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.
- **Automatic syringe adjusted to inject 0.1ml in each jet.**
- **Caliper .**
- **Curved scissors with rounded ends.**

• Tissue samples:

A. Organs:

All organs and tissues inspected from suspected tuberculous lesions including { lung, pleural membrane, liver, kidney, intestinal mucosa, and peritoneum membrane } were collected.

B. The lymph nodes:

Samples were collected from the following L.N. and tissues that showed tuberculous like lesions.

- ❑ Head L.N. : (retropharyngeal, submaxillary and parotid).
- ❑ Bronchial L.N. : (left, right mediastinal and pectoral L.N).
- ❑ Other L.N. :(Hepatic L.N., Mesentric L.N., Renal L.N., Prescapular L.N. ,Prefemoral and internal iliac L.N., Supramammary L.N.).

- **Materials used in processing of tissue samples (Marks,1972):**

  4% Sulphoric acid (H_2SO_4) -Sterile distilled water - Mortar with its head-Sterile sand.

- **Materials and Media Used For Isolation of Mycobacteria**

  Culture medium used for isolation of Mycobacteria:

  Lowenstein-Jensen medium (L-J) (Collee et al. 1996)

- **Stain used for staining of AFB:**

  Ziehl-Neelsen stain (Z.N.): (Collee et al. 1996).

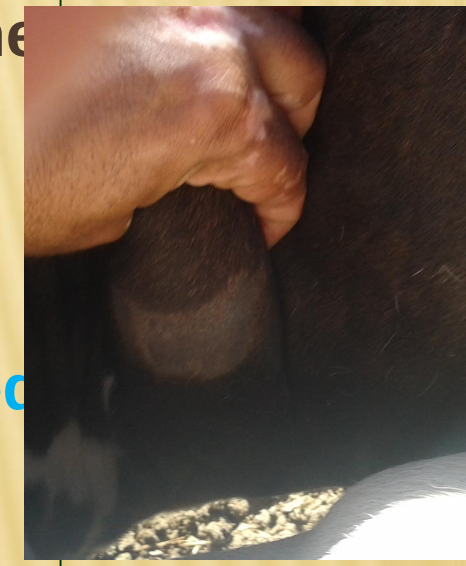
- **Antigen used for ELISA :**

  Bovine Purified Protein Derivative antigen : was obtained kindly from Bacteriological Diagnostic Products Department, (VSVRI), Abbasia, Cairo, Egypt .

METHOD:

Tuberculin test :

- A narrow zone at the middle third of the neck of the tested animals was marked by clipping the hair.
- The skin thickness was measured by using the caliper.
- Then 0.1 ml of bovine tuberculin was injected intradermally.
- The skin thickness was measured 72 hours post injection and the differences in mm were recorded in millimeter.



INTERPRETATION OF THE REACTION:

- **According to the Egyptian General Organization of Veterinary Services (GOVS): an increase in the skin thickness of 4 mm or more is considered positive , less than 3 mm is considered as negative and from 3 - 4 mm is considered as doubtful.**

