出國報告(類別:研習)

赴日本國立感染症研究所及北里大學參加 2017 年狂犬病診斷與監測研討會與技術交流

(2017 Rabies Diagnostic and Surveillance Conference in Japan)

服務機關: 行政院農業委員會家畜衛生試驗所

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派赴國家： 日本

出國期間： **2016** 年 **1** 月 **5** 日至 **1** 月 **17** 日

報告日期： **2016** 年 **3** 月 **30** 日

赴日本國立感染症研究所及北里大學參加 2017 年狂犬病診斷與監測研討會與技術交流

摘要

本(106)年 1 月 5 日至 17 日赴日本國立感染症研究所參加 2017 年狂犬病診斷與監測研討會，研討會期間與菲律賓、越南、蒙古及日本各都道府縣狂犬病診斷人員交流各國狂犬病疫情現況，並針對狂犬病診斷技術如即時反轉錄聚合酶鏈鎖反應(real-time reverse transcription polymerase chain reaction)、反轉錄恆溫環形核酸增幅法(reverse transcription loop-mediated isothermal amplification, RT-LAMP)、直接快速免疫組織化學染色法(direct rapid immunohistochemical test, DRIT)等進行研習。期間並赴北里大學朴天鎬教授實驗室進行參訪及交流狂犬病病理學研究。此次研習有助於了解與會國家狂犬病現況，並可增進狂犬病診斷技術與交流，並持續保持良好互動，以期未來建立長期研究合作關係。

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一、 行程綱要

日期	行程	地點
1 月 5 日(四)	搭機前往日本	臺灣-日本東京
1 月 6 日(五)	DRIT 研習	日本國立感染症研究所
1 月 9 日(一)	參加 2017 年狂犬病診斷與監測研討會	日本國立感染症研究所
1 月 10 日(二)	參加 2017 年狂犬病診斷與監測研討會 前往北里大學	日本國立感染症研究所 東京-青森
1 月 11 日(三)~13 日(五)	狂犬病技術研習與交流	北里大學
1 月 16 日(一)	搭乘新幹線前往東京	青森-東京
1 月 17 日(二)	搭機返回臺灣	日本-臺灣桃園

二、研習目的

應日本國立感染症研究所 Dr. Satoshi Inoue(井上智博士)邀請，前往參加 2017 年狂犬病診斷與監測研討會，與日本各都道府縣狂犬病診斷人員進行臺灣野生動物狂犬病診斷與監測現況分享，研討會亦邀請越南、蒙古、菲律賓等國外狂犬病實驗室診斷人員就其國內狂犬病疫情進行分享。赴日期間，前往青森縣北里大學參訪 Dr. Park Chun-Ho(朴天鎬博士)病理實驗室並交流狂犬病病理學研究。

三、　研習內容

(一)、2017 年狂犬病診斷與監測研討會

I. 日本:研討會邀請日本各都道府縣狂犬病診斷人員就轄內狂犬病監測及診斷進行分享。

1. 北海道：北海道境內有許多野生動物存在，可能存在狂犬病的野生動物主要為紅狐(red fox)、貉(raccoon dog)及浣熊(raccoon)。1993 年日本所有都道府縣野生動物路死數量統計上，紅狐路死數目以北海道居冠，貉路死數目於北海道為 0.5 例/公里。浣熊為一外來種，2008 年浣熊捕捉數目統計中，亦以北海道所捕捉到數目最多。但是目前北海道尚未開始進行針對野生動物進行狂犬病監測，目前僅針對疑似狂犬病犬隻進行監測。
2. 東京：自 2014 年開始針對野生動物進行狂犬病監測，主要監測野生動物為貉(受傷或生病)，平均每年監測數目為 1~2 例。
3. 千葉縣：千葉縣境內有成田國際機場及千葉港，為日本主要國際交通及運輸所在，因此自其他狂犬病疫區輸入狂犬病至千葉縣之可能性及風險性較高。因次每年於成田機場檢疫所進行狂犬病相關技術研修，並制定千葉縣狂犬病應變計畫，其分成 3 階段：(1)未發生時對應，進行犬隻晶片登錄、推廣狂犬病疫苗施打及定期舉辦研修會。(2)初步應對，若發生咬傷意外或有犬隻觀察到異常行為，將進行捕捉收容、隔離觀察。

檢查及診斷，必要時進行疫情調查、千葉縣衛生研究所進行犬隻解剖及狂犬病診斷，診斷結果呈現陽性或是偽陽性則將檢體後送至國立感染症研究所進行確認。(3)若是確認陽性，則將設立千葉縣健康危機管理對策總部，並將針對發病犬隻的感染源進行調查、調查與發病犬接觸的犬隻及人，依據調查結果進行處理以避免狂犬病疫情擴大。因應措施包含評估與發病犬接觸的人是否需要接受治療或進行暴露後疫苗免疫，與發病犬接觸的犬進行隔離觀察等。最後朝向狂犬病清淨的處理，該地區持續進行調查，並觀察 6 個月內是否有新的感染動物或人。2015 年千葉縣內發生一例體長 120 公分的紀州犬咬傷人案例，依據千葉縣狂犬病應變計畫進行相關診斷，犬隻進行剖檢，採集腦組織進行狂犬病診斷。2008 年 12 月至 2013 年 3 月共收集 194 犬隻血清樣本進行千葉縣犬隻狂犬病血清抗體陽性率調查，以 RFFIT(Rapid Fluorescent Focus Inhibition)進行檢測，抗體力價大於 0.5 IU/mL 判定為陽性，其血清抗體陽性率為 50%，數據分析顯示家犬抗體陽性率為 79%，流浪犬抗體陽性率為 13%。

4. 名古屋市：地方政府須提供快速及準確狂犬病診斷，因此於市內建立狂犬病診斷系統，由動物福利及管理中心進行解剖，採樣取腦組織，送至市內公衛機構進行狂犬病診斷。2012 年及 2016 年開設狂犬病診斷研習提供相關人員進行訓練。
5. 德島縣：境內已註冊犬隻數目約 40,500 隻，但實際數目約 55,000 隻，平均每年發生約 30 至 40 例動物咬傷人意外事件，平均每年被捕捉流浪犬數目約 700 至 800 隻。目前所遭遇的問題為境內未註冊的犬隻數目增加，且許多畜主不了解「狂犬病」，甚至有些獸醫也不清楚何謂「狂犬病」。因此與中央政府合作於德島縣舉行訓練課程，邀請鄰近都道府縣相關人員進行狂犬病訓練。
6. 熊本縣：狂犬病診斷由 Kumamoto Prefectural Institute of Public Health and Environmental Science (KIPHES)旗下的 Department of Microbiology 負責。熊本縣政府於 2012 年制定”Kumamoto prefectural manual for rabies

or suspected rabies outbreak”，主要分成三階段：第一階段為正常時期，將依據狂犬病防治法(Rabies Prevention Law)進行狂犬病預防計畫，包含提升犬隻註冊及免疫率、降低流浪犬數、輸入動物檢疫等。第二階段為疑似狂犬病爆發，疑似狂犬病動物將會送至 KIPHES 進行剖檢、採樣及狂犬病診斷，若初步診斷為陽性，則樣本將後送至國立感染症研究所進行確診。同時期將於公衛中心設立疫情調查小組(Epidemiological survey team)針對疑似狂犬病動物進行隔離檢疫並禁止運輸，並進行疫情調查，並將向熊本縣政府衛生危機管理部報告。熊本縣政府衛生危機管理部的職責則為協調及聯繫相關機構，舉行會議以決定如何處理此次疫情爆發。若確診為狂犬病則進行第三階段狂犬病暴發，將設立熊本縣狂犬病對策總部以因應疫情。

熊本縣於 2013 年開始執行疑似狂犬病動物的監測系統，並於 2013 年 12 月 18 日進行 1 例疑似狂犬病犬隻診斷。12 月 18 日晚間 11 點臨床醫師通報一名 60 歲男性被自己所飼養的犬隻咬傷左手小拇指，所飼養犬隻為 5 歲齡的大丹犬，平時為放養，該犬隻並未註冊且未進行狂犬病疫苗免疫，犬隻於 1 個月前持續出現前肢筋攣，並於咬傷畜主後不久即死亡。犬隻送至 KIPHES 進行剖檢，進行腦組織採樣，進行狂犬病診斷，並將樣本送至國立感染症研究所進行確診，該犬隻確診為狂犬病陰性，於剖檢發現造成急性死亡的原因應為心絲蟲症。

人員於案例發生前不久剛完成狂犬病相關訓練，因此遭遇本案例時，所有檢測完成得非常順利，但是因為疑似狂犬病案例於日本非常稀少，因此人員非常容易遺忘相關診斷流程，因此自 2016 年熊本縣政府將定期舉行狂犬病診斷的培訓。

7. 沖繩縣：沖繩縣針對食蛇獴(small Indian mongoose)進行狂犬病監測，針對食蛇獴的主要原因為食蛇獴為入侵種食肉目野生動物、棲息地分布有擴張趨勢、與人及家畜有接觸的機會、國外有食蛇獴罹患狂犬病的案例

等。因此沖繩縣政府與相關組織進行合作，若有發現死亡或捕捉的野生動物個體將通知動物福利及管理中心進行採樣，並將樣本送至 Institute of Health and Environment 進行狂犬病診斷。2014 年至 2015 年共計進行 9 例食蛇獴、2 例蝙蝠、8 例咬人犬及 2 例咬人貓的狂犬病檢測。

II. 越南:狂犬病疫情發生於 25 個省份共計 63 個城市，主要發生於山區及內陸地區。2014 年至 2016 年分別有 66 例、78 例、及 77 例人感染狂犬病案例。每年有超過 330,000 人接受暴露後狂犬病疫苗免疫。整年皆有狂犬病案例發生，犬隻為狂犬病主要宿主及傳播者，約有 95~97%人感染狂犬病案例是被家犬咬傷或與狂犬病犬隻或貓隻接觸所造成。尚無證據證實狂犬病死亡案例是由於暴露於其他狂犬病動物，但無法排除狂犬病病毒存在於其他動物。目前越南僅有第一基因型狂犬病病毒，每年人類死於狂犬病的數目統計是基於臨床診斷而非實驗室診斷。目前有針對人類感染狂犬病進行全國性控制及預防，但並未制訂動物狂犬病相關措施。

越南於 2005 年為利進行人類狂犬病診斷於河內國家衛生及流行病研究所(National Institute of hygiene and epidemiology, NIHE)建立第一個狂犬病診斷實驗室，並於 2006 年至 2015 年在日本國家感染症研究所協助下於 NIHE 從事狂犬病診斷及相關研究。目前於越南共計有 5 間實驗室有能力可進行狂犬病診斷，包含狂犬病直接免疫螢光染色法、反轉錄聚合酶鏈鎖反應(RT-PCR)、即時反轉錄聚合酶鏈鎖反應(real-time RT-PCR)及反轉錄恆溫環形核酸增幅法(RT-LAMP)。

2006 年至 2015 年造成人類及犬隻狂犬病案例皆為第一基因型的狂犬病病毒。親緣關係樹分析結果顯示，越南的狂犬病病毒可分為 2 群，第 1 群又可細分為 2 小群；sub-group 1a 的病毒主要分離自越南北部及中國，sub-group 1b 的病毒主要循環於高地(highland)及越南南部，第 2 群的病毒主要發現於越南北部及中國。自 2010 年至 2015 年所分離出病毒，皆屬於第 1 群。

越南在日本國立感染症研究所及 WHO 資助及技術協助下進行麗沙病毒研究，於越南北部 6 個省份收集了 1200 例蝙蝠血清樣本，以 FAVN(Fluorescent Antibody Virus Neutralization) test 進行 5 種麗沙病毒(狂犬病病毒、Lagos bat virus、Mokola virus、Duvenhage virus 及 European bat lyssavirus type 1)血清中和抗體力價測定，並以狂犬病直接免疫螢光抗體染色及 RT-PCR 於蝙蝠腦組織進行麗沙病毒抗原及核酸檢測。麗沙病毒血清抗體檢測結果顯示多種麗沙病毒(狂犬病病毒、Duvenhage virus、European bat lyssavirus type 1 及 Lagos bat virus)抗體陽性，可能是由於病毒間的抗體交叉反應，蝙蝠血清抗體陽性顯示蝙蝠曾暴露於所檢測的麗沙病毒或是未知的麗沙病毒。但是尚未有病毒於蝙蝠中被檢測出，因此無法斷定這些蝙蝠曾暴露於那種病毒。

III. 蒙古:蒙古地廣人稀，農業佔該國 GDP 的 16%，圈養動物(綿羊、山羊、牛、馬、駱駝)數目約 6 千萬。許多已知狂犬病可能存在的野生動物宿主分布於蒙古(狼，紅狐等)。自 1974 年至今，共計有 36 例人類感染狂犬病導致死亡案例。對於圈養動物的狂犬病控制策略為當發現圈樣動物被疑似狂犬病動物咬傷後，獸醫將檢查該農戶所圈養的動物，發現身上有傷口的動物直接進行撲殺，其他身上並未發現傷口的動物進行狂犬病疫苗免疫，並進行檢疫觀察。檢疫觀察期間若有動物出現異常症狀，則該動物進行撲殺。蒙古不易進行野生動物狂犬病控制，主要原因為國土面積太廣且食肉目野生動物族群數量不明，因此不易進行控制。

IV. 菲律賓:狂犬病於菲律賓平均每年造成 200 至 300 人死亡，每年動物狂犬病陽性率約 20~33%。動物狂犬病案例是依據實驗室診斷，但人類狂犬病案例主要是經由臨床診斷。因為菲律賓屬於多島嶼國家，雖然目前已設立 19 狂犬病診斷實驗室，但仍持續設置狂犬病診斷實驗室以利及時進行狂犬病診斷。

