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猪弓蟲病螢光抗體診斷法及組織病理變化

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一、緒　言

弓蟲病原蟲(Toxoplasma gondii)於哺乳動物，禽類，冷血動物，人類有自然感染的病例報告。本省屢有弓蟲病發生。曾報告從猪 (31.33.36)，猪肉 (34)、狗 (32)、野鼠 (37) 分離到原蟲。最近大規模企業化推廣養猪，猪弓蟲病在經濟及公共衛生的觀點，構成重要的問題。

血清學診斷弓蟲病計有色素反應 (25)、補體結合反應 (30) 補體結合阻止反應 (21)，凝集反應 (15.18)、螢光抗體間接法 (16.17.26.27.29)，血清學診斷法證明血清中抗體。最確實診斷弓蟲病仍是證明弓蟲蟲體。以一般染色方法的 Wright, Giemsa, H.E. 染色，可以證明蟲體，不過不容易判定，尤其組織中含少數蟲體之病例不易檢出病原，而以小白鼠接種方法需要數天，因此螢光抗體法利用螢光色素 (20.23) 標附抗體，檢查組織中的蟲體可得弓蟲病之確實診斷。

弓蟲對小白鼠具有很高的感受性，因此藉小白鼠從事弓蟲方面的研究，唯以小白鼠接種方法不易明瞭弓蟲性狀，最近組織培養法被利用以從事弓蟲研究 (1.3.5.11.19)。很少有以 PK—15 細胞株行組織培養，本試驗鑑於本省 PK—15 之普遍應用 (35)，故以 PK—15 接種弓蟲而以螢光抗體法檢查組織中之抗原。

Coons 等於1942年述及螢光抗體法 (6)，1950 年開始應用於病毒學，細菌學方面的診斷研究 (7)，1957年 Goldman 首次應用螢光抗體法檢出弓蟲 (10)，本省螢光抗體法雖應用於猪瘟診斷 (35)，迄未應用於弓蟲方面的研究。

本研究主爲研製弓蟲螢光標示抗體並測定製成標示抗體之染色力價，非特異性及保存性，並應用野外弓蟲病例。且探討以組織培養方法弓蟲株之在 PK—15 株化細胞上之增殖情形，並以送檢猪材料以組織病理檢查等等加以詳細研討，茲將所得成績報告於後。

二、試驗材料及方法

1。試驗材料

(1) 弓蟲 RH 强毒株：以接種小白鼠腹腔，5天繼代一次保存，供本實驗應用。

(2) PK—15 株化細胞：係由日本農林省家畜衛生試驗場分讓，採3天繼代一次方式繼代培養，供爲本試驗組織培養用細胞。

(3) 小白鼠：由本所 SPF 中心生產供應，不够數目由外面購入，每隻約15克左右健康良好者。

(4) 供試小猪：從臺糖公司購進三品種小猪，經弓蟲實驗耐過猪。

(5) 螢光顯微鏡：採用 Nikon 牌螢光顯微鏡，東芝牌 SH200 高壓水銀燈，PFM 型照相機鏡頭，暗視野聚光鏡，Kodak 牌 TRI--XPan 黑白軟牌，Kodak 牌 EIIB135—20 彩色軟片。

(6) 特殊藥品及組織切片儀器藥品：

螢光色素 Fluorescein isothiocyanate (FITC)：美國 BBL 製 100mg 裝。

Sephadex：G—25Coarse 瑞典 Pharmacia uppsala 製 100g 裝

DEAE：Cellulose Ion Exchanger Serva 西德製。

2。螢光標示抗體試製法

（1）高度免疫血清之製造

弓蟲 RH 株接種小白鼠腹腔，經四天之小白鼠經口餵飼供試猪，五天餵飼一次，第一次餵飼10隻，每次餵飼量增加10隻，經五次餵飼後放血，分離血清，經 56°C30分非働化，存放 -20°C 冰箱供螢光標示抗體之試製。

（2）螢光標示抗體試製過程

① r 球蛋白沉澱透析

血清加等量飽和硫酸銨，低溫攪拌1小時，置放冰箱一夜，經 8000 rpm 15 分遠心分離，以再蒸餾水溶解沉澱之球蛋白，重加硫酸銨遠心分離重覆三次，以 0.01M PH 7.2 PBS 冰室內透析2天。

② Folin 法測定蛋白質含量。或以電氣泳動法測定。

③ 標附螢光色素 FITC

r 球蛋白濃度測定後，FITC 以 1/50 球蛋白濃度之量先以 0.5 M PH 9.0 Carbonate —Bicarbonate 緩衝液溶解，於零下溫度標附球蛋白，攪拌一夜。

④ 精製標示抗體

通過 Sephadex Column 除去未結合色素，經 0.01M PH 7.2 PBS 透析一天，再通過 DEA E Column 除去非特異性物質。

3. 感染病材及送檢病料螢光抗體檢查

弓蟲 RH 株感染小白鼠第四天之內臟材料及地方送檢病材塗抹於 Matsunami's 玻片（薄片螢光用），依下列過程處置：

（1）塗抹片空中乾燥。

（2）丙酮固定15分，吹乾。

（3）滴下螢光標示抗體液。

（4）37°C 密封感作半小時。

（5）PBS 水洗15分（換液3～4次）。

（6）50% PBS—glycerine 封入。

（7）置於螢光顯微鏡鏡檢。

4. 試製螢光標示抗體液檢查

（1）檢查染色力價，非特異性，保存性

試製弓蟲標示抗體液，以 0.01M PH7.2 PBS 倍數稀釋，以稀釋之標示抗體液染色含弓蟲之內臟塗抹片，判定稀釋至何種倍數仍能清晰染出感染組織內弓蟲，而判定清晰染出弓蟲之最高稀釋度爲染色力價，同時以健康小白鼠內臟塗抹片當對照。同時觀察有無非弓蟲之發螢光物質，即觀察其非特異性。