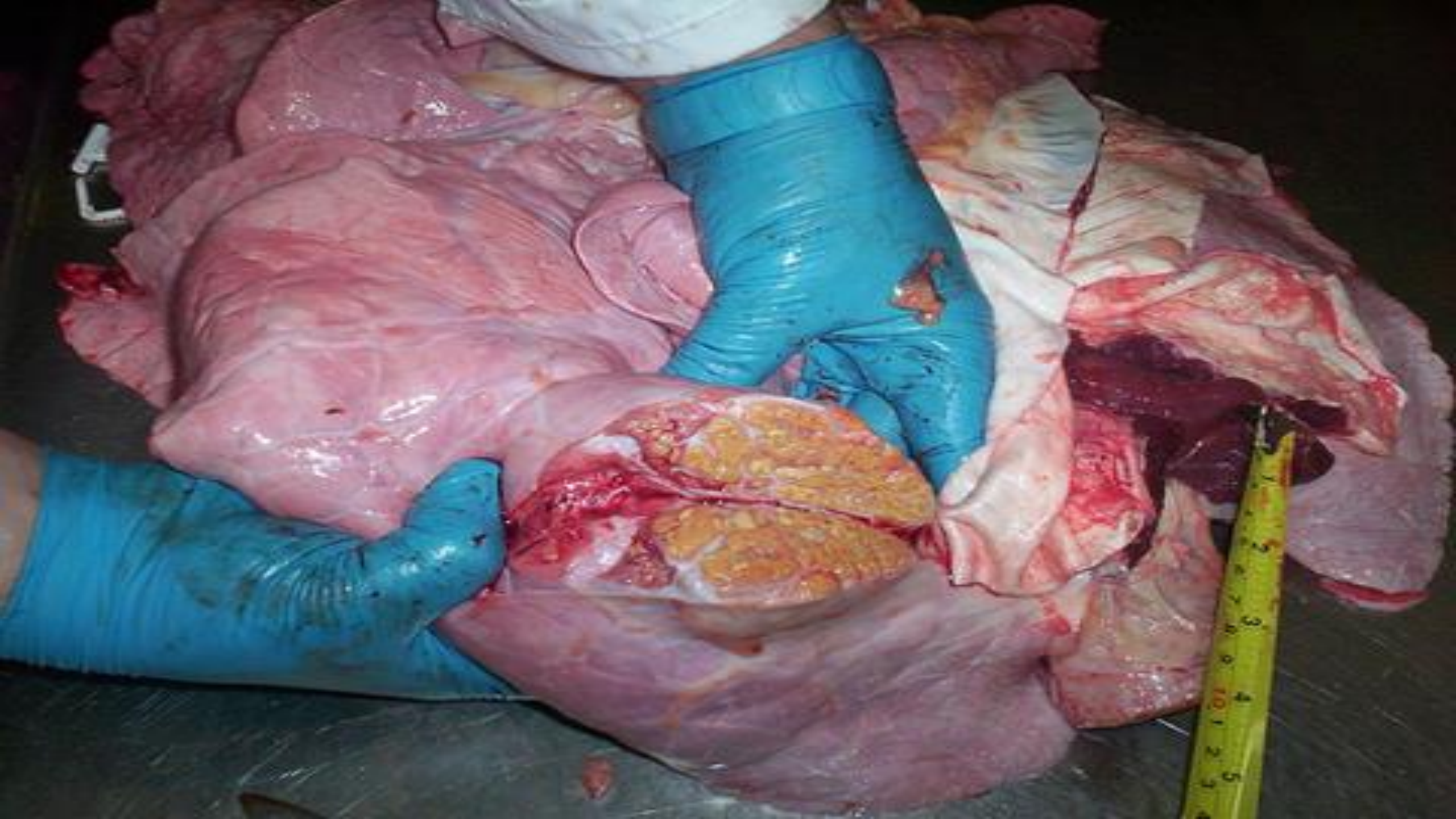
Skin Measurement before injection

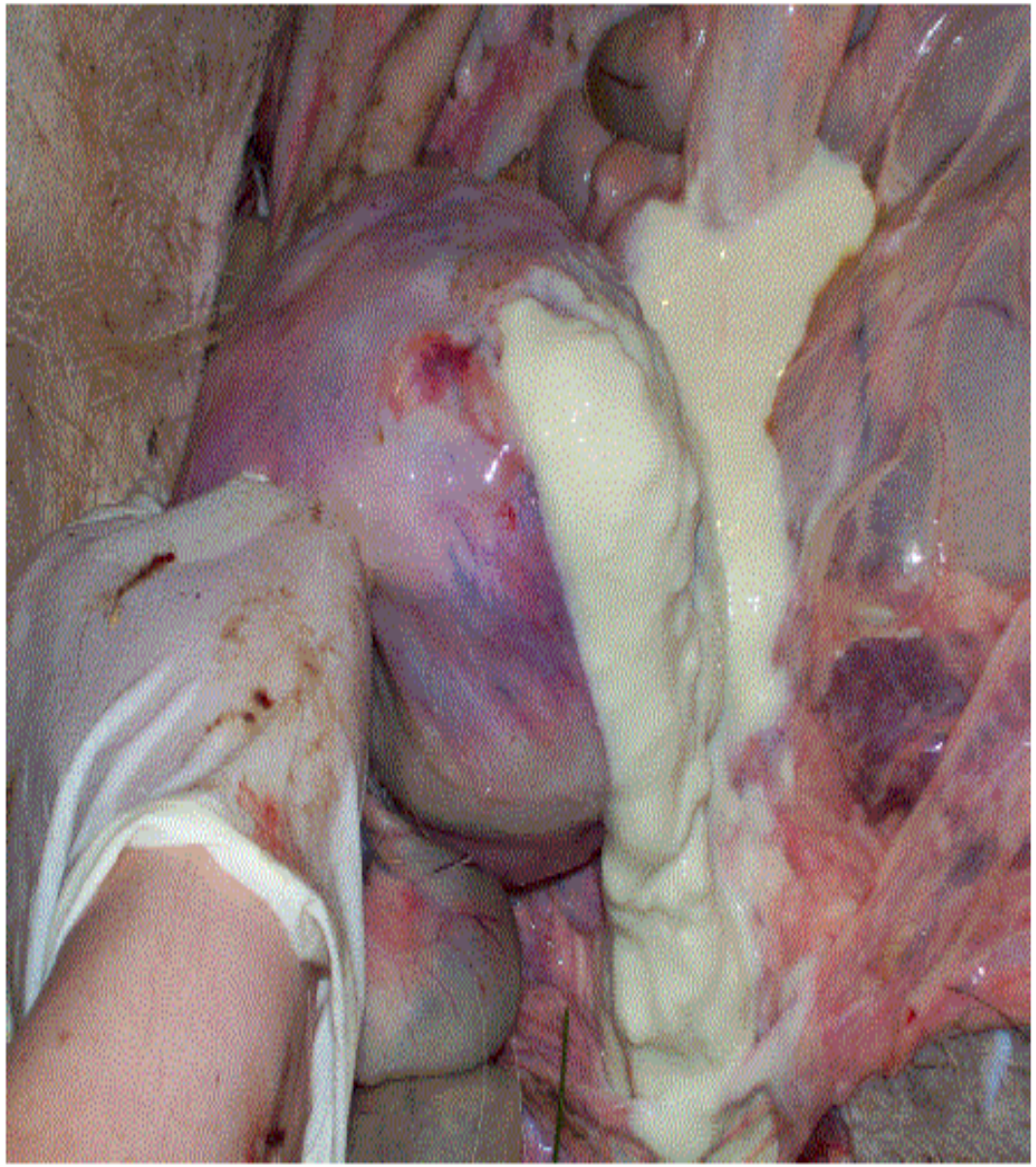
Skin Measurement after injection

Post mortem examination:

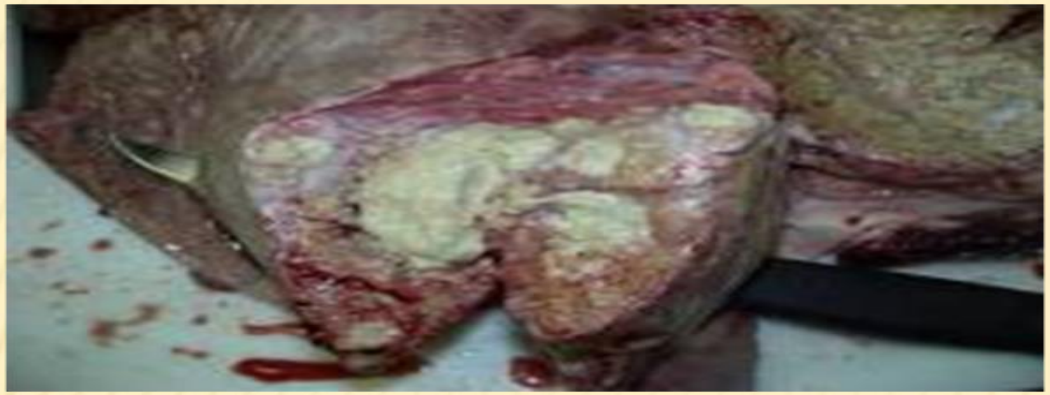
- Visual examination, palpation and incision of the lungs, liver and kidneys; lymph nodes of the thoracic and head regions; other lymph nodes and tissues/organs of the body to detect the presence of any suspected tuberculous lesions such as caseation, calcification and congestion .
- Not all infected animals present lesions at carcass inspection while gross visible lesions suggest that the disease is at an advanced or late stage.