V. 臺灣:邀請衛生福利部疾病管制署謝若郁針對臺灣狂犬病公共衛生議題進行演講，並邀請畜衛所胡書佳助理研究員針對臺灣野生動物狂犬病疫情現況及檢測進行演講。

圖 1、日本國立感染症研究所井上智博士進行研討會致詞及主持。

圖 2、日本各都道府縣狂犬病診斷人員進行自我介紹。

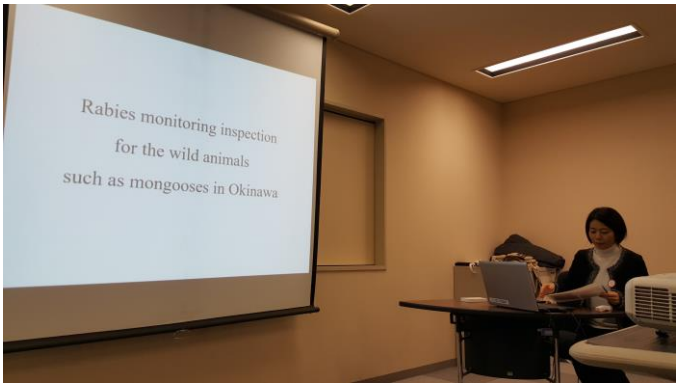


圖 3、日本沖繩縣狂犬病診斷人員進行簡報。

圖 4、越南專家進行越南狂犬病現況簡報。

圖 5、蒙古專家進行蒙古狂犬病現況簡報。

圖 6、菲律賓專家進行狂犬病現況及 DRIT 簡報。

圖 7、臺灣疾管署進行狂犬病公衛議題簡報。

圖 8、本所進行野生動物狂犬病現況簡報。

VI. 狂犬病檢測方法:

1. 即時反轉錄聚合酶鏈鎖反應(real-time RT-PCR):日本目前雖屬狂犬病非疫區，實驗室仍積極建立各種狂犬病診斷方法。日本國立感染症研究所目前依據 GeneBank 資料庫上所公開之亞洲狂犬病病毒株，設計亞洲區狂犬病檢測引子及探針，希望可與亞洲各狂犬病診斷實驗室合作，進行所設計之引子及探針的測試。
2. 反轉錄恆溫環形核酸增幅法(RT-LAMP):日本國立感染症研究所與蒙古 Institute of Veterinary Medicine 的 Dr. Boldbaatar Bazartseren 合作開發狂犬病 RT-LAMP 檢測方法。LAMP 檢測方法之優點在於檢測時間短(低於 1 小時)、不需要複雜的設備且檢測結果很容易判定(可直接用眼睛觀察)。目前依據不同地區的狂犬病病毒株序列設計 inner primer 並將不同的 inner primer 混合使用，可同時檢測出不同 lineage 的狂犬病病毒株。亦希望可與亞洲各狂犬病診斷實驗室合作，進行所設計之 LAMP 引子的測試。
3. 直接免疫組織化學染色(Direct Rapid Immunohistochemical Test, DRIT):目前 WHO 及 OIE 建議狂犬病診斷黃金標準為狂犬病直接免疫螢光染色法(direct flourescent antibody, DFA)，但對於開發中國家設立實驗室使用 DFA 進行檢測有困難，因為需要許多昂貴設備(包含螢光顯微鏡、培養箱、冰箱等)。DRIT 為美國疾病控制與預防中心所開發較便宜的診斷方

法，主要原理是利用免疫組織化學染色檢測狂犬病病毒，以 biotin 標記的狂犬病單株抗體與狂犬病抗原作用後，加入 streptavidin peroxidase 與 biotin 標記的狂犬病單株抗體作用後，加入 AEC 呈色，以光學顯微鏡進行觀察。亦有文獻發表認為 DRIT 其敏感度及特異度堪比 DFA。然而，biotin 標記的狂犬病單株抗體並非商業化產品，因此日本國立感染症研究所與菲律賓熱帶醫學研究所合作，由國立感染症研究所協助改良並進行狂犬病多株抗體(質體)的製備，由熱帶醫學研究所於菲律賓將質體多次免疫於兔子以製備多株抗體，及進行後續抗體純化及 biotin 標示，並以當地狂犬病檢測案例進行 DRIT 測試。

DRIT 試驗步驟:

a. 製備腦壓片 ，並以 10%福馬林固定 10 分鐘，以 TPBS 沖洗液潤洗玻片 2 次。
b. 玻片上加入以 3%過氧化氫作用 10 分鐘，以 TPBS 沖洗液潤洗玻片數次，將玻片周圍液體擦拭乾淨。
c. 玻片加入 biontin 標示的一次抗體，室溫作用 10 分鐘，以 TPBS 沖洗液潤洗玻片數次，將玻片周圍液體擦拭乾淨。
d. 玻片加入 streptavidin peroxidase，室溫作用 10 分鐘，以 TPBS 沖洗液潤洗玻片數次，將玻片周圍液體擦拭乾淨。
e. 準備 AEC 呈色劑，玻片加入 AEC 呈色劑，室溫作用 10 分鐘，以蒸餾水潤洗玻片數次，將玻片周圍液體擦拭乾淨。
f. 玻片加入蘇木紫染背景，室溫作用 2 分鐘，立即以蒸餾水潤洗玻片數次，將玻片周圍液體擦拭乾淨。
g. 以水溶性封片膠進行封片，玻片置於光學顯微鏡下進行觀察，陽性訊號將呈現紅色。

熱帶醫學研究所目前測試結果顯示，福馬林固定或是丙酮固定的腦壓片其 DRIT 測試結果與 DFA 結果一致。因此日本國立感染症研究所希望

與亞洲各狂犬病診斷實驗室合作，由國立感染症研究所提供抗體製備標準作業流程，供各實驗室自行生產抗體進行 DRIT 測試，希望藉由此種合作模式，了解 DRIT 應用狂犬病病例診斷上之可行性。

圖 9、狂犬病 real-time RT-PCR 簡報。

圖 10、狂犬病 RT-LAMP 簡報。

圖 11、參與研討會人員於國立感染症研究所前合影

(二)、參訪北里大學朴天鎬教授病理實驗室

本次參訪朴天鎬教授病理實驗室人員分別有來自蒙古 Dr. Boldbaatar Bazartseren、菲律賓 Dr. Daria Llenaresas-Manalo 及畜衛所胡書佳助理研究員。朴天鎬教授簡介病理實驗室人員，目前旗下有 2 名博士生，分別為志和希小

姐及君付和範先生，實驗室並有獸醫學部學生參與組織修片、切片製作、病例切片判讀診斷及動物剖檢等事務。參訪行程中並拜會北里大學獸醫學部學部長高井伸二博士。本次參訪內容摘要如下:

I. 參與動物剖檢及參觀解剖房:

1 月 11 日下午緊急解剖案例，為一 11 月齡 300 公斤重的日本雄性和牛(已去勢)，因治療後臨床症狀無改善並經大動物臨床醫師評估預後不佳，建議畜主進行安樂死。因病因不明，因此進行緊急解剖以釐清病因。動物安樂死後，由大動物臨床醫師進行該病例病史、臨床症狀、治療及相關檢查結果說明，並由大動物教授進行補充說明。全體人員對犧牲動物鞠躬後才開始進行解剖。本案例由朴天鎬教授主要負責解剖，病理實驗室獸醫學部學生協助進行肉眼病變拍攝及記錄，病理實驗室獸醫學部學生及博士生進行各臟器檢查及採集福馬林固定組織。

在本次解剖屬完全解剖，於解剖過程中可感受到日本人處事的細緻度及完整度，解剖過程中容易疏忽的腦下垂體、眼球等都有專人進行採樣，並在鋸開頭骨前，獸醫學部學生完整剃除頭部皮膚並善用工具進行頭部固定以便於鋸開頭骨。並於解剖完畢，由病理實驗室獸醫學部學生統整並說明案例所見肉眼病變，再由朴天鎬教授補充說明，並詢問現場臨床醫師是否有疑問並進行討論。

該解剖房設有焚化爐，因為北里大學位於日本北部的青森縣，因此冬季氣溫低於攝氏 0 度，故動物屍體肢解完畢後，可直接放入焚化爐內，等待下次焚燒。

圖 12、由臨床醫師進行病例資料說明。

圖 13、剖檢完畢由病理實驗室人員進行肉眼病變說明及討論。

II. 專題演講:

因應日本國內較少有惡性傳染病案例發生，因此朴天鎬教授邀請蒙古 Dr. Boldbaatar Bazartseren 針對蒙古曾發生的傳染病案例進行分享。Dr. Boldbaatar Bazartseren 介紹蒙古的獸醫體系及 Institute of Veterinary Medicine 主要研究，並針對羊痘及小反芻獸疫進行介紹。內容摘要如下:

1. 羊痘:屬於羊痘病毒屬(*Capripoxvirus*)，痘病毒科，臨床上皮膚可見斑(macules)或丘疹(papules)，在皮膚無毛區(如:腹部乳頭、尾根部)較易觀察，後期可見形成黑色痂皮。肉眼病變於消化道黏膜可見痘樣病變(pox lesion)，肺臟可見嚴重廣泛性的痘樣病變。
2. 小反芻獸疫:小反芻獸疫病毒屬於麻疹病毒屬，副黏液病毒科，僅有一種血清型，但病毒可分成 4 個 lineage。主要易感動物為綿羊及山羊，臨床症狀可見發燒、眼鼻分泌物增加(鼻分泌物一開始是清澈，逐漸變為黏稠)、口腔黏膜糜爛壞死，偶發可見下痢。肉眼病變可見舌、食道等組織黏膜糜爛或壞死，直腸因鬱血而出現斑馬狀條紋(zebra stripes)特徵性病變等。須與口蹄疫、藍舌病等進行區別診斷。2017 年 1 月於分布在蒙古西部瀕臨絕種的賽加羚羊(*Saiga tatarica mongolica*)族群中爆發小反芻獸疫疫情。

圖 14、蒙古 Dr. Boldbaatar Bazartseren 進行蒙古獸醫體系簡介。

圖 15、蒙古 Dr. Boldbaatar Bazartseren 進行傳染病簡介。

III. 研習狂犬病免疫組織化學染色法(Immunohistochemistry stain, IHC):

朴天鎬教授以狂犬病 anti-P 抗體進行狂犬病免疫組織化學染色並搭配蘇木紫-伊紅(H&E)染色法進行狂犬病病理學研究。

IHC 染色步驟:

a. 將玻片置於 4 缸 clear plus 及高濃度到低濃度酒精(2 缸 100%、95%、90%、80%及 70%)進行組織回水。
b. 玻片以清水清洗數次。
c. 玻片加入 0.25% trypsin，室溫作用 30 分鐘以進行抗原回復(enzymatic antigen retrieval)，作用後以清水清洗數次。
d. 玻片加入 0.3%過氧化氫-甲醇溶液，室溫作用 60 分鐘，作用後以清水清洗數次。
e. 玻片加入 10%山羊血清，室溫作用 60 分鐘以阻斷非特異性反應。
f. 玻片加入 1 次抗體(兔子 anti-P 狂犬病抗體 1000~1200 倍稀釋)，於 4℃作用隔夜。作用後以 PBS 清洗 3 次，每次 5 分鐘。
g. 玻片加入二次抗體 simple stain MAX-PO，室溫作用 30 分鐘，作用後以 PBS 清洗 3 次，每次 5 分鐘。
h. 玻片加入 DAB 呈色，玻片置於顯微鏡下觀察，當樣本出現陽性訊號後

尚未出現非特異性訊號前(約 1~2 分鐘)，以清水清洗中止反應。

i. 玻片加入蘇木紫以進行背景染色，並以流動清水清洗 5 分鐘。

j. 封片前，玻片分別置入低濃度到高濃度酒精(80%、90%、100%)、2 缸二甲苯及 3 缸 clear plus 脫去多餘水分後，再以 PARA mount-N 封片膠(非水溶性封片膠)進行封片

IV. IHC 觀察及狂犬病病理學研究交流:

比較狂犬病陽性腦組織於 IHC 染色及 H&E 染色法病變的差異，在 H&E 染色法可於腦部的神經元觀察到嗜酸性質內包涵體(Negri body)，但是頻率不一，有時不易觀察；但於 IHC 染色下，可在腦部神經元觀察到明顯且多量的紅色陽性訊號。朴天鎬教授長期於菲律賓熱帶醫學研究所合作，取得狂犬病犬腦組織在實驗室進行狂犬病病理學研究，以 IHC 進行狂犬病陽性犬隻唾液腺染色，可觀察到陽性訊號出現於顎下腺(mandibular gland)內的神經節神經元、黏液腺泡上皮細胞及肌上皮細胞(myoepithelium)，但於管道上皮細胞(duct epithelium)及 serous demilune 並未有明顯發現。耳下腺的腺泡細胞、肌上皮細胞及管道上皮細胞皆未出現陽性訊號。上述發現證實了狂犬病病毒從腦部增殖後向下經由顏面神經到達顎下腺神經節神經元，之後經由唾液腺肌上皮細胞後通往腺泡上皮細胞的路徑，相關內容已發表於期刊。

朴天鎬教授分享最新發表的發現，人類狂犬病生前檢測是使用皮膚 biopsy 檢體進行檢測，在哺乳動物的 muzzle skin 富含許多觸毛(tactile hair)或稱為 follicle-sinus complex (FSC)，每個觸毛有 2000 個以上的感覺神經末梢存在。朴天鎬教授於 muzzle skin 切片進行 IHC 染色，可於 FSC 觀察到陽性訊號，搭配其他抗體 IHC 檢測結果顯示染出陽性訊號的細胞可能是 Merkel cell，因此朴天鎬教授認為觸毛可作為狂犬病診斷的標的之一。相關內容已發表於期刊。朴天鎬教授樂於與我們分享他於狂犬病病理學上的發現，也希望與我們合作，期待我們一同驗證他的發現。

圖 16、朴天鎬教授介紹犬的 muzzle skin。

圖 17、朴天鎬教授觸毛組織修片方法。

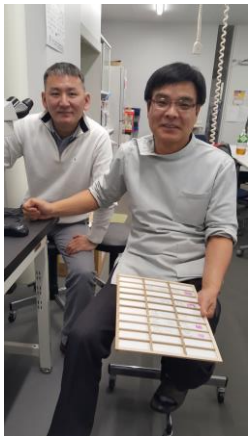


圖 18、朴天鎬教授分享狂犬病病理學上的發現。

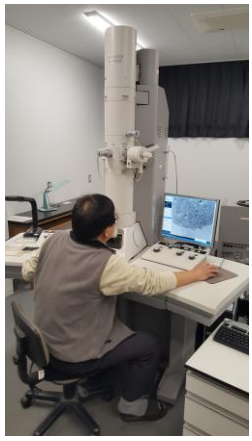


圖 19、朴天鎬教授使用電子顯微鏡進行狂犬病病理學研究。

圖 20、實驗室所張貼狂犬病病理研究海報。

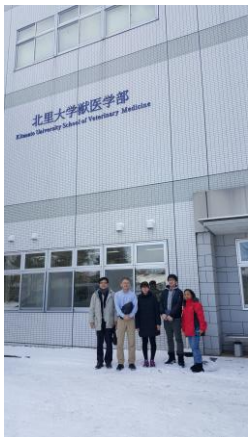


圖 21、與朴天鎬教授及其研究生、蒙古及菲律賓專家於北里大學獸醫學部外合影。

四、　心得與建議

感謝日本國立感染症研究所邀請及長官提供本次珍貴機會參加本次研討會及研習，感謝井上智博士於行程安排的協助及赴日期間的照顧，並感謝朴天鎬教授及其博士生志和希小姐及君付和範先生於赴北里大學參訪時的照顧及經驗交流分享。