試製之標示抗體液並經冰箱保存一段時間後，染色感染組織內弓蟲，觀查標示抗體是否會經保存而失去作用。

（2）臟器抗原性保存

感染弓蟲之小白鼠內臟塗抹片，保存 4°C 及 —20°C ，經保存一段時間，觀查保存時間對抗原性檢出之影響。

（3）封入標本螢光保存性

染色封入含弓蟲之陽性臟器塗抹標本，放置室溫及冰箱內，保存一段時間，檢查封入標本螢光保存情形。

5. 接種 PK—15 株化細胞，實施螢光抗體法檢查

PK—15株化細胞以 Earles 溶液當做培養液（含山羊血清10%，7%$NaHCO_3$2%，抗生素液0.5～

1%而抗生素液含 Penicillin 200u/ml，Streptomycin 200r/ml， Kanamycin 20r/ml) 。PK—15細胞經 TV 液 (0.1%Trypsin 及 0.01%EDTA 混合) 消化溶解加 Earles 溶液培養於 3cm 之小燒皿，經 1 天培養形成單層細胞，接種弓蟲 RH 株腹水液，每皿接種 0.5ml. 接種之弓蟲力價經稀釋至以螢光能檢出 PK—15 組織弓蟲之前 10^{-1} 稀釋倍數爲接種力價，每皿接種 0.5ml 後置放 37°C 感作半小時，抽去弓蟲 RH 株腹水液，加入 2ml Earles 氏液經 3，6，9，12，24，36，48小時 3 天、4 天、5 天培養；經上述時間培養取出蓋玻片 (18mm×18mm)，以 0.01M PH 7.2 PBS 洗滌→吹乾→丙酮固定15分→滴下螢光標示抗體液→ 37°C 密封感作半小時→ PBS 水洗15分 (換液 3～4 次) →50%PBS—glycerine 封入→螢光顯微鏡鏡檢。

上述接種弓蟲腹水液，並以其 10^{-1}，10^{-2} 稀釋度接種 PK—15 細胞，經12小時、24小時、3 天計算弓蟲螢光染色數目，其弓蟲數計算採 2 次檢查 400×顯微鏡10視野下之螢光染色弓蟲數目，而以平均數表示之。觀察稀釋量和檢出弓蟲數的關係。

6. 送檢病材組織病理檢查

送檢田間猪病材，以內臟塗抹片實施螢光抗體染色，將染出陽性病材，採取內臟及腦組織，以10%福馬林固定，經石臘包埋，以 Harris Hematoxylin Eosin 染色，置放顯微鏡下觀查組織病理變化。

三、試驗成績

1. 螢光標示抗體之試製

感染弓蟲之小白鼠，經口餵飼弓蟲感染耐過材料猪，5 次餵飼，分離血清，經螢光標示抗體試製過程，分三段收集標示抗體液。收集量及染色力價，非特異性檢查成績如表 1、表 2 所示。

TABLE 1. Results of staining with various dilutions of manufactured FAb (Lot.I)
(Aceton fixation time : 15 min ; staining : 30 min)

Fraction	Collected FAb (ml)	Dilution of FAb	Reaction	Infected mice				Uninfected mice			
				Liver	Spleen	Lung	Kidney	Liver	Spleen	Lung	Kidney
1	65	1	Specific	++	++	++	++				
			Non-Spec.	−	−	−	−	−	−	−	−
		5	Specific	++	++	++	++				
			Non-Spec.	−	−	−	−	−	−	−	−
		10	Specific	++	++	++	++				
			Non-Spec.	−	−	−	−	−	−	−	−
		20	Specific	+	+	+	+				
			Non-Spec.	−	−	−	−	−	−	−	−
		40	Specific	−	−	+	±				
			Non-Spec.	−	−	−	−	−	−	−	−
		80	Specific	−	−	−	±				
			Non-Spec.	−	−	−	−	−	−	−	−
		160	Specific	−	−	−	−				
			Non-Spec.	−	−	−	−	−	−	−	−

Legend, − : unstained, ± : stained slightly, + : stained clearly, ++ : stained in extreme brightness
FAb : fluorescein—conjugated antibody.

TABLE 2. Results of prepared FAb and stain titer

Lot	Fraction	Collected FA (ml)	Non-specific	Stain titer
1	1	65	- -	20 ×
	2	55	—	20 ×
	3	40	—	20 ×
2	1	50	—	16 ×
	2	35	—	16 ×
3	1	50	- -	12 ×
	2	48	- - -	12 ×

弓虫免疫血清 50ml ，試製螢光標示抗體 Lot 1分 3 段收集 65ml，55ml，40ml 其力價皆爲 20×。此種免疫血清保存—20°C 1年，製成螢光標示抗體液 Lot 2 ，其染色力價爲至16×，並以重新免疫得之血清，製成螢光標示抗體液 Lot 3，其染色力價爲12×。