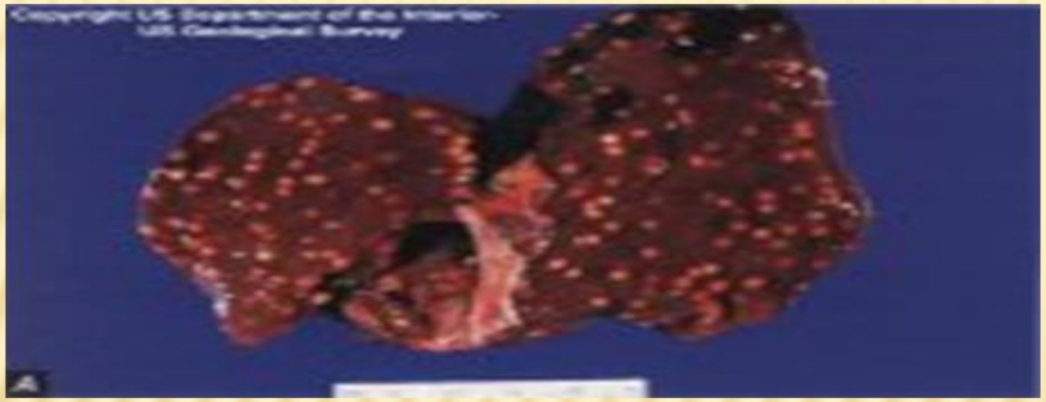
PM LESIONS





Large abscess within abdominal cavity may be large (grapefruit-sized) or very small



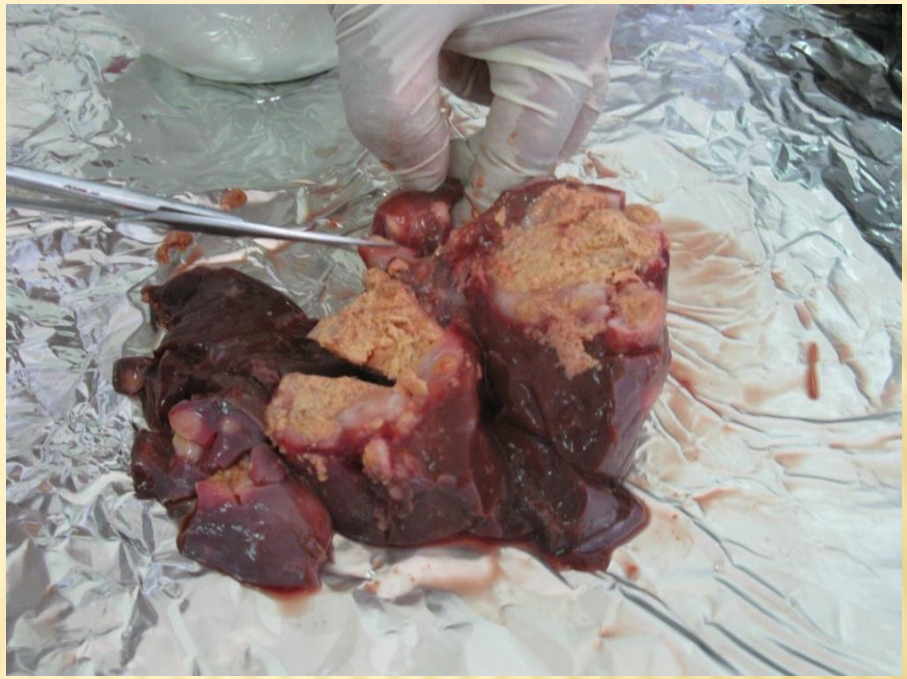


Tuberculosis nodules lying against ribs associated with avian Tb

L.N shows the tuberculous calcification

Liver shows the tuberculous lesions

the tuberculous like lesions in L.N.



