

## 出國報告（出國類別：其他）

# 赴墨西哥參加美洲狂犬病研討會

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# 摘要

「美洲狂犬病研討會」是全球狂犬病防治及研究領域的年度盛會，自 1999 年起每年由美洲各國輪流舉辦一次。包含研究人員、公衛專家、防疫官員、野生動物學家及實驗室人員等各個領域，遍及五大洲、20 多個國家的代表皆會出席並發表研究成果。2014 年應美國疾病管制局專家邀請，參加於墨西哥坎昆舉辦之第 25 屆美洲狂犬病研討會並發表我國鼬獾狂犬病病例報告，藉由參與本會議與國際學者交流，並攜回各國狂犬病防疫經驗與研究資料，供作防疫策略擬定之參考。

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# 壹、目的

狂犬病是全球最重要的人畜共通傳染病之一，據世界衛生組織(WHO)統計，全世界每年死於狂犬病的人數高達55,000人，其中又以亞洲的印度、中國及一些非洲國家疫情最嚴重，且絕大多數與遭犬抓傷或咬傷有關。近年來，在WHO、世界動物衛生組織(OIE)及一些非政府組織(NGO)的努力之下，由犬媒介的狂犬病病例逐年減少，但由野生動物所媒介的狂犬病確有增加的趨勢。

「美洲狂犬病研討會(Rabies In The Americas; RITA)」是全球狂犬病防治及研究領域的年度盛會，自1999年起每年由美洲各國輪流舉辦一次。包含研究人員、公衛專家、防疫官員、野生動物學家及實驗室人員等各個領域，遍及五大洲、20多個國家的代表皆會出席並發表研究成果。

2013年7月家畜衛生試驗所確診3例鼬獾狂犬病病例，引起國人及國際高度關注，自2013年7月至2014年9月，本所已完成400餘例動物狂犬病病例診斷與研究。鼬獾為近5年才被發現的狂犬病保毒動物，目前全世界僅中國大陸及台灣有病例發生，鼬獾感染狂犬病之研究資料極為缺乏，許多國家尤其是目前為狂犬病非疫區的國家對我國野生動物狂犬病監測及防治有高度興趣。

本次於美洲狂犬病研討會發表我國鼬獾狂犬病病例報告，內容包括台灣鼬獾狂犬病病毒核酸序列分析及病理學研究成果，對各國從事野生動物狂犬病研究人員提供鼬獾狂犬病研究第一手資料，藉由參與本會議與國際學者交流，並攜回各國狂犬病防疫經驗與研究資料，供作防疫策略擬定之參考。

## 貳、行程及研討會議程

日期	行程 / 議程
10/24 - 10/25	搭機離台-美國轉機-抵達墨西哥坎昆(Cancun)
10/26 (日)	第 25 屆美洲狂犬病研討會(RITA)開幕式
10/27 (一)	1. 「George Bear 拉丁美洲研究人員獎」專題演講 2. 主題一、 <b>人類狂犬病</b> <ul style="list-style-type: none"> <li>• (1) 流行病學</li> <li>• (2) 預防措施與治療</li> <li>• (3) 圓桌會議(專家演講): 狂犬病治療發展進程</li> </ul>
10/28 (二)	1. 「美洲狂犬病研討會暨世界動物保護研究人員獎」專題演講 2. 主題二、 <b>動物狂犬病</b> <ul style="list-style-type: none"> <li>• (1) 城市型狂犬病(Urban rabies)</li> <li>• (2) 圓桌會議(專家演講): 野生動物狂犬病</li> <li>• (3) 由蝙蝠傳播之狂犬病</li> </ul>
10/29 (三)	1. 主題二、動物狂犬病(續) <ul style="list-style-type: none"> <li>• (4) 小型食肉目野生動物狂犬病</li> </ul> 2. 主題三、 <b>狂犬病最新研究現況</b> <ul style="list-style-type: none"> <li>• (1) 致病機轉</li> <li>• (2) 診斷</li> </ul>
10/30 (四)	1. 主題三、狂犬病最新研究現況(續) <ul style="list-style-type: none"> <li>• (3) 免疫學</li> <li>• (4) 新型狂犬病疫苗研發概況及抗病毒藥</li> </ul> 2. 閉幕式暨第 26 屆 RITA 宣傳
10/31 - 11/2	搭機離開墨西哥坎昆(Cancun)-美國轉機-返台

## 參、研討會內容摘要報告

**研討會簡介** 1990年代全球狂犬病疫情達到高峰，為喚起人們對狂犬病的重視及提升狂犬病跨國合作，由美國、加拿大及墨西哥等3個美洲國家發起，於美國喬治亞州亞特蘭大(Atlanta)舉辦首屆美洲狂犬病研討會，邀請所有美洲地區國家的防治/研究人員參與，共同為控制狂犬病而努力。由於首屆美洲狂犬病研討會辦理獲得空前成功，此後每年皆舉辦一次、由美洲各國輪流擔任主辦國。研討會除發表最新狂犬病研究概況，更針對各國的狂犬病防疫現況做及時的成果報告及檢討，因此吸引越來越多歐洲、亞洲及非洲等國家的專家學者與會，逐漸演變成為全球狂犬病的年度盛會。