2.試製螢光標示抗體液檢查。

（1）試製螢光標示抗體液保存性

試製螢光標示抗體液 Lot.I 經 4°C 冰箱內保存後稀釋20倍，染色感染弓蟲之內臟塗抹片，其結果如表 3 。

TABLE 3. Preservation of manufactured FAb at 4°C

Infected mice	2 wks			1 mon			1.5 mons			2 mons		
	lot 1	lot 2	lot 3	lot 1	lot 2	lot 3	lot 1	lot 2	lot 3	lot 1	lot 2	lot 3
Liver	±	±	±	±	±	±	±	±	±	±	±	±
Spleen	±	±	±	±	±	±	±	±	±	±	±	±
Kidney	±	±	±	±	±	±	±	±	±	±	±	±

試製標示抗體液經 4°C 保存 2 個月力價未見降低。

（2）封入標本之螢光保存性

染色後經 50%PBS—Glycerine 封埋之陽性材料，放置室溫及冰箱其螢光保存情形如表 4 。

TABLE 4. Preservation of Toxoplasma microscopic specimen after staining

Temperature	Infected mice	1 wk	2 wks	3 wks	4 wks	5 wks
4°C	Liver	±	±	±	±	
	Spleen	+	+	+	±	
Room temp.	Liver	+	±			
	Spleen	+	±			

Legend ± : Stained slightly, + : Stained clearly,

染色封入標本保存室溫 7 天， 4°C 3 週仍可見螢光。放置時間愈久螢光強度愈弱。

（3）臟器抗原保存性

含弓蟲之經 Acetone 固定臟器塗抹片保存 4°C 及 −20°C，以試製螢光標示抗體液染色，測定塗抹片之保存性，成績如表 5。

TABLE 5. Preservation of Toxoplasma microscopic specimen after fixation

Temperature	Infected mice	1 wk	2 wks	3 wks	4 wks	2mons
4°C	Liver	±	±	+	±	±
	Spleen	±	±	±	±	±
−20°C	Liver	±	±	±	±	+
	Spleen	+	+	+	+	+

Legend ± : Stained clearly,

含弓蟲之臟器塗抹片經 4°C 及 −20°C 保存 2 個月，以螢光標示抗體液仍可染出塗抹片所含抗原。

（4）標示抗體液一凍結乾燥

將製成力價×12 Lot 3 標示抗體液，加入 madium 及未加 medium 每支分裝 0,4ml 以 acetone 預備凍結，實施急速眞空乾燥，所得乾燥成品以 PBS PH 7.2 稀釋後，以陽性小白鼠病材實施螢光染色結果如表 6。

TABLE 6. Results of staining with frozen-dried products

Fraction	Frozen-dried medium	Resuets		
1	1% glycine	$\ominus_1$ $\oplus_1$	$\ominus_2$ $\circledcirc_2$	
1	5% glycine	✿ ✿	$\ominus_2$ ✿	
1	1% lactse	✿ ✿	$\oplus_2$ $\circledcirc_2$	
1	5% lactose	✿ $\oplus_1$	✿ $\oplus_2$	
1	no medium	$\oplus_1$ $\oplus_1$	$\oplus_2$ $\oplus_2$	$\oplus_4$ $\oplus_4$
2	no medium	$\oplus_2$ $\oplus_2$	$\oplus_2$ $\oplus_2$	

✿ : showed nonspecific $\circ_2$: FAb was diluted 2×

未加 medium 之標示抗體一凍結乾燥成品，染色無非特異性，且力價未見降低，較加入 medium 理想並實施 chamber 方式眞空處理大量製造，以陽性病材染色結果，甚爲理想。

3. 感染小白鼠病材及送檢猪病材螢光抗體檢查

自弓蟲感染小白鼠之肝、脾、肺、腎、腸淋巴、腋以試製螢光標示抗體染色檢出多量之弓蟲蟲體，至於卵巢、睾丸、腎上腺、心、腸亦能檢出弓蟲。

送檢田間猪病材中 3 病例檢查肝、肺、淋巴、腎、胯檢出發黃綠螢光弓蟲蟲體，田間猪病材比人工感染小白鼠病材中之弓蟲數量爲少。

4. PK—15 株化細胞，組織培養弓蟲株螢光檢查

於 3 小時即能染出弓蟲（照片 3） 3 小時之弓蟲一般以單一弓蟲存在，六小時亦染出弓蟲，弓蟲數目於鏡下無增加情形，於六小時中發現有弓蟲成對侵入核內，此因螢光很強而核及細胞質細胞膜著螢光可看見（照片 4）， 9 小時及 12 小時弓蟲數無明顯增加（照片 5.照片 6），12 小時見縱分裂弓蟲，24 小時後弓蟲成對數目開始增加（照片 7.8）。

36小時後弓蟲成對數目增加很多（照片 9）至第 3 天檢查到弓蟲之處即發現一堆成區域分佈之弓蟲蟲體（照片10），弓蟲成堆聚集成很多小區，每一蟲體聚集區之中間很少發現有單一及少數弓蟲分佈，第 4 天及第 5 天檢查和第3天相似（照片11.照片12）。

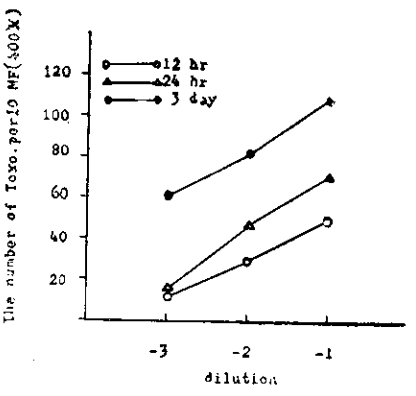


Fig. 1 Relation between inoculation quantities and examinated Toxo.in PK-15 cell line with fluorescent antibody technique.