ISOLATION OF MYCOBACTERIA

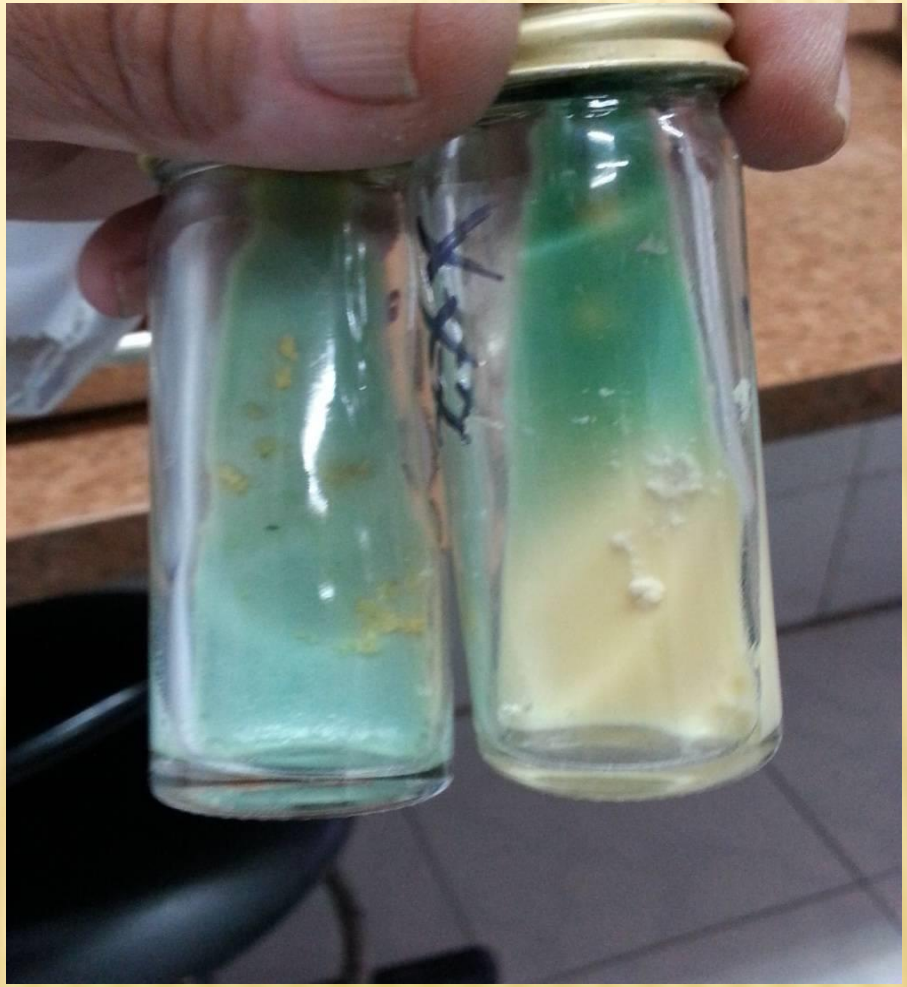
1) Processing of samples for isolation of acid fast bacilli :-

Organs and tissues showing gross lesion or congestion were transferred to a sterile mortar containing washed sterile sand. The fat was trimmed and the suspected material was cut into small pieces. Two ml of sterile distilled water were added to the crushed tissue, homogenized and ground till suspension was obtained. Two ml of 4% H_2SO_4 acid were added to the mixture, and then incubated at 37 ^{o}C for 30 minutes. The mixture was diluted with 16 ml of sterile distilled water and centrifuged at 3000 rpm for 20 minutes.