相關心得整理如下：

- 對於可能發生的疫情抱持著嚴陣以待的精神

參與 2017 年狂犬病診斷與監測研討會，了解日本目前各單位對於狂犬病防疫所作的努力，雖然日本目前為狂犬病非疫區，但自從臺灣(維持狂犬病非疫區多年)於 2013 年爆發野生動物狂犬病疫情以來，日本學習到了就算目前尚未有狂犬病疫情發生於日本，但不見得相同的情況不會發生在日本身上。因此相關單位積極針對可能發生的疫情作準備，建立狂犬病疫情發生的緊急應變措施、加強相關人員教育訓練、積極開發狂犬病診斷方法等作為，對於可能發生的疫情抱持著嚴陣以待的精神來面對。

同樣的精神亦可用在其他疾病的防治上，臺灣為海島型國家，目前雖有許多海外惡性傳染病並未波及至我國，但隨著國際運輸的便利，人員、動物及貨品往來頻繁更有可能造成疾病的傳播，因此在疾病診斷方面，我們更需要做好萬全準備，以供緊急狀況時可提供診斷服務。

- 對於生命的尊重及作業的精緻化

本次赴北里大學參訪，全程參與了病例解剖，在解剖過程中可以看得出北里大學獸醫學部學生及教授對於生命的尊重，適當的安樂死方法盡可能避免動物感受到苦痛。從進行安樂死到完成解剖的過程中，許多處理方式都可以看出對於作業精緻化的要求。例如:動物放血時的處理，始將引流管插入頸動脈，將引流管

導入排水通道，因當地氣溫偏低，適時加入熱水以避免血液凝固造成阻塞，如此可以避免解剖現場的髒亂並使後續現場清潔更容易。剖檢移除消化道時，會以繩子先將消化道綁緊後再剪斷，以避免汙染及造成現場髒亂。另以容器盛裝消化道，以避免剪開消化道進行檢查時，消化道內容物溢出造成影響。採集的樣本放入福馬林固定前，以帶有小紙片的細繩標記所採集的樣本。腦組織採樣易遺漏的腦下垂體，也會有專人進行採集。

許多我們認為理所當然的程序，只需要再加入一些簡單的小步驟，就可以使整個過程更臻於完美。

- 未來的持續合作

日本為狂犬病非疫區，但仍積極針對狂犬病進行新興診斷方法的開發及研究，並協助東南亞開發中國家設置狂犬病實驗室及建立診斷方法，並積極與亞洲各國狂犬病實驗室合作。日本希望可以藉由日本開發狂犬病診斷方法，亞洲各狂犬病診斷實驗室應用該國狂犬病案例驗證日本所開發的狂犬病診斷方法，並回饋其結果，以供日本實驗室進行方法改進，並希望可藉由此模式提升亞洲各國狂犬病診斷實驗室診斷能力。日本國立感染症研究所及北里大學皆希望未來可持續於本所進行狂犬病研究的合作，也希望未來在與本所 MOU 簽訂後，可逐步開展相關合作計畫。

Workshop for molecular diagnosis of rabies and technical training
NIID, Tokyo, Japan at Jan. 9th and 10th in 2017

Action plan, molecular diagnosis, \wildlife survey in Japan

What preparedness we need?

Satoshi INOUE, PhD DVM

Laboratory of Transmission Control of Zoonosis,
Department of Veterinary Science,
National Institute of Infectious Diseases, Japan

The last indigenous rabies cases in Japan.

1956 Human

1957 Cat

The imported rabies cases in Japan.

1970 Student infected at Nepal

2006 Retired men infcted at Philippines

Three ferret-badgers (*Melogale moschata*) were confirmed as rabies and reported to OIE on July 17, 2013.

TAIPEI TIMES 2013 July 25 Thu

Ferret-badger with rabies bit man

FIRST BLOOD: The man was bitten on the finger by a sick ferret-badger, but the Centers for Disease Control said first tests have found no sign of rabies in the man

By Lee I-chia / Staff reporter, with CNA

A dead ferret-badger was found in Taitung County's Donghe Township yesterday. Photo: CNA

The first case of a person being bitten by a wild Formosan ferret-badger infected with rabies was confirmed in Taitung County's Donghe Township (東河) on Tuesday night, the Council of Agriculture said.

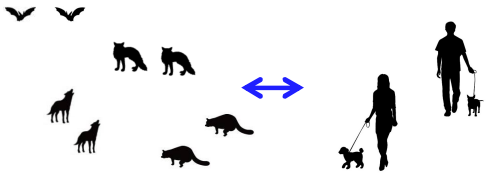
The incident came in the wake of the council's announcement last week that three cases of rabies infection were found in dead wild ferret-badgers in Yunlin County's Gukeng (古坑) and Nantou County's Yuchi (魚池) and Lugu (鹿谷) last year, causing Taiwan to fall off the list of rabies-free countries after 54 years.

FB = Ferret badger

Strong impact in Japan for the rabies free status.

Wildlife Rabies

What preparedness we need!



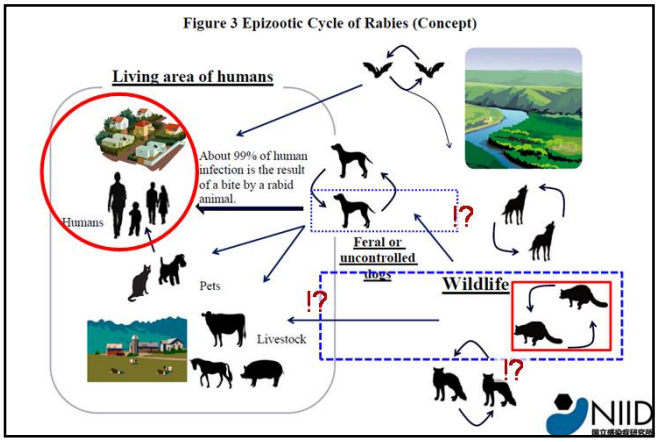
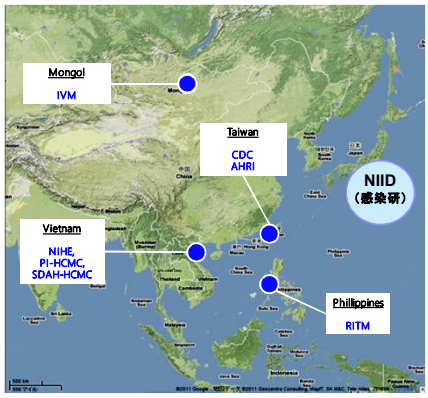


Figure 3 Epizootic Cycle of Rabies (Concept)

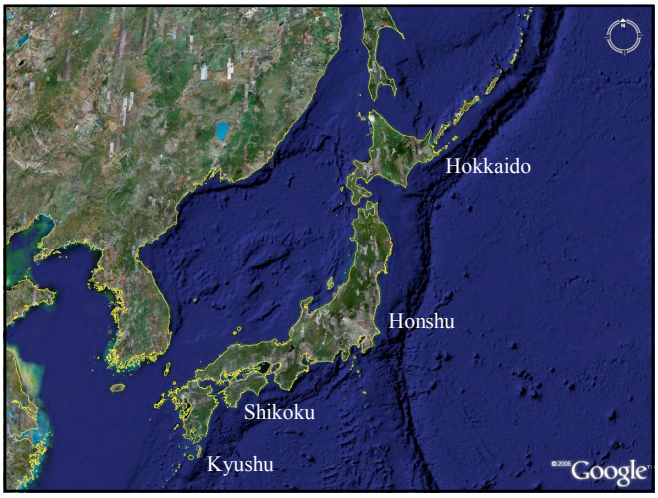
Keys for Strengthening Rabies Control

1. **Laboratory network**
2. **Evaluation of diagnostics**
3. **Establishing free zones**
4. **Border control by the cooperation**


Mongol
IVM
Taiwan
CDC
AHRI
NIID
（感染研）
Vietnam
NIHE,
PI-HCMC,
SDAH-HCMC
Phillippines
RITM

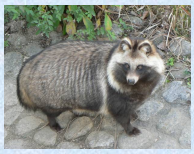
Surveillance of animal rabies in Hokkaido, JAPAN

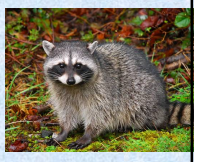
Kohji URAGUCHI
Hokkaido Institute of Public Health



Major (potential) vectors for rabies in Hokkaido

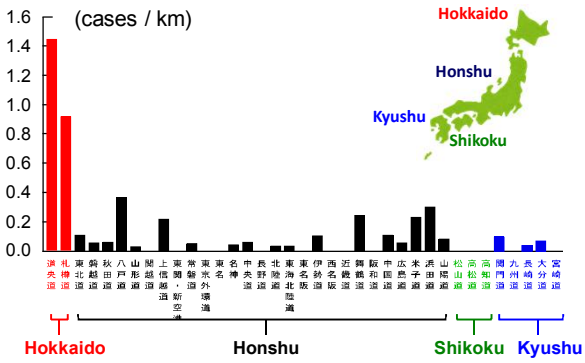

Red fox
very common


Raccoon dog
common


Raccoon
Alien species

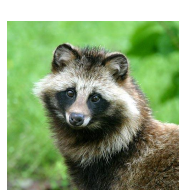
Red fox

Distribution of Red fox in Japan

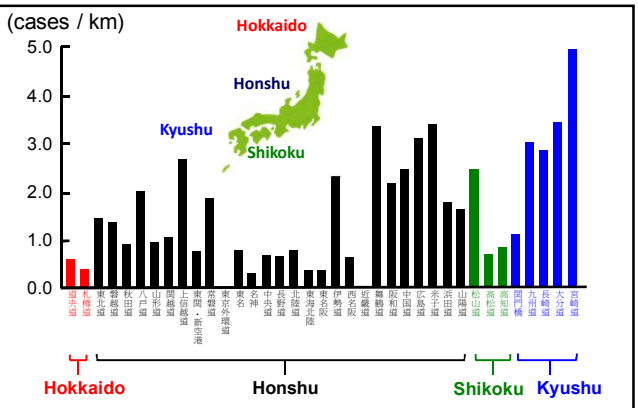


Number of road-killed foxes on each freeway in Japan (1993) (modified from Otaishi, Ibe & Masuda 1998)

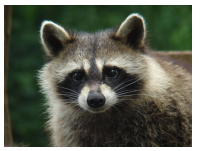
Two foxes begging tourists for food in Hokkaido, Japan



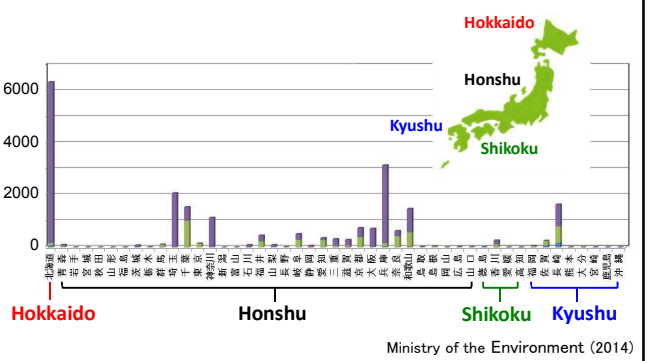
Distribution of Raccoon dog in Japan



Number of road-killed Raccoon dogs on each freeway in Japan (1993) (modified from Otaishi, Ibe & Masuda 1998)



Distribution of Raccoon in Japan

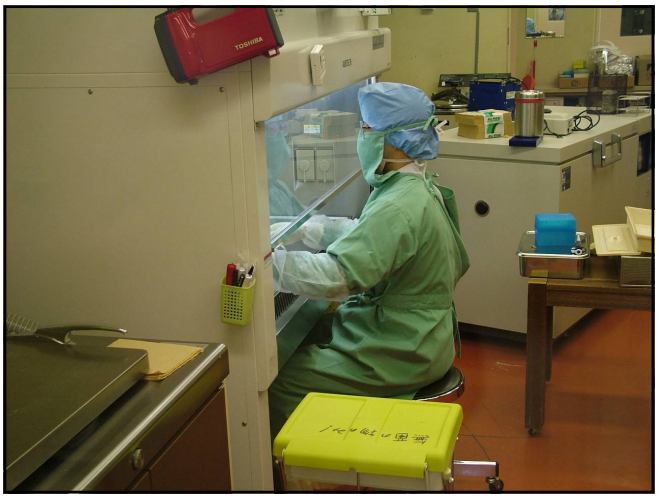


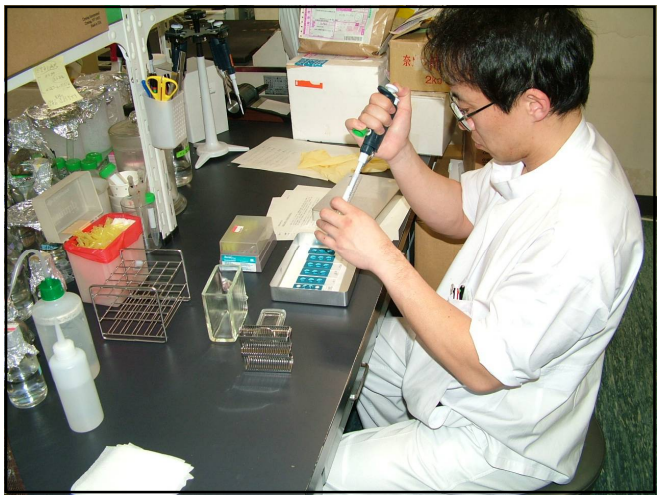
Ministry of the Environment (2014)

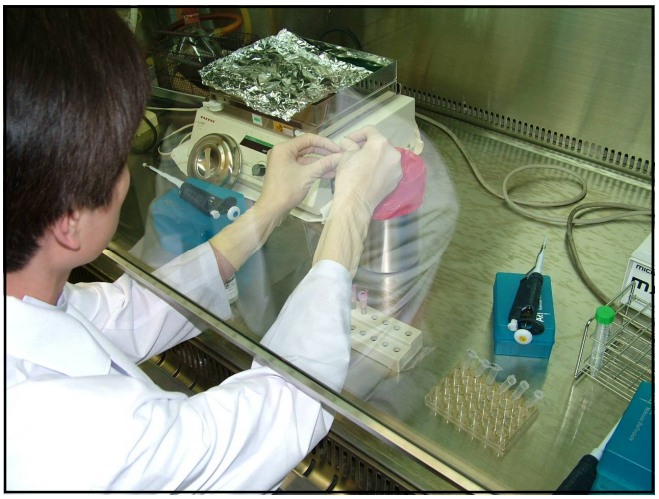
Number of captured Raccoon in each prefecture in Japan (2008)

But
surveillance of wildlife has not started in Hokkaido.