上述接種腹水液，並以其 10^{-1} ，10^{-2} 稀釋度接種 PK-15 細胞，經12小時，24小時， 3 天計算弓蟲螢光染色數目，檢討弓蟲接種量和檢出弓蟲數之關係其結果如圖 1 所示。

弓蟲腹水液接種 PK—15 細胞，接種量少，則於 24 小時弓蟲數較12小時增加有限，接種量較多則12小時至24小時中增加較多，弓蟲量接種多寡於 3 天即呈明顯增加。

5. 送檢病材組織病理檢查

送檢田間猪病材，螢光檢查陽性病例之內臟及腦組織，以10%福馬林固定，經石臘包埋，HE 染色結果如下：

肉眼病變～

肺臟：呈間質性水腫，其炎性浸潤及小葉間結締組織水腫嚴重病例，呈現如大理石狀紋理。

淋巴腺：肝門、胃門、脾門、肺門、腸間膜、頸下等淋巴腺呈髓樣腫脹，有時可見淋巴腺呈灰白色之坏死點。

脾臟、肝臟：肉眼病變不顯着，肝臟偶而可見到灰白色坏死點。

腎臟：包膜剝離有不良情形。

腹水：常呈黃色澤濁增加。

組織鏡下病變～

肺臟：肺臟呈間質性肺炎。肺泡壁增厚，富於細胞，肺壁增厚蔓延全肺。肺壁有小圓球細胞及單核球侵入，細胞浸潤亦見於肺泡腔中，單核球（Monocyte）中可見吞噬之弓蟲蟲體，肺有水腫情形。（照片13.）。

肝臟：局部坏死巢（照片14）之分佈，局部坏死巢中有時可見小圓球細胞及組織球之浸潤。

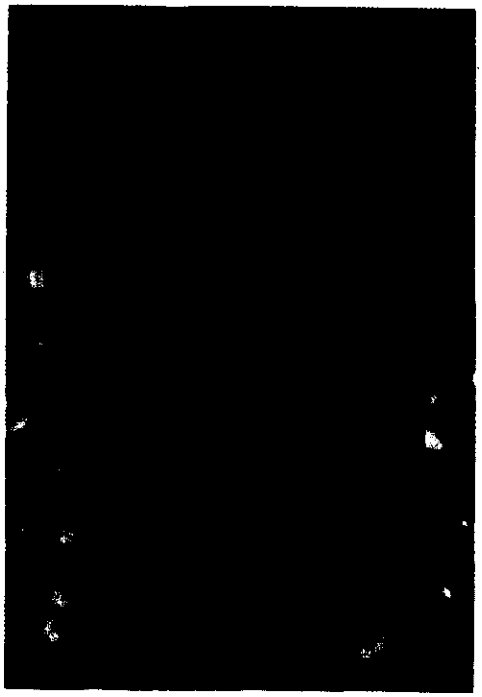
淋巴腺：皮質部及髓質部有局部坏死巢之分佈（照片15），淋巴小節之萎縮。

脾臟：濾泡之萎縮，淋巴球數目減少及局部坏死巢之散發。

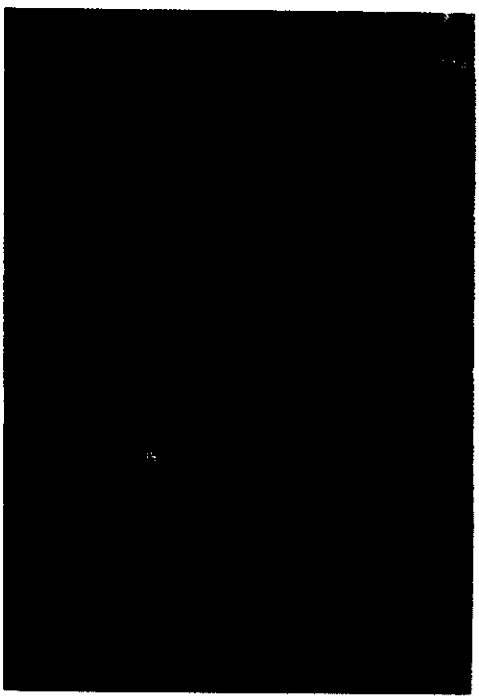
腎臟：小圓球細胞浸潤（照片16）及見腎小管坏死病變。

中樞神經，血管壁有小圓球細胞浸潤（照片17）及 Gliosis。（照片18）。

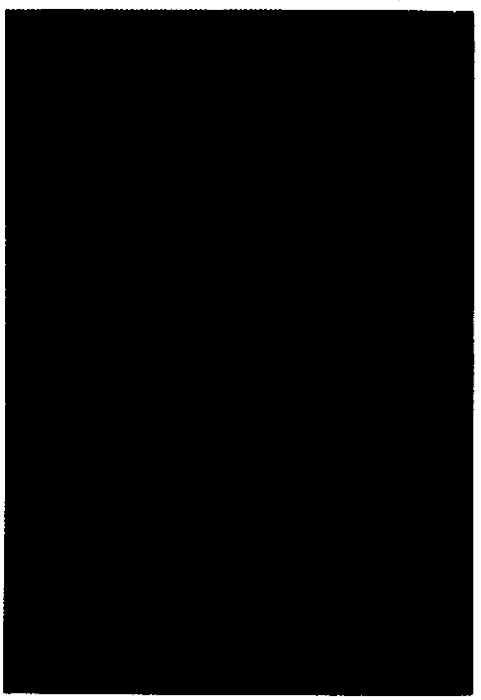
1) Toxoplasma organisms in smeared liver tissue of infected mice stained by fluorescent antibody (20x).