The supernatant fluid was poured off into disinfectant (5% phenol solution) and the obtained sediment was inoculated into four Lowenstein- Jensen slants. Then incubated at 37^{o}C in inclined position for overnight ,then vertically for at least 6 - 8 weeks and examined daily over a week period then once each week. The obtained growths were observed for morphological character and for pigment production.

Processing of samples for isolation of acid fast bacilli.

Positive culture for M. Bovis on L . J .Media



Lateral flow kits tested.

Anigen Rapid Bovine TB Ab test Kit:-

Each kit contains certain number of test devices, each sealed in a foil pouch with three items inside:

a. One cassette device. b. One plastic dropper.

c. One desiccant.

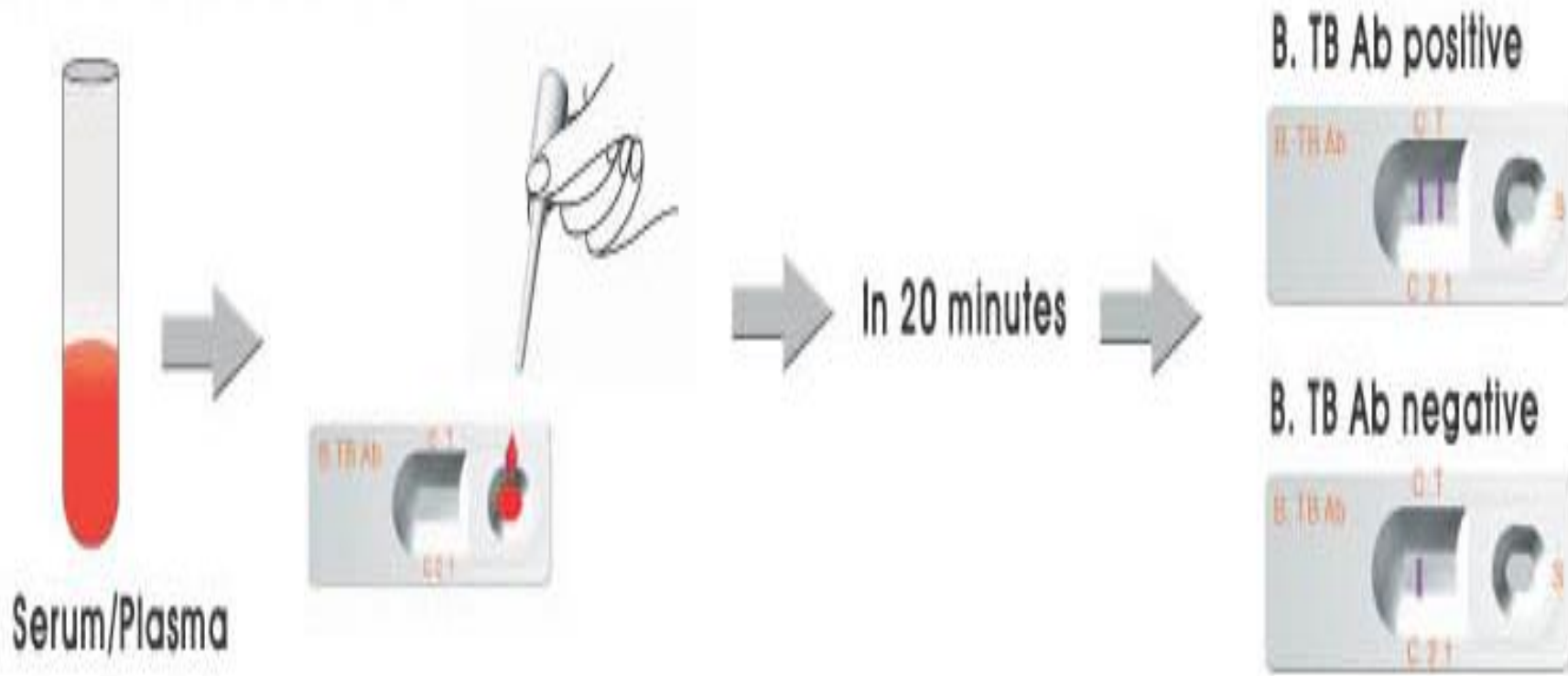
2. One package insert (instruction for use).