Hokkaido pref. government has a surveillance system for only suspected rabid dogs.








Thank you

Workshop for molecular diagnosis of rabies and
technical training at NIID from January 9th to 10th, 2017

Tokyo Metropolitan Institute of Public Health

Department of Microbiology
Laboratory of Parasitics and Zoonoses

Kaoru Hatakeyama

TOKYO 2020

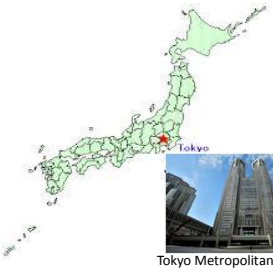
TOKYO 2020 PARALYMPIC GAMES

Tokyo : area (Honshu + 24 Islands)

Tokyo City

The area of Tokyo is 0.6% of the country.
（Tokyo area 2,122km^2 ／ Japan 372,972km^2

But, the 11% of population lives in Tokyo.
population of Tokyo : 13,636,222 persons
population of Japan 126,963,398 persons



The Imperial Palace

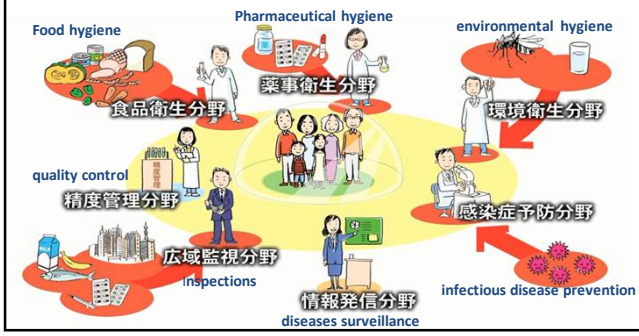
Akihabara

Tokyo Metropolitan government

Asakusa

Tokyo Metropolitan Institute of Public Health

Number of members :423 persons
clerical staffs : 66 persons , Inspectors :127persons, Laboratory staffs 230persons



Rabies control in Tokyo

Number of registered and inoculated Dogs in Tokyo (2009-2014)

year	Number of registered	Number of inoculations	percentage of inoculations(%)
2014	518,121	379,512	73.2%
2013	516,567	380,188	73.6%
2012	513,250	378,123	73.7%
2011	506,986	375,505	74.1%
2010	500,646	370,648	74.0%
2009	487,902	366,352	75.1%

http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou10/01.html

Tokyo Metropolitan Government

Bureau of Social Welfare and Public Health

Health and Safety Division

Environmental Health and Sanitation Section

Institute of Public Health

Animal care and Consultation Center

Head office
Tama branch
Jonajima branch

Rabies control
Animal Welfare

Inspection request

Sheltering Dogs and Cats in 2015 in Tokyo

	Dog		Cat		others	total
	Adult	Puppy	Adult	kitty		
Shelter from owners	41	0	79	3	—	123
Shelter from finders	269	11	11	709	—	1,000
Shelter	60	0	—	—	—	60
Shelter (injury)	22	0	366	31	3	422
Stray dogs	0	0	—	—	—	0
rabies diagnosis	0	0	0	0	—	0
total	392	11	456	743	3	1,605

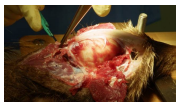
http://www.fukushihoken.metro.tokyo.jp/douso/shiryou/toriatsukai.html

Inspecting rabies in wild animals since 2014

Target of inspection: raccoon dog (injuries and sickness)
Number of inspection : 1〜2 head /year





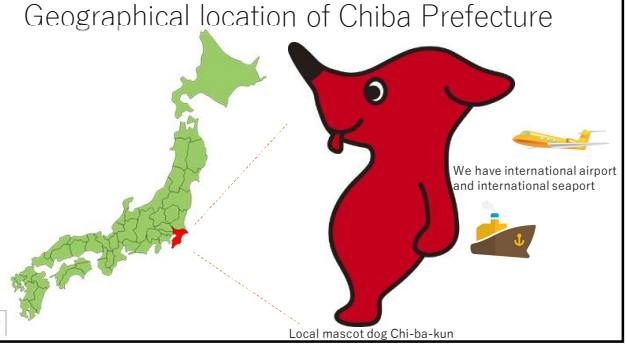


Workshop for molecular diagnosis of rabies and technical training at NIID
Jan. 9th 2017 Shinjuku Tokyo

千葉県の狂犬病対策について
Rabies measure in Chiba Prefecture

Masakatsu Taira, DVM
Chiba Prefectural Institute of Public Health
Virology Division

Geographical location of Chiba Prefecture



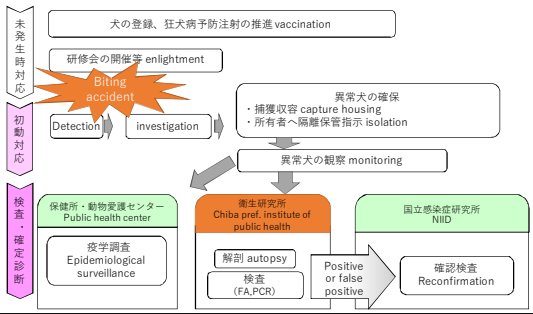
Narita international airport

成田空港や千葉港があり、
空や海から日本の玄関口となってます。それに伴い人も物も感染症も外国から入り込むリスクが高い県です。

Chiba international seaport

成田空港検疫所での研修
Technical training @ Narita international airport animal quarantine service office

千葉県狂犬病対応マニュアルの概要 Overview
the Chiba prefectural Rabies contingency plan



千葉県狂犬病対応マニュアルの概要

確定検査の結果が「**陽性**」だった場合

千葉県健康危機管理対策本部の設置	・千葉県健康危機管理対策本部 ・保健所（現地連絡会議）
適切な対策を講ずるための調査	・発症犬への感染源の調査・・・発症犬の履歴、現状調査 ・発症犬等との接触犬の調査・・・接触犬の履歴、現状調査 ・発症犬等との接触者の調査・・・接触者の履歴、現状調査 ・対策を講ずる地域の区分設定・・・第１～第４エリア
調査結果に基づく狂犬病発生の拡大防止のための措置	・発症犬等との接触者への対応・・治療要否判定、ＰＥＰの実施 ・発症犬等との接触犬への対応・・・感染リスクの評価、隔離観察 ・対策を講ずる地域における対応・・・抑留、登録・注射の徹底、モニタリング
清浄化に向けての調査・措置	・継続調査等・・・モニタリング調査、放浪犬の抑留等 ・事案対応の終息・・・約６ヶ月間新たな感染動物（人）がない場合

産経ニュース 東京 24℃ 関西 オピニオン フォト

ホーム スポーツ エンタメ ライフ 地方 GQ WEB限定 日本力

速報 事件 政治 国際 経済 コラム 特集 写真 ランキング

犯罪・疑惑 事故・災害 裁判

青汁、まだ信じてる？知らない人はショックかも…サントリー [PR]

2015.9.14 18:21

文字の大きさ 小 中 大 印刷

警察官が人襲った体長１２０センチ「紀州犬」に１３発発砲し射殺 千葉県警「拳銃使用は適正」…住民「パンパンパンの銃声、テレビ番組かと…」 千葉・松戸

ブログに書く 7 Tweet (1/4ページ)

１４日午前２時ごろ、千葉県松戸市日暮の路上で、同市に住むアルバイトの女性（２３）が犬にかまれたとする１１０番通報が、一緒にいた友人の女性からあった。約３０分後には同市稔台の路上で、同市に住む飼い主の男性（７１）も襲われてかまれ、一緒に犬を捜索していた県警松戸署地域課の男性署員３人が拳銃計１３発を発砲し犬を射殺した。かまれた２人はそれぞれ左腕に軽傷。日暮では１３日夜に１０代後半の男性も犬に左腕をかまれて軽傷を

犬が射殺された現場周辺を調べる警察官＝１４日、松戸市稔台（江田隆一撮影）

Case：松戸市咬傷事故の犬
Biting accident case at Matsudo Chiba

千葉県の犬における抗体保有状況
Seroepidemiology on Rabies at Chiba pref.

Purpose

➢狂犬病ウイルスが外国から侵入した場合、千葉県内の犬は狂犬病の感染拡大を防げるのか？
If a Rabies dog invade in Chiba prefecture, will we able to prevent the infection from spreading？

Material and Method

Material
Canine serum：Dec.2008～Mar.2013
194 samples

Method
Rapid Fluorescent Focus inhibition
(RFFIT)test (@NIID)

Positive range (0.5 < IU/ml)

Result

Positive rate of rabies virus

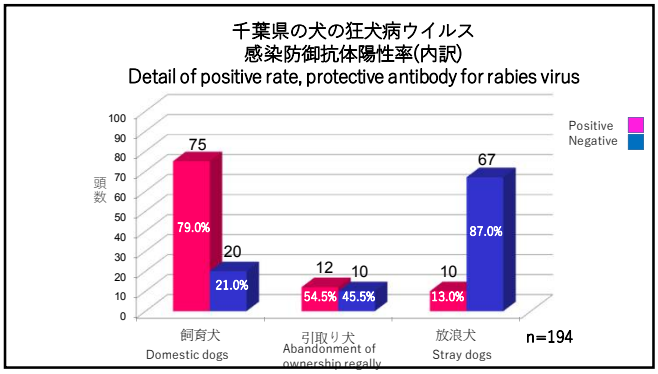
(*)positive: 0.5< IU/ml

Protective Antibody
positive ：97heads
negative：97

Positive rate
５０％

➢WHO recommended over 70% to prevent the infection from spreading.

This data showed that we didn't have antibody enough for prevent infection spreading.



Discussion

WHO の見解をもとに･･･
Based on WHO recommendation･･･

もし･･･千葉県に狂犬病が侵入したら･･･
飼い犬（良識ある飼い主の）は感染防御は出来るが、
放浪犬を中心に野外で感染拡大する可能性がある。

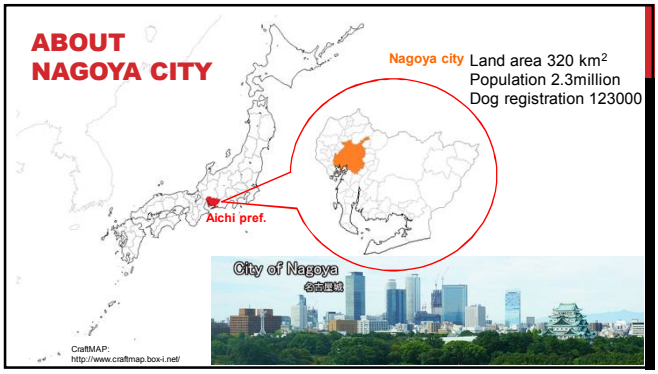
Domestic dogs will protect rabies each other. But…
Stray dog will not able to prevent the Rabies infection from spreading.

Thank you very much for your kind attention !

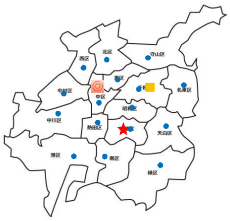
TRAINING OF RABIES DIAGNOSIS IN NAGOYA CITY

NAGOYA CITY INSTITUTE OF PUBLIC HEALTH
AKARI KODAIRA

ABOUT NAGOYA CITY



CITY GOVERNMENT OFFICES



- City Government Office
- 16 Health Center (16 wards)
- Animal Welfare and Management Center
- ★City Institute of Public Health
  - Microbiology department
  - Virus section

LABORATORY DIAGNOSIS OF RABIES IN NAGOYA CITY

laboratory diagnosis in past

Outsource work to Vet. University in the surrounding prefecture

problems **Need quickness**

Need to take responsibility for diagnosis as a local government

Establish a reliable diagnosis system in city

- Animal Welfare and Management Center
  Cut open bones of skull, take a brain
- City Institute of Public Health
  FA, PCR

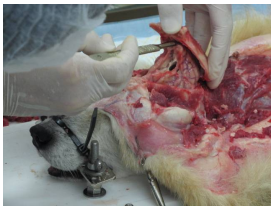
TRAINING FOR RABIES DIAGNOSIS（1ST）

Date : Oct. 25, and 26, 2012

Target : Vet. staff
(Animal Welfare and Management Center, City Institute of Public Health, Health Center)

Contents : Lecture about basic knowledge of Rabies
Practical work to cut open a dog head and take off a brain

TRAINING FOR RABIES DIAGNOSIS（1ST）



The refrigerator was broken → Mild autolysis of a brain

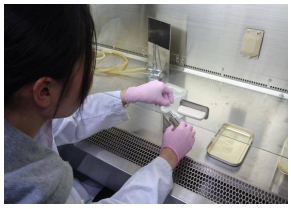
TRAINING FOR RABIES DIAGNOSIS（2ND）

Date : Feb. 3, and 12, 2016

Target : Vet. staff
(Animal Welfare and Management Center, City Institute of Public Health, Health Center)

Contents : Practical work to cut open a cat head and take off a brain
→ Rabies diagnosis (FA, PCR)
At a later date,
Review the practice using movies and photos
Report about my experience on training course in Philippines

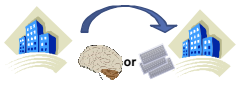
TRAINING FOR RABIES DIAGNOSIS（2ND）



ISSUES

The number of dogs get put down is decreasing (good news !)
→ Difficult to get a dog for scheduled diagnosis training

Need role-sharing rearrangement between Animal Welfare and Management Center and City Institute of Public Health



Building Crisis control system about Rabies in Tokushima pref.

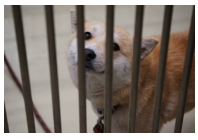
徳島県危機管理部県民くらし安全局安全衛生課
（Tokushima Prefectural Government, food＆health management）
矢野　さやか
SAYAKA　YANO

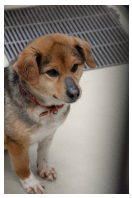
Tokushima Pref. Info.