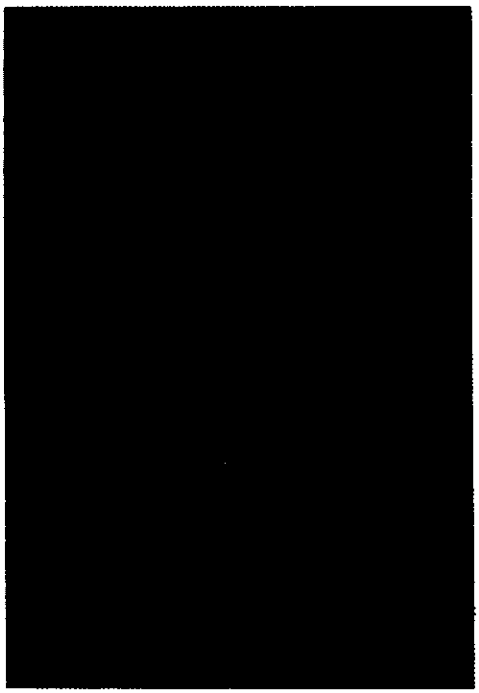
2) Toxoplasma organisms in smeared spleen tissue of infected mice stained by fluorescent antibody (20x).



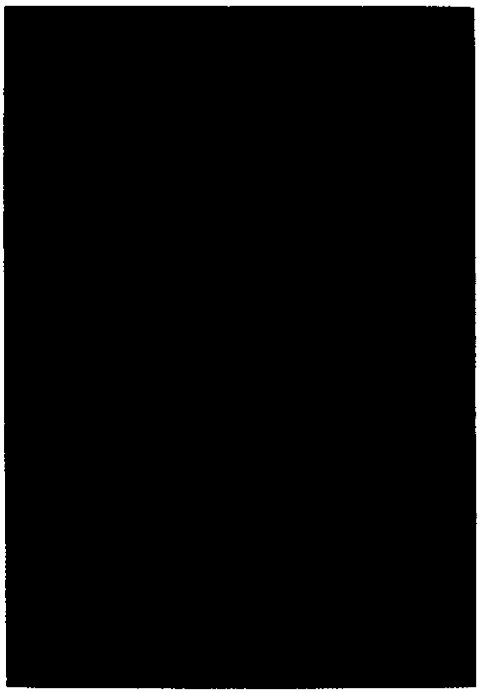
3) Toxoplasma organism in PK-15 cell line at 3 hrs after inoculation.



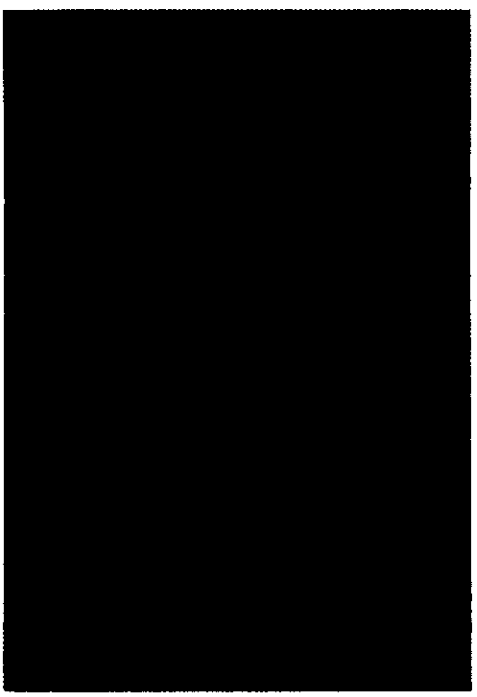
4) One paired-formed toxoplasma was found in nucleus and one single toxoplasma in cytoplasm at 6 hrs after inoculation. (PK-15cell)



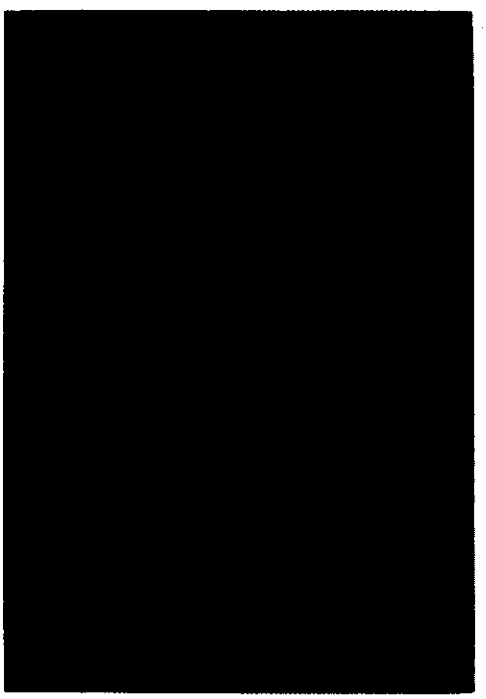
5) Toxoplasma organisms in PK-15 cell line at 9 hrs after inoculation.