3. Positive and negative controls

The TB IgG / IgM Rapid Test is limited to the qualitative detection of IgG and IgM anti-*M.TB* in serum or plasma and also recognizes antibodies to *M. bovis* and *M. africanum* .

Anigen Rapid Bovine TB Ab test Kit

Test Procedures



Interpretation of the test:

- **Negative result:** The presence of only one color band within the result window.

- **Positive result:** The presence of tow color bands (T band and C band) within the result window. (Even if the intensity of the band color is faint it should be consider as positive).

- **Invalid:** If the color band was not visible within the result window after performing the test, the result was considered invalid and the specimen was re-tested.

Results

TABLE (1): RESULTS OF TUBERCULIN SKIN TEST AND POST MORTEM FINDING OF SLAUGHTERED TUBERCULIN POSITIVE CATTLE.

No. of tested cattle	Reactor cattle		PM finding			
			Visible Lesion		Non Visible Lesion	
	No.	%	No.	%	No.	%
1900	50	2.6	45	90	5	10

PM: Postmortem. No.: Number.

TABLE (2) RESULTS OF POSTMORTEM FINDING IN 50 SLAUGHTERED TUBERCULIN REACTOR CATTLE ACCORDING TO THE SITE OF LESION.

Reactor cattle		PM finding									
		VL (45)								NVL(5)	
		Head		Pulmonary		Digestive		Generalized			
No.	%	No.	%	No.	%	No.	%	No.	%	No	%
50	2.6	6	12	24	48	10	20	5	10	5	10

VL: Visible Lesion NVL: Non Visible Lesion PM: Postmortem.

TABLE (3): RESULTS OF ANIGEN RAPID BOVINE TB AB TEST KIT FROM TUBERCULIN REACTOR CATTLE IN COMPARISON TO THE TYPE OF LESIONS.

PM finding	Number of positive tuberculin reactor	Anigen Rapid Bovine TB kit			
		Positive		Negative	
		No.	%	No.	%
Visible	45	20	44.4	25	55.6
Non visible	5	1	20	4	80
Total	50	21	42	29	58