Population　750,000
Registered Dog 40,500 (Real 55,000)
Preventive injection rate 62%
Bite accident 30-40/year
Capture Dogs(walk around free) 700-800/year
Vet. Charge staff(rabies) 12
Vet clinic 54

Stray dogs

Lost dogs

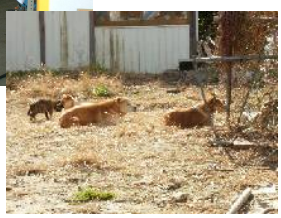




Tokushima Animal Welfare Center

Protect lost dog&cat
Capture Dogs(walk around free)
Adoption Event
Enlightenment、Education
Zoonosis surveillance

Patrol car



Increased stray dogs
Just only given food

worry about if .....

Problems

No Registered Dog increased
many pet owners don't know "RABIES" correctly , and also some Vet. & Dr.
Poor skill for distinction
Uncertain the duties of each section (no manual)

「RABIES」building crisis control system

2004～ "Zoonosis Examination meeting"

2006 Correspondence Manual about rabies（Tokushima personal）

2006～ "Health Center conference"

2007 Training when Rabies occurs

2007～ Inspection Manual , Skill rise Diagnostic testing

2018～ Surveillance in dog, cat, wild animals in Tokushima

Inspection → Training → Revise

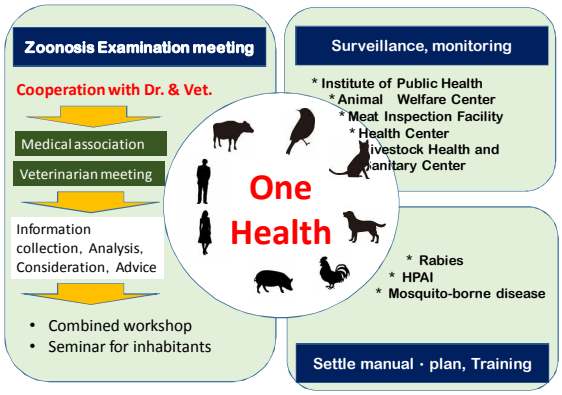
Zoonosis Examination meeting

Control RABIES
= common topic in all sections

（**Background**）
Infection Law was revised
→ obligation of Vet.
→ needed cooperation Dr. & Vet.

（**Member**）
Doctors , Veterinarians, Chairperson of infection section(Dr.), Advisors

（**Administration=Working group**）
Section
human health
food health,zoonosis（vet.）
Animal Welfare Center
Meat Inspection Facility
Health Center
Control wild animals
Livestock health
Institute of Public Health



Training in Tokushima

neighbor prefectures combined workshop by national government

Tokushima Prefectural Government= Rabies reference center

Group discussion about correspondence

Virtual practice in Health Center

Participants

prefectural government , municipality village office , medical association, veterinarian meeting, police station, fire department, report(Newspaper,TV) professional person …

Purpose of Virtual practice

Building Crisis control systems about Zoonosis

① Reconfirm each duties
② Inspect Manual
③ Extract problems

- **Awareness against Rabies(Zoonosis)**
- **Make it possible "Prompt and Appropriate actions"**

Rabies Surveillance

Health Center (living dog, cat)

municipality village office (dead animal)

Animal Welfare Center — Observation,Analysis, Extraction,Stamp

Examination Immunofluorescence method,RT-PCR — **Institute of Public Health**

To deside each rules, burdens are reduced cooperate usually

Animal : wild animals
Racoon dog, Weasel,Fox,Masked palm civet,Racoon et.

Surveillance in Animal Welfare Center

Skill rise training and Surveillance

Training : 1~2 times/ y
Surveillance: 10~20bodies /y

Building Crisis control system about Rabies

Important things

○The persons in charge are recognized about them duties.
○The persons concerned have same will & consciousness.
○Try !
○Continue !

Thank you for having me.

Workshop for molecular diagnosis of rabies and technical training

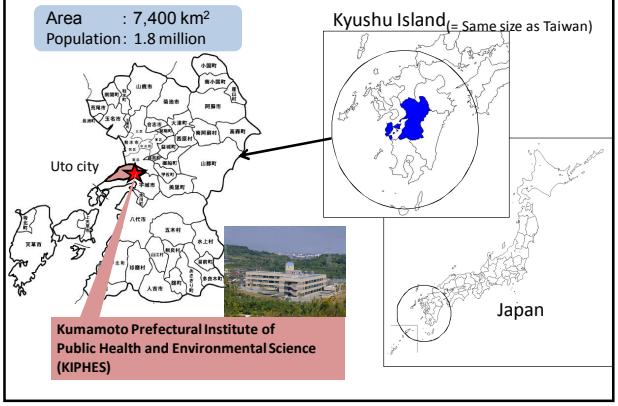
Countermeasures for rabies in Kumamoto Prefecture

Junko Toda,
D. V. M, Ph. D.

Department of Microbiology,
Kumamoto Prefectural Institute of
Public Health and Environmental Science (KIPHES)

Kumamoto Prefecture

Area : 7,400 km²
Population : 1.8 million



Organization chart of KIPHES

Seven staffs:
3 veterinarians, 3 pharmacists, and 1 clinical laboratory technician

Countermeasures for rabies in Kumamoto Prefecture

1. Establishment of rabies contingency plan in Kumamoto Prefecture

2. Incident of animal bite by a rabies suspected dog in 2013

Countermeasures for rabies in Kumamoto Prefecture

1. Establishment of rabies contingency plan in Kumamoto Prefecture

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Establishment of rabies contingency plan in Kumamoto Prefecture

March 2012

- Published "Kumamoto prefectural manual for rabies or suspected rabies outbreak"
- Based on "The 2001 Guidelines on Rabies Countermeasures" (MHLW Notification, November 2001, and supplement, January 2003)

"Kumamoto prefectural manual for rabies or suspected rabies outbreak"

Stage 1 (normal)

Preventive activities of rabies under the Rabies Prevention Law

1) [Dog owners, Public health centers] Register and vaccinate pet dogs
2) [Public health centers] Detain stray dogs
3) [Animal quarantine service offices] Quarantine susceptible imported animals

Stage 2 (suspected rabies outbreak)

1) Epidemiological survey team at a public health center
(1) Hearing the detector
(2) Report to the Hearth Crisis Management Division
(3) Quarantine the suspect rabid animal and prohibit the transport of it
(4) Investigate an epidemiological survey

2) Health Crisis Management Division, Kumamoto Prefectural Government
(1) Liaise and coordinate with related organization
(2) Head a meeting on how to deal with the outbreak

Suspect rabid animals → KIPHES: Specimen collections & Rabies examination

Suspect rabid samples → NIID: Rabies examination

Rabies is diagnosed !

Stage 3 (Rabies outbreak)

Level 1: in Japan
Deal with the media

Level 2: in Kyushu Island
Establish Kumamoto prefectural headquarter for rabies monitoring

Level 3: in Kumamoto pref.
Establish Kumamoto prefectural headquarter for rabies countermeasures

Establishment of rabies contingency plan in Kumamoto Prefecture

2013

・Implemented an inspection system for suspect rabid animals in our government.

1) Conducted the first responder training to prepare for catching a suspected rabid stray dog at a public health center

2) Participated in Health and labor science research, "The urgent study of monitoring method for animal rabies included wildlife in Japan"

December 18, 2013
Encountered an opportunity to examine a suspect rabid dog

Hideo Osako,
Head of Department of Microbiology

Countermeasures for rabies in Kumamoto Prefecture

1. Establishment of rabies contingency plan in Kumamoto Prefecture

2. Incident of animal bite by a rabies suspected dog in 2013

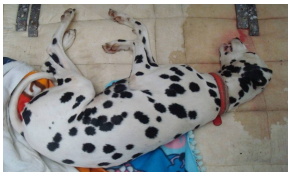
Situation investigation

- Report: at 23:00, December 18, 2013, from a doctor who examined a patient bitten by his dog
- Place: Yatsushiro City, Kumamoto prefecture
- Patient: male, 60 years old, self-employed, no history of travelling abroad
- Bitten body part: the fifth finger of the left hand
- Wound: punctured by canine teeth, bleeding
- Dog: Dalmatian, born in 2008, 5 years old, outside keeping, bought from the pet store at the largest shopping mall
- Medical examination history after keeping: no
- Registration of dog: no
- Rabies vaccination history : no
- Dog's Symptom: front leg spasms lasting repeatedly from a month ago

Site photographs

Keeping place (the house was located rice paddy area)

The appearance of the dog



The fifth finger of the left hand bitten by the dog

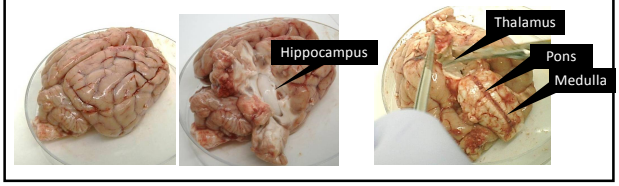
Identification of rabies

- Opening of the skull
- Collection of brain samples : cerebellum, hippocampus, thalamus, pons, medulla
- Rabies detection
  1) Direct fluorescent antibody test (DFA)
  2) Nested RT-PCR test

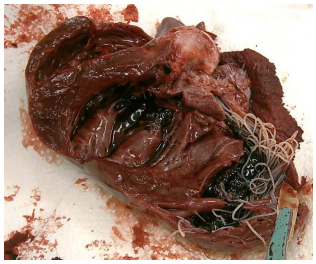
  } Retest at NIID

Opening of the skull （started at 11:30）

Collection of brain samples（finished at 12:10）



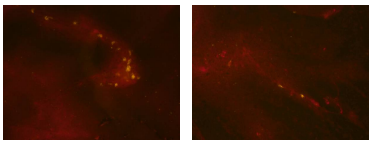
Finding at autopsy



Heartworms were founded in the right ventricle

Result of DFA

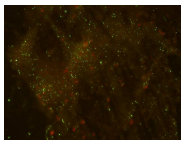
Samples



The left hippocampus (x200)

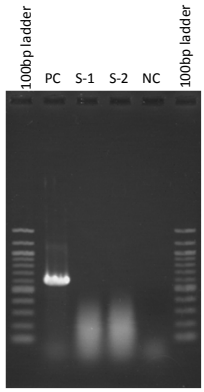
Medulla (x200)

Positive control
performed at NIID



CVS11/mouse brain (x200)

Result of nested RT-PCR



OneStep RT-PCR (N7/JW6)

PC: positive control

NC: negative control (DW)

S-1: pooled sample 1
(the pooled sample of thalamus, pons, and medulla)

S-2: pooled sample 2
(the pooled sample of cerebellum, hippocampus)

Results

	FDA	Nested RT-PCR
KIPHES	Negative*	Negative
NIID	Negative	Negative

* No positive controlled experiment

Rabies was not detected from the dog

The accidental death of the dog was caused by filariosis.

Lessons we learned from the incident

- Our department stuff was trained to diagnose rabies just before the incident happened, and fortunately the rabies tests were carried out smoothly.
- However, the rabies examination procedure may be easily forgotten, because rabies is rare in our country.

→ We conduct the training of rabies examination for public health veterinarians in Kumamoto Prefectural Government on regular basis from 2016.

- A high-resolution camera system is required to have a confirmation from NIID that our FDA result is correct.

The collaboration with NIID and other related organizations is necessary for rabies prevention !

Rabies monitoring inspection

for the wild animals

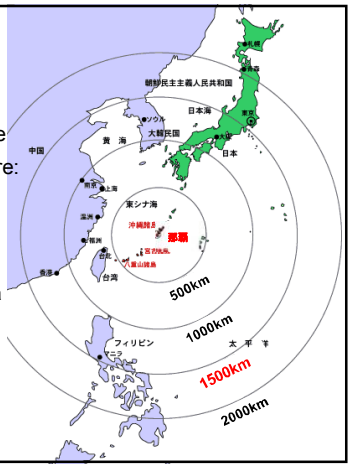
such as mongooses in Okinawa

About Okinawa

- It consists of 160 islands (49 manned islands)
- Subtropical maritime climate
- Annual average temperature: **20～25℃**
- Annual rainfall ：約**2000㎜**
- typhoon：6 in 2015
- Draw a circle around Okinawa

Taipei	630Km
Shanghai	820Km
Seoul	1260Km
Manila	1500Km
Tokyo	1550Km

- Major industries: 7 million tourists a year



Okinawa is a Treasure House of Endemic Species

Okinawa spiny rat　Okinawa rail　Okinawa woodpecker　Ryukyu tip-nosed frog

Ishikawa's frog　Okinawa black-breasted leaf turtle　Ryukyu robin　Matrona dragonfly

Nature Conservation and Afforestation Promotion Division, Okinawa Prefectural Government

Invasive Alien Species

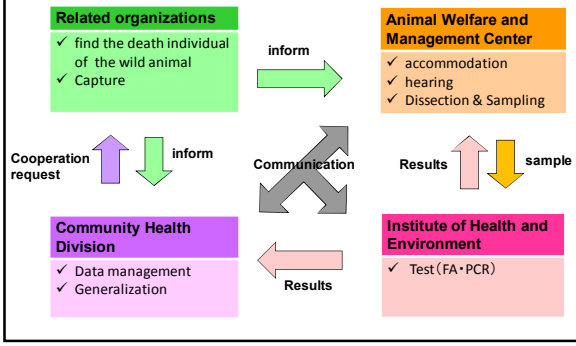
Mongoose (small Indian mongoose)

- original distribution　West Asia to Southeast Asia
- Length & weight　Male about 60cm, 0.5-1.0kg
  Female about 50cm, 0.3-0.6kg
- Diet　insects, mammals, birds, amphibians, reptiles
- breeding　1-2times/year, 1-5heads/time (Av.2.26 heads)
- Ecology　diurnal, solitary, life span is 1-3years.