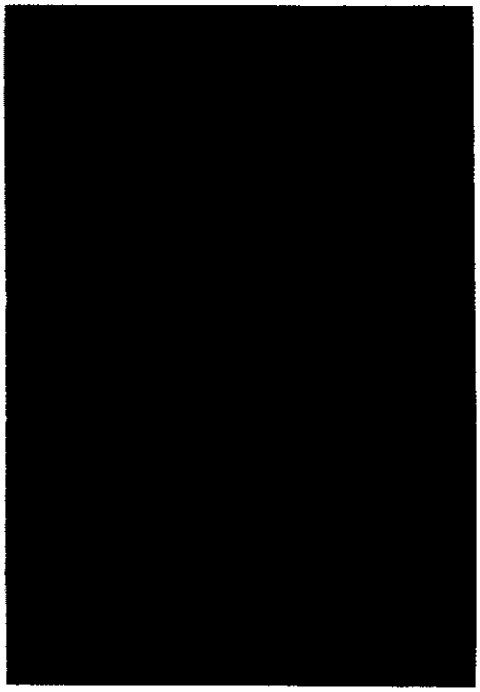
6) One pair-formed toxoplasma was found at 12 hrs after inoculation. (PK-15cell)



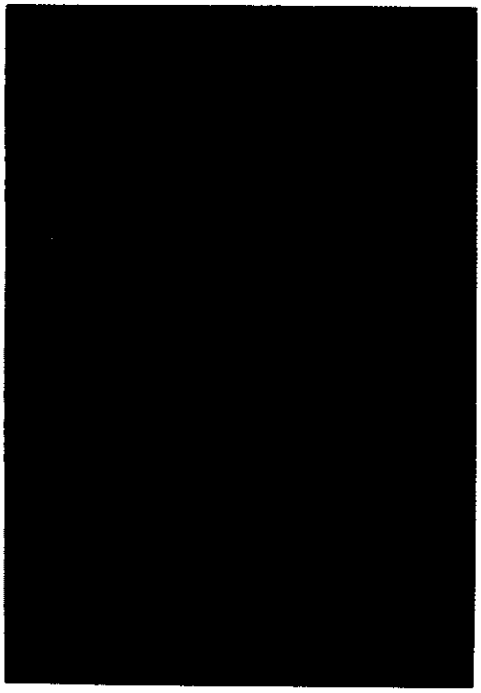
7) Rosette-formed toxoplasma was found at 24 hrs after inoculation. (PK-15cell)



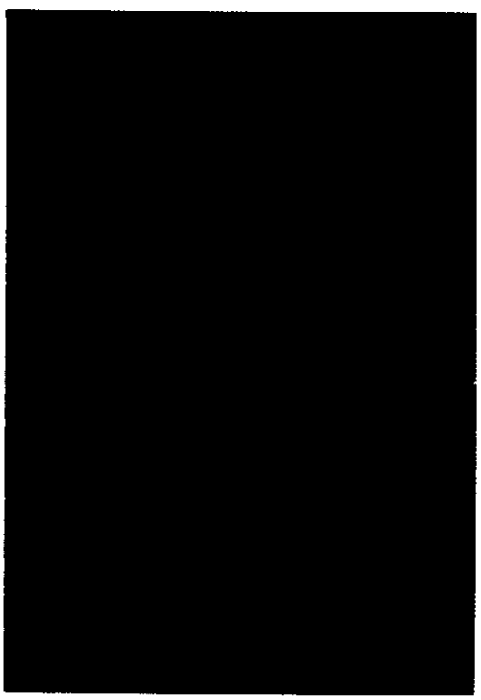
8) Pair-formed toxoplasma showed a slight increase at 24 hrs after inoculation. (PK-15cell)



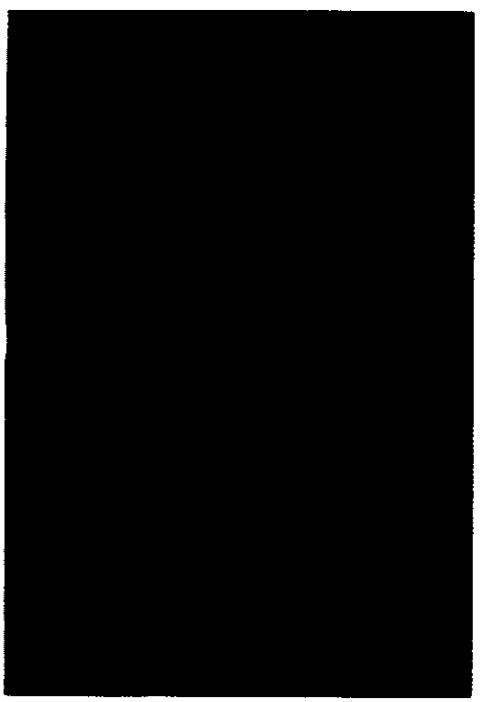
9) A large amount of paired toxoplasma was noted at 36 hrs after inoculation. (PK-15cell)



10) Focal accumulation of toxoplasma was observed at 3 days after inoculation. (PK-15cell)



11) Focal accumulation of toxoplasma was observed at 4 days after inoculation. (PK-15cell)



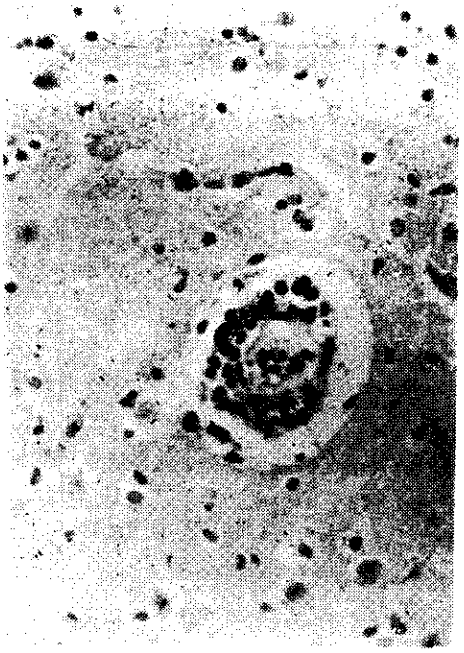
12) Focal accumulation of toxoplasma was observed at 5 days after inoculation. (PK-15cell)

(13) Interstitial pneumonia of toxoplasmosis in pig. H. E.40x.