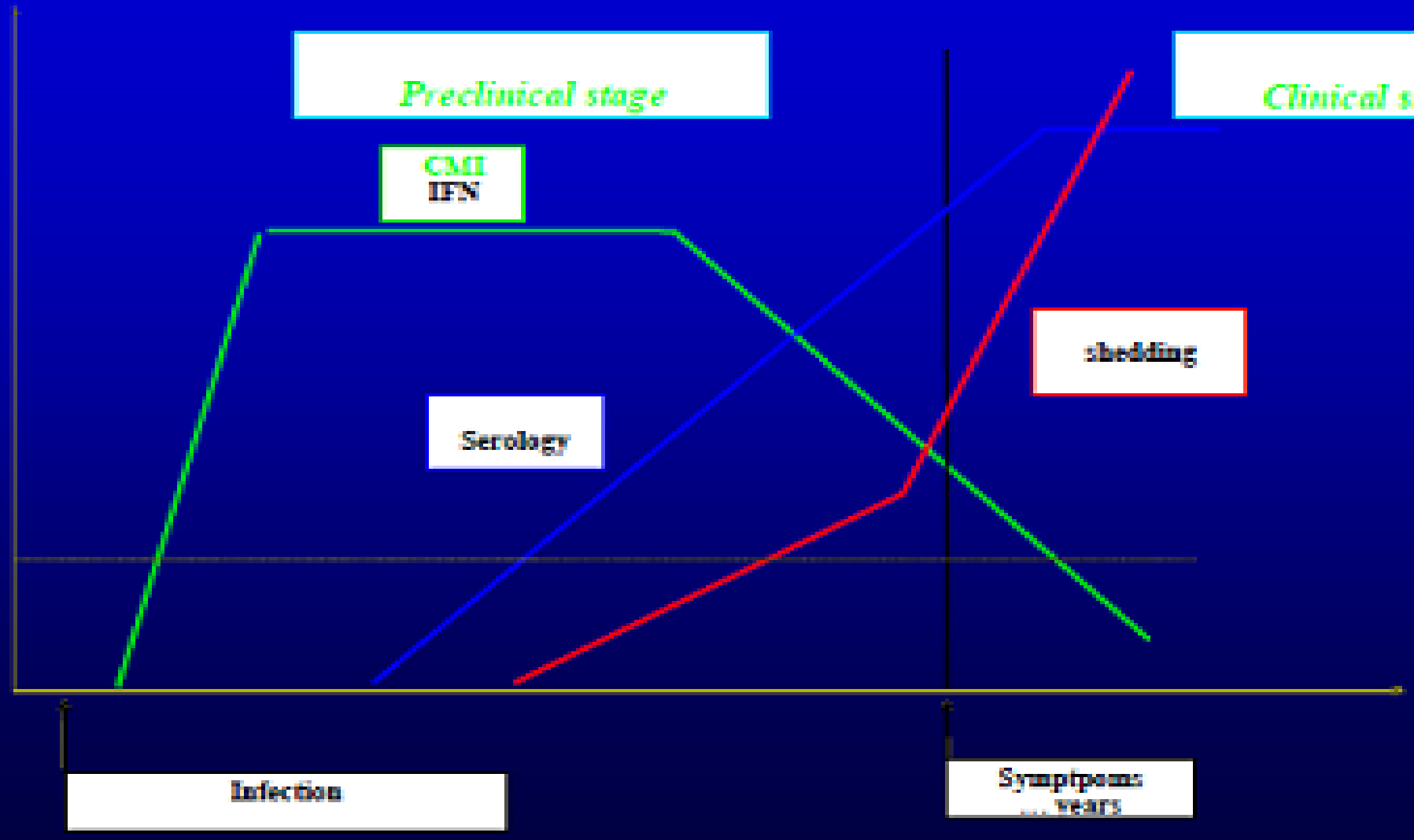
PM: Postmortem. TB: Tuberculosis.

TABLE (4) COMPARISON BETWEEN THE RESULTS OF BACTERIOLOGICAL ISOLATION, ELISA AND ANIGEN RAPID BOVINE TB AB TEST KIT ON SAMPLES OBTAINED FROM TUBERCULIN POSITIVE ANIMALS.

PM finding	Sites of lesions	N0	*M. bovis* isolates		ELISA		Ani gen Rapid Bovine TB kit	
			No.	%	No.	%	No.	%
I. Visible lesions	1. Local							
	a. Head	6	4	66.7	2	33.3	2	33.3
	b. Pulmonary	24	22	91.7	12	50	10	41.7
	c. Digestive	10	7	70	4	40	3	30
	2. Generalized	5	5	100	5	100	5	100
Sub total		**45**	**38**	**84.4**	**23**	**51.1**	**20**	**44.4**
II. Non visible lesions	Congestion in L.N.	5	2	40	1	20	1	20
Total		**50**	**40**	**80**	**24**	**48**	**21**	**42**

B-PPD: Bovine Protein Purified Derivatives. TB: Tuberculosis.

Host "responses" to *Mycobacterium bovis* infection



CONCLUSION AND RECOMMENDATION

- The true prevalence of bovine tuberculosis in a herd to be established the single intradermal tuberculin test and bacteriological culture and isolation should be done.
- ELISA serodiagnosis of bovine tuberculosis can be applied as a complementary test in farms positive for TST but not as an basic test.
- The sensitivity of lateral flow kits in diagnosis of bovine tuberculosis is not only low but also differ according to source of the kit and the type of capture antigen used in the kits.
- A national program to test all the dairy farms at least annually should be planned.

Thank You

شكرا للسادة
لحضور