Why rabies surveillance for a mongoose in Okinawa

- ✓ Representative Carnivora
- ✓ Extended tendency of the habitat distribution
- ✓ Contact opportunity with a human and the domestic animal
- ✓ Involvement with rabies outbreak in foreign countries
- ✓ Designated as "Invasive Alien Species "
- ✓ Subject of the harmful capture
- ✓ Available continuously

Cooperation of the rabies monitoring in the wild animal



Number of Rabies tested animals by species

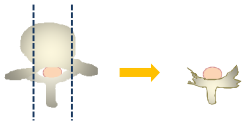
	2014	2015	total	Remarks
Wild animals				
mongoose	5	4	9	Capture 6 traffic accidents 3
bat	2		2	death unknown
Pet animals				
dog	4	4	8	Bite dog
cat	2		2	Bite cat
total	12	9	21	

When the brain damage is remarkable due to a traffic accident or the passage of time, it can not be sampled…

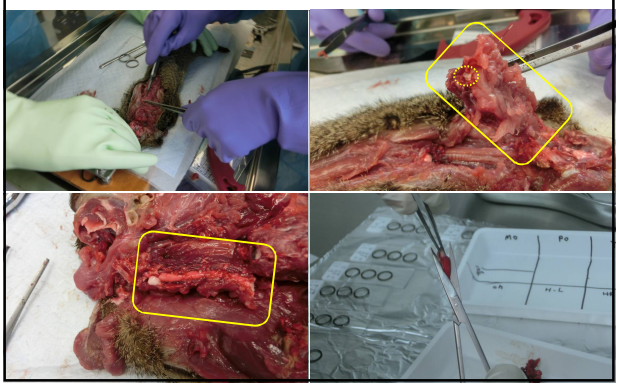
Use the spinal cord as a material

- How to collect spinal cord -

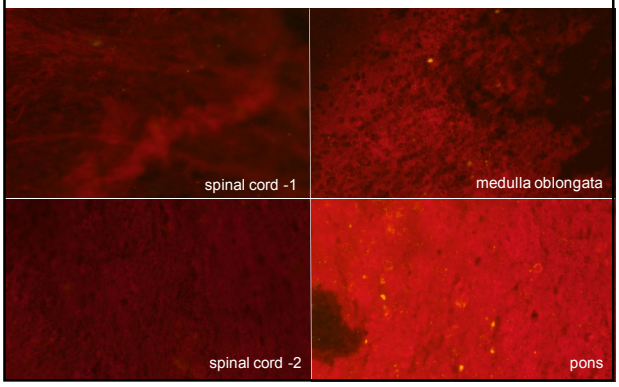
1. Cut the spine 5-6 cm below the first cervical vertebra
2. Removing muscle
3. Incision of vertebrae
4. Exposed spinal cord
5. The spinal cord creates a new section And Smear on slide glass



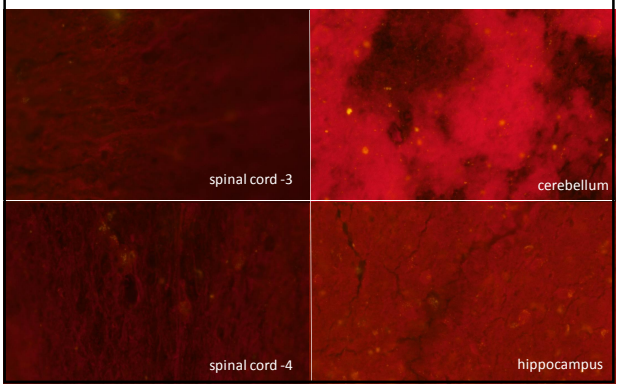
Spinal cord sampling



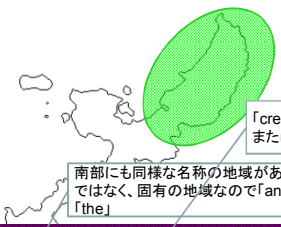
Comparison of FA images of spinal cord and brain -1



Comparison of FA images of spinal cord and brain -2



About Yambaru in Okinawa Island



「creature」は怪物のニュアンスがあるので→または「wild organism」

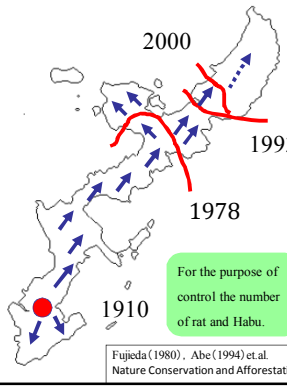
南部にも同様な名称の地域があるわけではなく、固有の地域なので「an」→「the」

「part」→「area」

- There is an expanse of pasania tree forest in the northern part of Okinawa.
- Many of Okinawa's creatures came to the Okinawa island in ancient times when the islands were still connected to the Asian continent. The ancient creatures evolved into the many endemic species of Okinawa.
- There was no carnivorous animals.

沖縄県動物愛護管理センター病理解剖室

How mongooses spread to Okinawa?



30,000 mangooses (2001)

Invation into Yambaru

The entrance of Yambaru

Nago city and environs
(spreaded 2km / yaer)

「part」→「area」

For the purpose of control the number of rat and Habu.

17 mangooses were released in Southern part of Okinawa Island

Fujieda(1980)、Abe(1994)et al.
Nature Conservation and Afforestation Promotion Division, Okinawa Prefectural Government

Mortality data of the mongoose

1・The number of traffic accidents

from Six places of roads Administration Office

	northern	Central	southern	Express way
2012	9	21	2	46
2013	4	11	1	
Total	13	32	3	46

2・mongoose eradication projects

The worker did not find the unidentified death individual of the mongoose in Yambaru.

3・The number of consultation from The local government

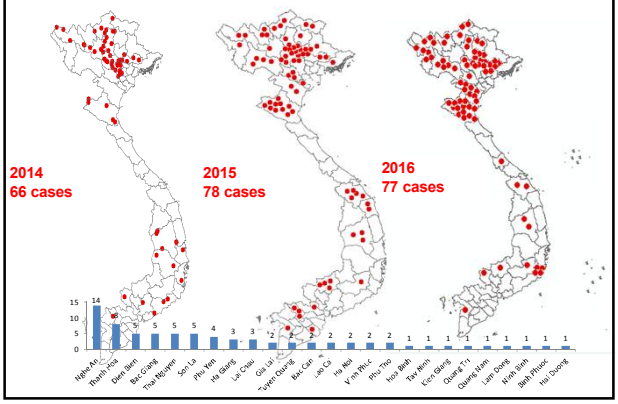
	northern	Central	southern
2012	0	13	1
2013	0	14	2
Total	0	27	3

Rabies situation in Vietnam

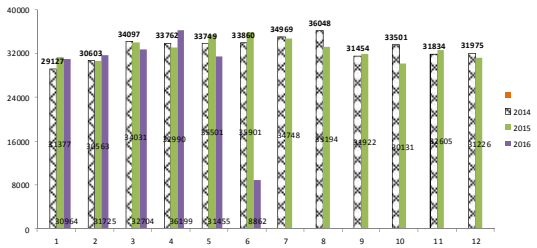
Rabies in Vietnam

- In Vietnam, the epidemic of rabies occurred in a large extent of 25/63 provinces/cities , particularly in the mountainous and midland areas. 76 human rabies cases were reported in 2016
- Every year, more than 330.000 people have to be received post-bite vaccination.
- Rabies can occur all the year round
- Dogs are main reservoirs and transmitters. 95-97% of human rabies cases are bitten by domestic dogs, or infected via contact with rabid dogs, following is cat.
- No clear evidence of rabies deaths due to exposure to other rabid animals. However, it does not exclude the existence of rabies in other animals/reservoirs.
- Only rabies virus (RABV), genotype 1 has been recognized and reported .
- The annual data of human deaths due to rabies reported has been based only on the clinical diagnosis, not by laboratory confirmation.

EPIDEMIOLOGICAL MAP OF RABIES CASE DISTRIBUTION IN HUMAN ACROSS THE COUNTRY IN 2014, 2015 AND 2016



NUMBER OF PEP BY MONTH IN 2014, 2015 AND 2016

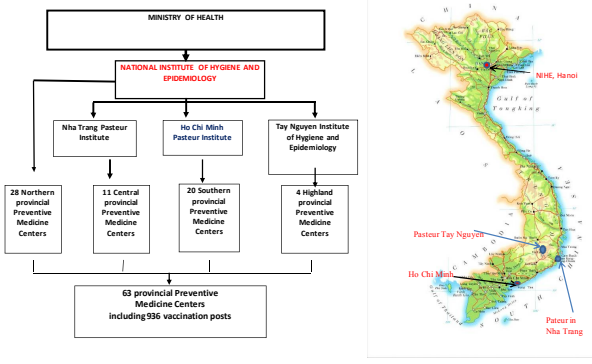


TOTAL NUMBER OF PEP
2014: *394.979*
2015: 394.186
UP TO 6/2016 : *171.899*

Rabies surveillance and control

- **Has a national program** for human rabies control and prevention, animal rabies has not been established
- The most annual data of human deaths due to rabies reported has been based only on the clinical diagnosis, not by laboratory confirmation.

The system for human rabies surveillance and control in Vietnam

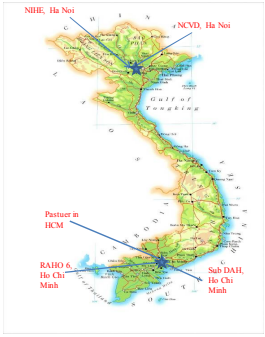


Rabies diagnosis

- 2005, the first rabies lab for human was established in NIHE
- 2006-2015, methods for rabies diagnosis and research had been transferred by NIID and implemented in NIHE under projects of JICA, NIID.
- Lab-based data for human and animal rabies has been reported by NIHE since 2007, PI in HCM from 2013. From 2015, NCVD stared to confirm rabies animal cases
- To step by step set-up the net work for rabies diagnosis, from 2010 training courses on biosafety and rabies diagnosis funded by JICA, WHO, NIID, FAO had conducted by NIHE for NCVD, Regional Pasteur Institutes, RAHOs and some Sub DAHs and Provincial Preventive Medicine Centers to built the capacity for rabies diagnosis and research.
- There are 5 laboratories have capacity and do rabies diagnosis in Vietnam so far. FAT. RT-PCR, Real-time PCR, RT-LAMP were employed routinely for diagnosis in these labs

Rabies laboratories in Vietnam

- Rabies lab in NIHE
- National Center for Veterinary Diagnosis
- Pasteur in Ho Chi Minh city
- Regional Animal Health Office
- HCM Sub Department of Animal Health



Diagnostic capabilities

Name of laboratory	Type	Location	FAT	Mol	ELISA	MI	CC	FAVN	RFFIT
National Institute of Hygiene and Epidemiology	National	Hanoi	✓	✓	✓	✓	✓	✓	✓
Ho Chi Minh City Pasteur Institute	Regional	Ho Chi Minh City	✓	✓	✓	✓	✓		
NCVD	National	Hanoi	✓	✓	✓				
RAHO 6	Regional	Ho Chi Minh City	✓	✓	✓				
Ho Chi Minh City Sub-Department of Animal Health	Provincial	Ho Chi Minh City	✓	✓	✓				

Lab network - challenges

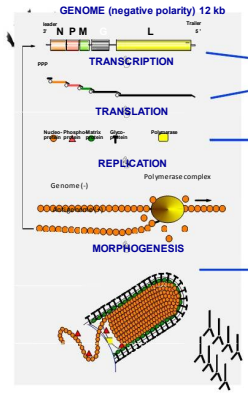
- Both rabies laboratory at NIHE and PIHCMC are currently capable of rabies diagnosis and research but need to improve the working space, equipments and techniques to be the lead laboratory and will be responsible for developing and implementing a standardized methodology for specimen handling and testing and a quality assurance system for specimen collection, testing, cataloguing and storage
- Currently no rabies diagnostic testing undertaken at the Pasteur Institutes Nha Trang and Central Highlands. Rabies diagnostic capability needs to be established at these centers.
- All PPMCs need to be trained and equipped materials for specimen collection
- To have inter-sectors agreement documents and SOPs on mechanism of sharing laboratory material and information among sectors from both animal and human health

DIAGNOSIS/RESEARCH RABIES/ LYSSAVIRUS IN NIHE

Samples:

- **Animals:**
  - **Dog/cat/bat head**
  - **Sera**
- **Human:**
  - **CSF;**
  - **SLV;**
  - **Sera;**
  - **Skin biopsy**
- **Methods:**

Rabies diagnosis



RT-PCR, RT-LAMP, Real-time PCR

FAT, ELISA

Mouse/cell inoculation

FAVN,RFFIT,ELISA

INSTITUT PASTEUR

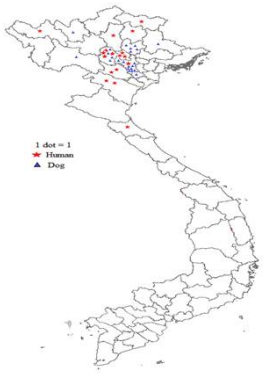
Diagnostic results of rabies suspected cases of human and animals (2013–2016)

	Humans	Animals
Provinces	*Positive/total*	*Positive/total*
Hanoi	1/2	10/12
Phu Tho	5/5	4/4
Hoa Binh	2/2	-
Thai Nguyen	1/2	5/6
Son La	1/2	1/1
Tuyen Quang	2/3	-
Vinh Phuc	3/4	-
Thai Binh	0/1	-
Ha Nam	-	1/1
Hai Phong	-	0/1
Lai Chau	1/2	-
Bac Kan	1/1	-
Cao Bang	1/1	-
Lao Cai	0/1	1/1
Lang Son	-	1/1
Ha Tinh	1/1	-
Thanh Hoa	3/4	-
Ha Giang	1/2	
Bac Giang	2/3	
Ninh Binh	1/2	
Quang Nam	1/2	
Total	27/43 (63%)	23/27 (85%)

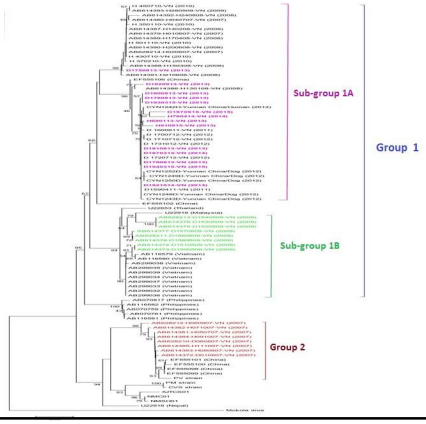
RESEARCH ON MOLECULAR EPIDEMIOLOGY OF RABIES VIRUSES

Distribution of rabies virus strains isolated in Vietnam 2013 – 2015.

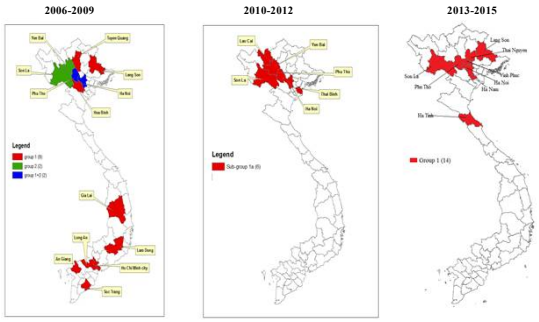
	North	Centre	Total
Human	14	3	17
Dog	23	0	23
Total	37	3	40



Phylogenetic structure of RABVs in Vietnam and neighbor countries



Circulation of rabies virus groups isolated in Vietnam by geographical regions (2006-2015)



Conclusions

1. Rabies virus, genotype 1 were the cause of epidemic in dogs and humans in Vietnam 2006-2015
2. Rabies viruses in Vietnam, 2006 – 2015 were divided into 2 groups, group 1 further divided into two sub – groups.