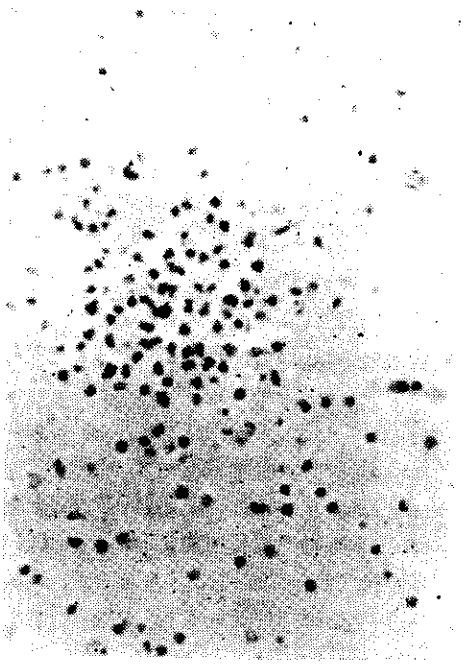
(14) Focal necrosis of the liver, H.E. 400x.

(15) Toxoplasma located in endothelial cell of lymph node, H.E.400x.

(16) Infiltration of small round cell in renal tubule.100x.



(17) Perivascular cuffing in the brain. 400x.



(18) Glial nodule in the brain.400x.

四、討　　論

Coons 等於1942年述及螢光抗體法(6)，1950年開始應用於疾病診斷研究(7)，1957年 Goldman 首次應用螢光抗體法檢出弓蟲(10)，隨後相繼應用螢光抗體直接法，藉螢光色素標附抗體而和抗原作用發出特異性螢光而確實診斷弓蟲病（2,4,12,13,14,24,28）。本省過去對弓蟲病診斷研究一向利用 Giemsa,wright,H.E. 染色及小白鼠接種（31,32,33,36,37）而未應用螢光抗體法從事弓蟲病診斷研究。螢光抗體法性特異高比其他診斷法理想，雖非抗原在視野下消失，難確立抗原和宿主間的關係，然抗原和標示抗體發出螢光特性，應用疾病診斷及研究方面仍是很理想的方法。

本試驗以小白鼠感染弓蟲經口餵飼供試豬，經數次免疫得到高度免疫血清，以螢光色素（FITC）標附通過 Sephadex, DEAE 除去未結合色素及非特異性物質，此種試製標示抗體染色感染弓蟲病材及野外病材皆檢出發黃綠螢光弓形之抗原，弓蟲之週圍二邊地區螢光清晰，中間核螢光不顯著，無螢光漏出情形；並經保存 4°C 冰箱 2 個月仍無非特異性出現，較 Ito 等(12)以內臟粉末除去非特異性結果理想。染色後材料保存於室溫 7 天，4°C 3 週仍可見螢光，臟器塗抹片保存 4°C 及 −20°C 2 個月仍可染出弓蟲和 Ito 等報告相似(12)。臟器塗抹片於 4°C 及 −20°C 可保存 2 個月結果以應用野外弓蟲病無螢光診斷設備時，可先實施塗抹保存再轉送具螢光診斷設備機構予以診斷。

弓蟲接種 PK—15 株化細胞組織培養螢光染色結果，3 小時即檢出單一弓蟲抗原，於 6 小時發現有成對弓蟲侵入核情形，此 Rumington (22)亦報告弓蟲可於核增殖。12小時，24小時成對弓虫數漸增，而36小時成對弓虫數增殖最旺盛，此由於弓蟲行縱分裂所致，以螢光組織培養染色證實了成對弓蟲仍分裂增殖的結果；接種後 3 、 4 、 5 天檢出成堆狀聚集數量很多弓蟲，此顯示弓蟲分裂增殖分離後又重新感染鄰近細胞。由所得結果，提示今後可利用 PK—15 株代細胞實施弓蟲接種增殖及繼代保存。

本省自民國59年六縣市家畜疾病防治所購置螢光顯微鏡，並經召開技術人員講習兩次且已結束，螢光抗體法已應用本省豬瘟診斷，本着即有基礎設備，當可應用於本省弓蟲病螢光抗體診斷，應用 PK—15 株化細胞實施弓蟲繼代保存增殖。

五、結　　論

1. 試製標示抗體以感染弓蟲小白鼠病材及野外送檢豬病材及病材接種 PK—15 株化細胞，皆容易染出發黃綠螢光之弓蟲抗原。
2. 試製標示抗體液保存 4°C 力價未見降低。經 50% PBS—glycerine 封埋標本，可保存室溫 7 天，4°C 3 星期。經丙酮固定之臟器塗抹片保存 4°C及 −20°C 2 個月仍不喪失其抗原性。不添加 medium 之標示抗體—冷凍乾燥成品。，無非異性，且染色力價未見降低。
3. 小白鼠組織應用螢光抗體塗抹法從肝、脾、肺、腎、腸、淋巴，胰臟檢出多量弓蟲，於卵巢、睪丸、腎上腺、心、腸檢出略少之弓蟲。從送檢豬肝、肺、腎、淋巴檢出較少量弓蟲。
4. 弓蟲 RH 强毒株接種 PK—15 株代細胞，實施組織培養螢光抗體法染色。接種感作後作後 3 小時檢出弓蟲，6 小時有發現弓蟲侵入核情形，12小時見成對縱分裂弓蟲，24小時成對之弓蟲數略增，36小時成對弓蟲數目增加最多，3、4、5 天後弓蟲成堆狀聚集數目很多。