- Sub-group 1a: viruses isolated in the North VN, China
- Sub-group 1b: viruses circulated in the highland and south
- Group 2 was found only in North VN and China

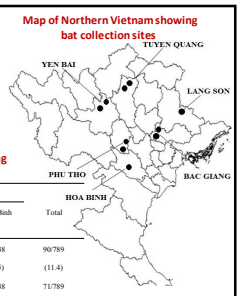
3. From 2010 – 2015, no virus strain belongs to group 2 was found.

Research on bat lyssavirus

- Financial and technical supported by NIID and WHO, Vietnam country office.
- 1200 bat serum samples collected in 6 northern provinces of Vietnam.
- FAVN used for titration, neutralization antibodies against *lyssavirus* in bat sera using 5 rabies/*lyssaviruses:* Rabies virus (RABV); Lagos bat virus (LBV); Mokola virus (MOKV); 4, Duvenhage virus (DUVV); 5, European bat *lyssavirus* type 1 (EBLV-1) supported by NIID.
- FAT and RT-PCR used for detecting *lyssavirus* antigen and gene in bat brain samples

Hunting bats for food

Bat sera screened and positive for neutralizing antibodies against Lyssaviruses



Virus strains[a]	No. positive/No. tested (percentage positive)						
	Tuyen Quang	Yen Bai	Lang Son	Bac Giang	Phu Tho	Hoa Binh	Total
RABV	8/141 (5.7)	16/179 (8.9)	8/121 (6.6)	39/108 (36.1)	10/102 (9.8)	9/138 (6.5)	90/789 (11.4)
DUVV	3/141 (2.1)	45/179 (25.3)	0/121 (0)	0/108 (0)	23/102 (22.5)	0/138 (0)	71/789 (9)
EBLV-1	15/141 (10.6)	45/179 (25.3)	15/121 (12.4)	39/108 (36.1)	20/102 (19.6)	8/138 (5.8)	142/789 (18)
LBV	0/79 (0)	0/97 (0)	0/99 (0)	4/108 (3.7)	0/47 (0)	0/105 (0)	4/535 (0.75)
MOKV	0/79 (0)	0/97 (0)	0/99 (0)	0/108 (0)	0/47 (0)	0/105 (0)	0/535 (0)
Total[b]	24/141 (17)	54/179 (30.2)	17/121 (11.2)	62/108 (57.4)	27/102 (26.5)	9/138 (6.5)	193/789 (24.4)

Conclusions

- This study provides serological evidence of the presence of *lyssavirus* neutralizing antibodies in bats from Northern Vietnam.
- As no virus was isolated, we could not conclude to which virus or viruses these bats had been exposed.
- The presence of antibodies to multiple *lyssaviruses* including anti RABV found in some individual bats may be the result of cross neutralization with other viruses. The absence of consistent reactivity patterns suggests exposure of these bats to the tested *lyssaviruses*, or another unknown *lyssavirus*

Thank you for attention!

Rabies in wild life

Boldbaatar Bazartseren (PhD, D.V.M)
Chief of laboratory of Virology
Institute of Veterinary Medicine (IVM)
Mongolia
boldoomglvet@yahoo.com

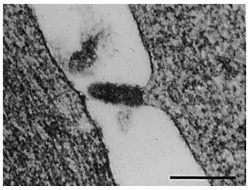
Contents of presentation

- *Rabies*
- *Reservoir animals*
- *Isolated viruses from wild animals*
- *Rabies situation in Mongolia*
- *Rabies control strategy in Mongolia*
- *Rabies diagnosis in Mongolia*

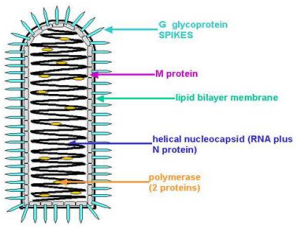
Rabies virus

- *Virus structure*
- *Virus genome*

Structure of Rabies virus



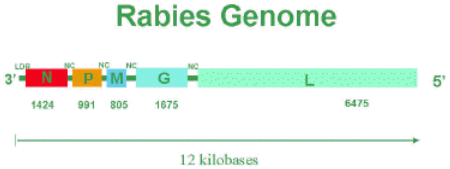
(Scale bar = 200 nm.)
Pierre-Emmanuel Ceccaldi,
Pasteur Institute, Paris



Dr. Richard Hunt, University of South Carolina

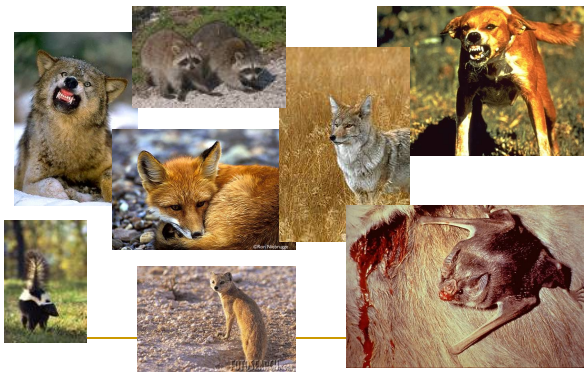
Rabies virus genome

- Single-stranded RNA
- Negative-sense genome
- Linear

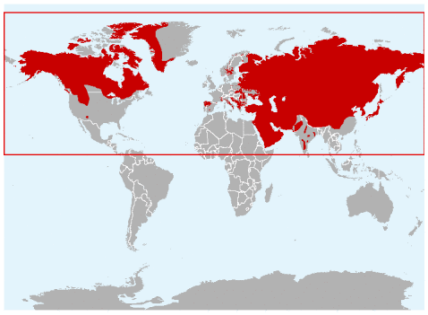


Dr. Richard Hunt, University of South Carolina

Reservoir animals (world)

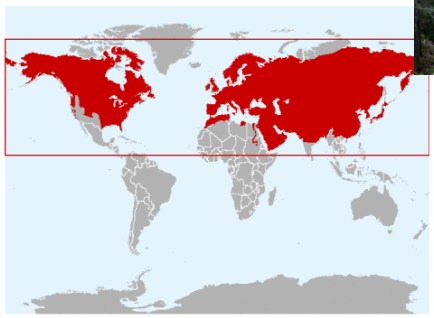


Wolf (Canis lupus)



http://www.canids.org/

Red fox (Vulpes vulpes)

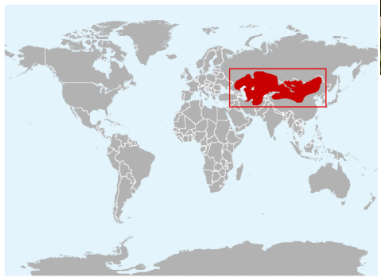


http://www.canids.org/

Corsac fox (Vulpes vulpes)

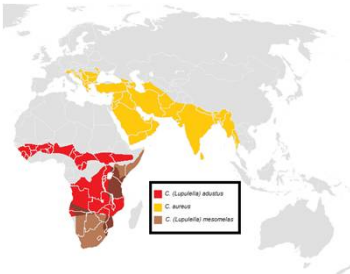
http://www.canids.org/

Manul cat or Pallas's cat(Felus manul)



http://www.wikipedia.org/

Jackals (Canis adustus) (Canis aureus)

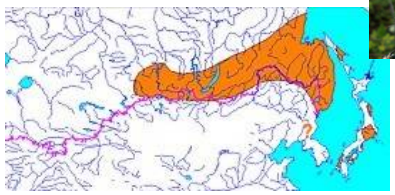


Canis adustus

Canis aureus

http://www.canids.org/

Greater Tube-nosed Bat (Murina leucogaster)



Dr. Botwinkin succeeded Irkiut virus from this bat

http://zmmu.msu.ru/bats/rusbats/e_guide/

Isolated viruses from wild animals (Rhabdoviridae members)

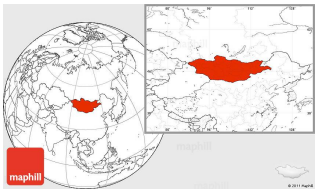
Lyssavirus genus

1 **Rabies (domestic and wild carnivores)**
2 Lagos bat virus
3 Mokola virus
4 Duvenhage virus
5 European bat lyssavirus I
6 European bat lyssavirus II
7 Australian lyssaviruses
Several unclassified Lyssaviruses (Irkut, Khujand)

Rabies situation in Mongolia

- *Animal husbandry in Mongolia*
- *Rabies human cases*
- *Rabies diagnosis*
- *Rabies control in Mongolia*

Overview of Mongolia



Landscape: 1,564,116 km2 (19th)

Human population: 3,042,511 (138th)

Domestic animal population: ~60mln

Agriculture sector in GDP: 16%

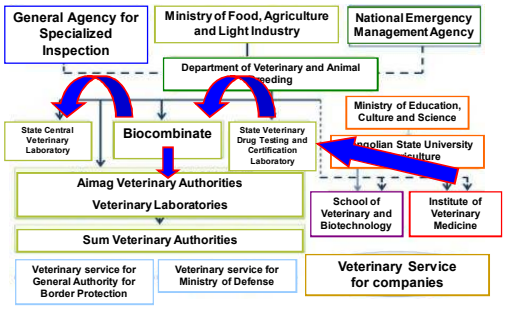
Land boundaries: 8,220 km
China 4,677 km
Russia 3,543 km

Domestic animal number between 2010 – 2015 (millions)

Year	Sheep	Goat	Cattle	Horse	Camel	Total
2010	14,480	13,883	2,176	1,920	0,269	32,728
2011	15,509	15,809	2,315	2.093	0,279	36,005
2012	18,141	17,558	2,584	2,330	0,305	40,920
2013	20,060	19,218	2,908	2,618	0,321	45,127
2014	23,209	22,003	3,412	2,995	0,349	51,9
2015	24,933	23,582	3,779	3,294	0,367	55,957

Animal husbandry in Mongolia

Veterinary organizations of Mongolia

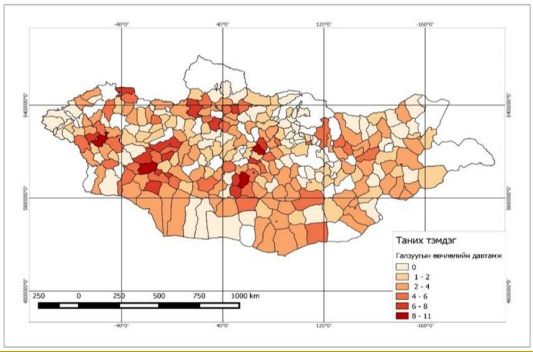


Vaccinated human population in Mongolia

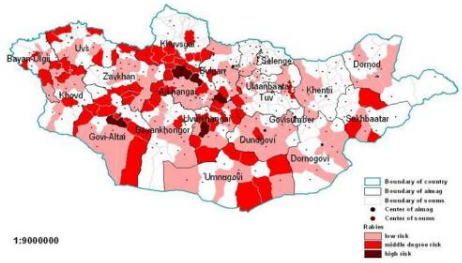
2011	2012	2013	2014	2015	2016 (half)
7192	6346	7015	3067	8015	3797

Total 36 human death has been reported since 1974

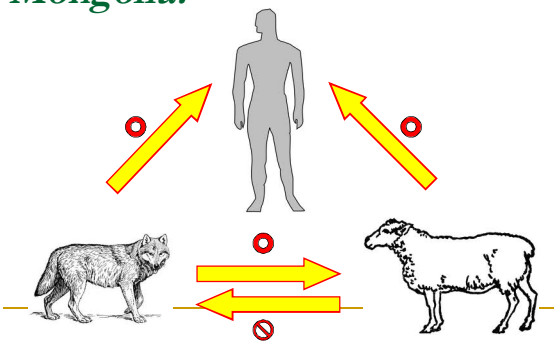
Rabies animal cases in Mongolia



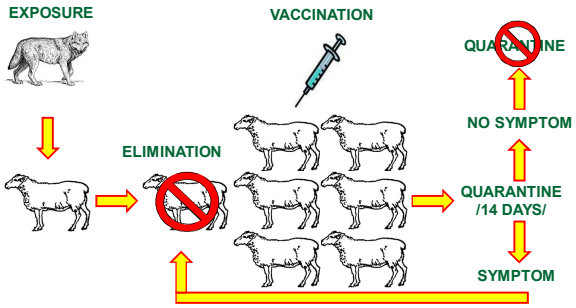
Rabies endemic areas in Mongolia



How does occur rabies in Mongolia?



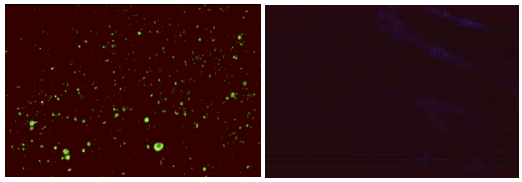
Rabies control strategy in domestic animals



Rabies diagnosis

- *Direct fluorescent antibody test (DFA)*
- *RT-PCR*
- *Immunochromathography test (ICT)*

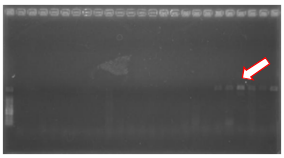
Direct fluorescent antibody test (DFA)



Positive result | Negative result

http://www.cdc.gov/rabies/diagnosis.html

RT-PCR (full N gene)



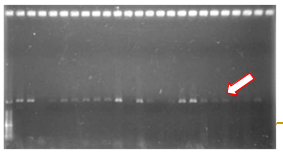
Primers- P1 and 304 primer set

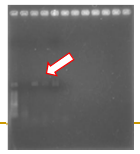
RT primer- P1

RT enzyme- AMV (Promega, USA)

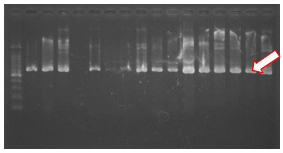
Product size- 1.5kb (full length N gene)

DNA ladder- 100bp (Takara, Japan)





RT-PCR (partial N gene)



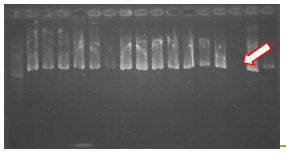
Primers- P1 and JW6 primer set

RT primer- P1

RT enzyme- AMV (Promega, USA)

Product size- 660pb (partial N gene)

DNA ladder- 100bp (Takara, Japan)



Rabies vaccines in Mongolia

Veterinary vaccines

- PV (1969-1996) vaccine strain (discontinued)
- ERA (since 1981) vaccine strain (1.5mln doses)

Human vaccines and immunoglobulin

- Cell culture vaccine and equine immunoglobulin are being imported from Russia and China
- Local Fermi vaccine (discontinued; human usage is not recommended by WHO since 1950's)

Considerable issues of rabies in Mongolia

Registration of pet dogs

Dog rabies vaccination

Reduce population of free-ranging animals

Reduce pet contact with wild animals

Control of rabies in wild animals of Mongolia is impossible!!!!!