   弓蟲稀釋量影響 PK—15 弓蟲增殖量，弓蟲接種量多寡於第 3 天皆呈明顯增加。
5. 豬弓蟲病肺臟呈現間質性水腫，嚴重病例呈現大理石狀紋理，淋巴腺呈髓樣腫脹，腹水呈微黃之昏濁增加。組織病變肺呈間質性肺炎、肝、脾、淋巴、腎呈局部壞死病變，腦呈輕度非化膿性腦炎。

誌　　謝

本研究之完成得國家經費補助，謹致衷心之謝忱。並蒙農復會李秘書長崇道，余組長如桐，林技正本欽、劉技正永和及本所陳所長守仁諸先生鼓勵指導，實驗進行亦蒙本所豬瘟研究股，寄生蟲研究股提供材料協助，謹此一併致謝。

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STUDIES ON THE FLUORESCENT ANTIBODY TECHNIQUE AND HISTOPATHOLOGICAL CHANGES OF SWINE TOXOPLASMOSIS

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English Summary

The infection of Toxoplasma gondii is demonstrable among man and animals. In Taiwan, Toxoplasma gondii has been isolated from swine, pork, canine, and wild rat. Therefore, toxoplasmosis has been become an important problem in the view of public health point of view.

It was used to detect the toxoplasma in organs with Giemsa ,Wright, Hematoxylin and Eosin,when a sample of infected tissue contains a few organisms, these method was then insufficient, a most reliable method must be established.

The fluorescent antibody technique was first described by Coons et al in 1942 and almost completed by them in 1950. It was first applied to the detection of Toxoplasma gondii by Goldman in 1957.

The present study was to manufacture fluorescent antibody (FAb) and to examine the stain titer, nonspecific, and preservation of the manufactured FAb and also applied this FAb to examine the infected organisms, field cases, the multiplication of Toxoplasma gondii on PK—15 cell line, and the pathological changes of swine toxo. The results obtained are summaried as follows :

1. Artificially immuneds erum was collected from experimental pigs which previously were orally infected with mice containing toxoplasma. The serum was fractionated with ammonium sulfate in order to obtain globulin. The globulin then was conjugated with fluorescein Isothiocynate (FITC) , for the purpose of removing the unconjugated fluorescent materials(UFM)and nonspecific staining substance, the fluorescein conjugated antibody was absorbed with Sephadex and DEAE, so manufactured FAb was applied to examine the various organ of infected mice, field swine cases and inoculated PK—15 cell. The specific fluorescent Toxoplasma was easily recognized.
2. The manufactured FAb after absorption with DEAE was usable, when preserved at 4°C for 2 months. The toxoplasma contained in specimen of organs could be preserved without loss of antigenicity at 4°C and —20°C for at least 2 months after fixation and at room temperature for one week, at 4°C for 3 weeks after staining. The frozen-dried products of FAd retain original stain titer and show ideal results.
3. The process controlled the fixation with aceton for 15min and staining with manufactuted for 30 min. was applied to examine the the infected mouse organs. Toxoplasma was

specifically recognized in the liver, spleen, lung, kidney, lymph nodes. and pancreas in large numbers and in ovary, testis, adrenal gland ,heart, intestine in small numbers. From the submitted field swine cases, the toxoplasma also was identified in the liver, lung, kidney, lymph nodes.

4. The PK—15 cell line was inoculated with toxoplasma RH strain. After the period of cultivation for 3, 6, 9, 12, 24, 36, 48 th hours and 3, 4, 5th day, the infected PK—15 was stained with manufactured FAb The results showed that the toxoplasma was recognized after 3 hrs—cultivation. At 6 hrs after inoculation, paired toxoplasma was found in the nucleus. After incubation with 12 hrs, the number of toxoplasma did not show increasing alternation. After 24hrs—incubation, pair—formed toxoplasma showed a slight increase. After 36 hrs—incubation,a large amount of paired toxoplasma were noted. While incubation from 3-5 days, a focal accumulation of toxoplasma was observed, this showed a rapid multiplication. In this experiment, the serial dilution of toxo. was inoculated on PK—15 cell, the quantity of toxoplasma stained with fluorescein—labelled antibody was also studied, The results showed when the quantity of toxoplasma inoculated on PK—15 cell was small, the rate of multiplication was slow, the toxoplasma showed distinct multiplication on the third day with the serial dilution.
5. The pathological examination of the submitted pigs died of toxoplasmosis was carried out. Through gross examination, the lung was watery, it appeared interstitial edema, in the more severe cases, the out surfaces of it appeared as marble structure. The. lymph nodes at different location were swollen moderately with watery appearance. The ascite with slightly cloudy appearance was found in the peritoneum.

The alveolar walls of lung on microscopic examimtion were rich in cells consisted of small round cell and monocyte, the edema in lung was often found. while the focal necrosis of the liver, spleen, lymph nodes Kidney could be found. The brain was found with slightly nonsuppurative encephalitis.