Rabies reservoir population reduction

Trap-Vaccinate-Release (TVR) programs

Oral rabies vaccination (ORV) of wildlife

Land size is big, wild carnivore population is not clear

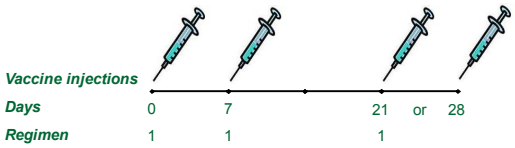
A bait vaccine for wild carnivores

Rabies re-examined.
C. Rupprecht, C. Hanlon, T. Hemachudha
Lancet Infect Dis. 2002 Jun;2(6):327-43.

Prevention of rabies in humans

- ***Pre-exposure vaccination***
- ***Post-exposure vaccination***

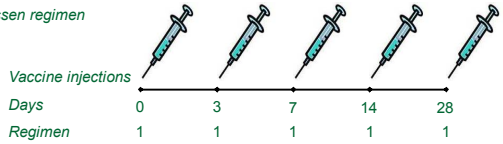
Pre-exposure vaccination



Check neutralizing antibody level every 6 months by Rapid Focus Forming Inhibition Test (RFFIT)
<0.5IU/ml booster dose

Post-exposure vaccination

Essen regimen



ERIG (equine rabies immunoglobulin)

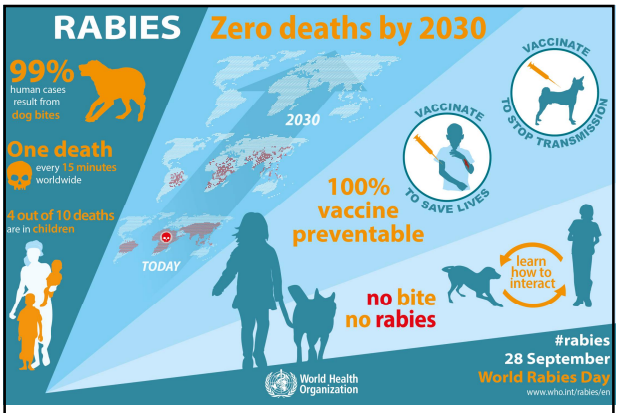
Isolated viruses from wild animals (Rhabdoviridae members)

Lyssavirus

1 **Rabies**
2 Lagos bat virus
3 Mokola virus
4 Duvenhage virus
5 European bat lyssavirus I
6 European bat lyssavirus II
7 Australian lyssaviruses
Several unclassified Lyssaviruses (Irkut, Khujand)

Vesiculovirus

Vesicular stomatitis Indiana (VSI)
Vesicular stomatitis New Jersey (VSNJ)
Vesicular stomatitis Alagoas (VSA) about 50 less important viruses



Anne-Marie Labouche (WHO)

Unique points of rabies in Mongolia

- Wild carnivore originated (dog, wolf, red fox, corsac and manul cat)
- Seasonal change (spring, especially February, March is high, btu July, August low)

DEPARTMENT OF HEALTH
RESEARCH INSTITUTE FOR TROPICAL MEDICINE

Direct Rapid Immunohistochemical Test (DRIT)

Dr. Daria Llenaresas-Manalo
Supervising Science Research Specialist
Veterinary Research Department
Research Institute for Tropical Medicine
Philippines

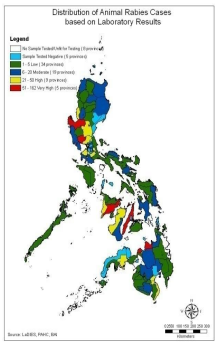
Background

RABIES

- A fatal neurologic disease caused by rabies virus
- More than 55,000 people die of rabies every year worldwide
- Dogs remains the principal cause of animal bites and rabies cases
- Endemic in the Philippines
- 200-300 human deaths per year
- 20-33 % animal rabies positivity rate per year
- Animal rabies is laboratory diagnosed, human rabies clinically diagnosed
- Laboratory confirmation is essential in countries whose goal is to eliminate rabies

Rabies Laboratories in the Philippines

- Around 19 (4 LGU funded) rabies diagnostics laboratories all over the country
- Additional laboratories need to be set up



Direct Fluorescent Antibody Test (dFAT)

- Currently being used diagnostic test
- Gold standard (WHO 2005)
- Setting up a laboratory network using dFAT as the diagnostic test for rabies is quite challenging for developing countries
  - Needs expensive equipment (FA microscope, incubator, freezer).
  - Reagent not locally available

Hence, a need for more economical yet reliable diagnostic test.

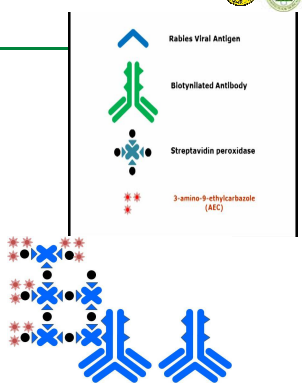
Direct Rapid Immunohistochemical Test

- Center for Disease Control and Prevention (CDC) developed new diagnostic method as a cheaper alternative to Direct Fluorescent Antibody Test (dFAT)
- Employs an immunohistochemical test to detect rabies virus by incorporating various components of existing immunoperoxidase techniques

The reduced cost suggest high potential for making rabies diagnosis available in other provinces for which a capacity for diagnosis will contribute to rabies control.

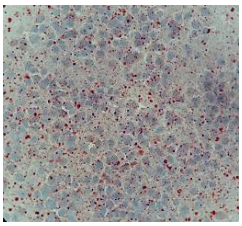
Principle of DRIT

- uses highly concentrated, purified biotinylated monoclonal/polyclonal antibody to rabies virus antigen.
- the **biotin labeled antibody** binds to the antigen.
- addition of **enzyme-labeled streptavidin** results in the binding to each biotinylated antibody.
- after the antigen-antibody reaction, the enzyme label is reacted with a **substrate (AEC)** to yeild an intensely colored product that can be visualized with an ordinary light microscope.

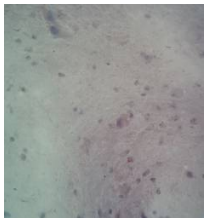


Rabies viral antigen

Microscopic Observation



A. Positive 400x magnification



B. Negative 400x magnification

The direct rapid immunohistochemical test (DRIT) on fresh brain touch impressions
Positive: red specific staining identification of rabies virus nucleoprotein
Negative : Blue background: hematoxylin counterstain

Advantages of the DRIT

- Rapid (<1 hour), simple, requires no specialized equipment and can be successfully performed on samples preserved in glycerol solution for 15 months or frozen for 24 months
- The DRIT permits for rabies diagnosis under field conditions
- .
- DRIT does not require an expensive fluorescent microscope
- Initial studies shows it is as sensitive and specific as dFAT (Lembo et al.,2006)

..however

- The reagent, rabies biotinylated antibody is not commercially available
- Only available in selected laboratory
  - CDC which is a monoclonal antibody
  - Wistar Institute - USA
  - University of Pretoria –South Africa
- Need to locally produced rabies polyclonal antibodies or biotinylated polyclonal antibodies

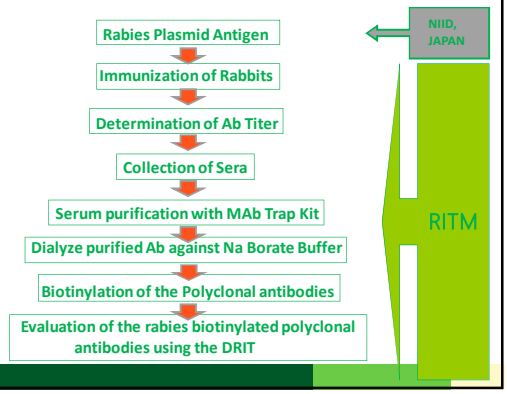
Objectives

- To produce biotinylated polyclonal antibodies using rabies N plasmid DNA antigen to be used as reagent for DRIT
- To evaluate (preliminary) the reagent using banked brain samples at the RITM rabies laboratory

Specific objectives

- To determine the sensitivity and specificity DRIT against gold standard dFAT
- To compare the two methods of fixation, 10 % buffered formalin and cold acetone

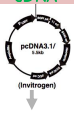
Methodology



Immunization Procedure

RABV-N cDNA

DNA vaccination



Plasmid DNA expressing target protein

Shima-jet injector

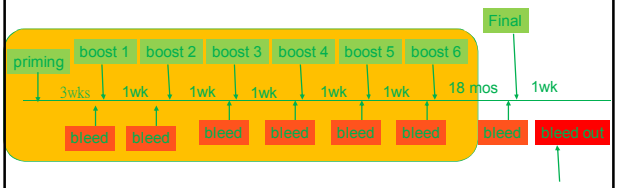
plasmid DNA

120 ul of Ag (400 ug/ul) + 750 ul PBS
Injected to 4 sites

Comparison of the syringe and Shima-jet injection

shimadjet

Schedule of immunization and bleeding



Antibody purification

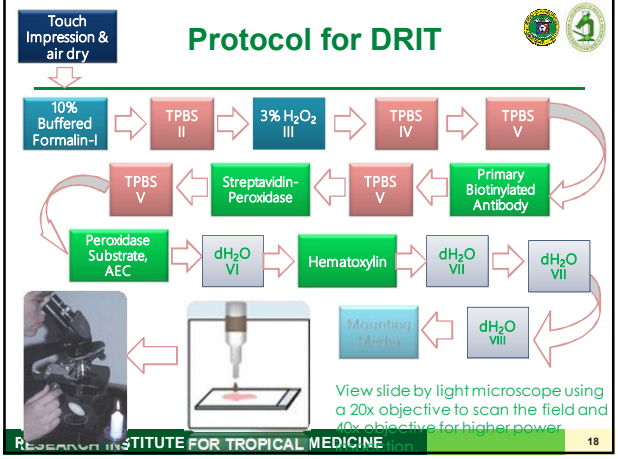
Mab Trap Kit
- Is an affinity chromatography kit for fast and effective purification of monoclonal and polyclonal IgG.

- includes one Hi-trap column, prepacked with Protein G Sepharose High Performance.

Biotinylation of antibody

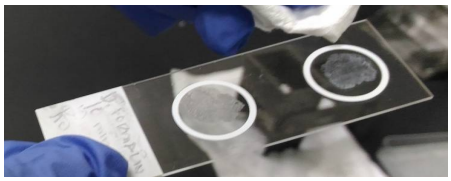
The purified polyclonal antibodies (IgG) was biotinylated with NHS-LC Biotin

Protocol for DRIT



View slide by light microscope using a 20x objective to scan the field and 40x objective for higher power examination.

Procedure of DRIT



Touch impressions of the brain

Fixed with either cold acetone at least 30 mins
or 10% buffered formalin 10 mins

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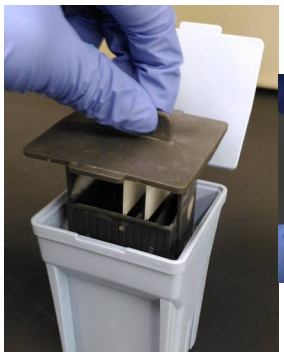

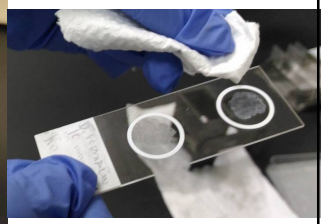

Wash with Tween PBS

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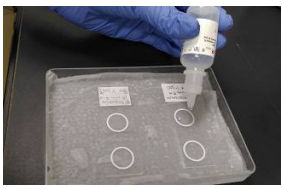

Add proteinase K 10 mins humid chamber
room temperature

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Hydrogen peroxide 10 mins

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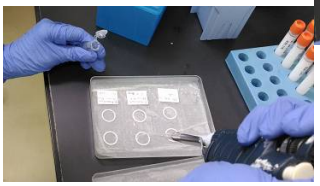

Rabies antibody/biotinylated rabies antibody (10 mins)

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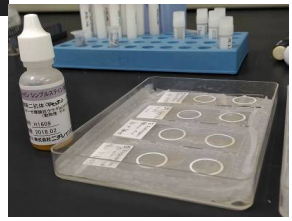
Secondary antibody
(FITC anti-rabbit) 10 mins



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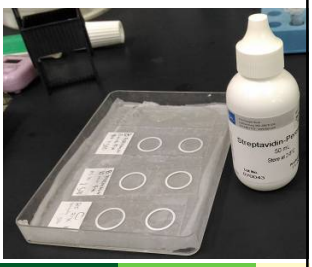
Strepavidin peroxidase10 mins



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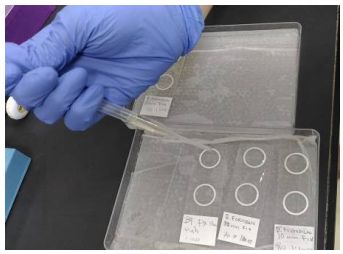

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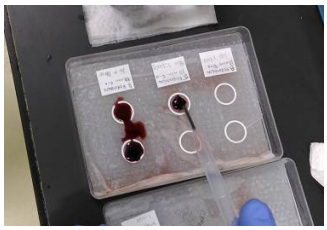

3-Amino-9-carbamizole (AEC) 10 mins, wash water

Counter stain with Gills-haematoxillin 2 mins

Mount with water soluble mounting media

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Conclusion

The DRIT can be used as an alternative test for rabies antigen detection

Both 10% formalin and acetone can be used as fixation for DRIT

Acknowledgement

Research Institute for Tropical Medicine
Laarni Tuason, RMT
Luz P. Acosta, DrPh
Blanca Jarilla, PhD
Eleonor Avenido, MSc
Plebeian B. Medina, RMT, MS
Catalino S. Demetria,DVM
Fidelino Malbas, DVM, MPH
Beatriz P. Quiambao, MD
VRD and Immunology staff

National Institute for Infectious Diseases, Japan
Inoue Satoshi, DVM, PhD

School of Veterinary Medicine, Kitasato University, Aamori, Japan
Chun-Ho, Park , DVM, PhD`

THANK YOU