本次2014年10月26日~10月30日於墨西哥坎昆(Cancun)舉辦，由墨西哥國家農業健康安全管理局(SENASICA)主辦，並由美國農部(USDA)擔任協辦單位並贊助全程同步翻譯(英文↔西班牙文)。此次研討會的目的在於提供各國狂犬病研究學者及防治專家一個交流平台，另外WHO、OIE、跨國狂犬病防治機構及美洲各國代表亦會透過參加本會議檢討過去一年在狂犬病防治上的進展及困境，最終期望在各界的努力之下能控制甚至撲滅狂犬病。本次總計有20國，超過300位專家學者與會。研討會主題包括：人類狂犬病、動物狂犬病及狂犬病最新研究現況等三大主題，每個主題之下又細分為：(1)狂犬病流行病學；(2)狂犬病預防措施與治療；(3)狂犬病治療發展進程；(4)城市型動物狂犬病(Urban rabies)；(5)野生動物狂犬病；(6)蝙蝠媒介之狂犬病；(7)小型食肉目野生動物狂犬病；(8)狂犬病致病機轉；(9)狂犬病診斷方法；(10)狂犬病免疫學；(11)新型狂犬病疫苗研發概況及抗病毒藥物等11項子題，共68個口頭論文報告及45個壁報論文發表，以下就研討會各大主題摘要報告如下：

## 一、人類狂犬病

(一) **人類狂犬病的趨勢與挑戰** 美國自 1960 年代迄今，共有 115 人不幸死於狂犬病，平均每年檢出 6,000 至 10,000 例動物狂犬病病例。在人類的死亡病例中，其中有 83 例經 RT-PCR 檢測及病毒序列分析，確認病人是由何種途徑、在哪個國家被感染。相反地，其中有 3 例以 RT-PCR 檢測為陰性。在這 3 個特殊病例中，有 2 例僅憑藉單一方法做診斷，另 1 例則是美國第一例人感染狂犬病後存活的病例，這個病例則是經由多種不同方法檢測血清以及腦及腦脊髓液最終確診為狂犬病。自然感染(遭狂犬病動物抓傷或咬傷)及醫源性感染(器官移植)的病例都曾經發生過。美國本土最近一個感染狂犬病病例發生在一位居住於美東地區的 47 歲女性，因疾病正接受免疫抑制劑的治療，住院的前 5 週因無法檢出狂犬病抗體而未被確診，直到第 6 週才確認該名婦人是遭感染狂犬病的灰狐咬傷而罹病，此病人目前還在住院觀察中，本病例的發生凸顯出現階段預防人類狂犬病的困境。回顧近 55 年來的人類病例，我們所面臨的挑戰是顯而易見的，在美國，因政府財政困窘不得不刪減公共衛生的預算，但也同時影響了重要疾病的診斷、監測，甚至是專業人員的養成教育。

## 二、動物狂犬病

(一) 狂犬病是否會入侵甚至持續存在拉丁美洲的陸生野生動物族群？ 2009 年的統計顯示，拉丁美洲各國的人感染狂犬病的病例數顯著減少，但不幸的是，2009-2012 年的統計顯示，各國狂犬病防治似乎遇到瓶頸，深究其原因，我們發現：如果不採取積極措施控制野生動物狂犬病疫情，人類的病例很難再繼續減少。歸納已發表的野生動物狂犬病監測資料，在拉丁美洲目前地區已知的保毒動物包括：灰狐、食蟹狐、臭鼬、浣熊及靈長類動物，但是各國的狂犬病監測似乎都有一個共同的盲點，那就是"只監測已有病例被報告的物種"，缺乏完整的野生動物狂犬病監測體系，例如美國已確認會攜帶狂犬病的獾，就應該納入狂犬病監測。上述野生動物究竟是狂犬病的保毒動物還是偶發感染？也須進行大規模的研究計畫來釐清。自 1992 年以來，拉丁美洲每年平均檢出的犬狂犬病病例僅數十例且逐年減少，但是野生動物狂犬病病例數每年約 5,000 - 9,000 例，欲控制甚至撲滅野生動物狂犬病，惟有大規模投與口服狂犬病疫苗一途，並搭配教育民眾：(1)哪些動物具有狂犬病風險；(2)帶寵物施打疫苗；(3)為寵物絕育。

(二) 由蝙蝠傳播之狂犬病 分析由蝙蝠攜帶的狂犬病病毒序列可發現由於蝙蝠具備高度飛行能力，病毒的傳播速度很快且不受地理環境的限制。研究顯示吸血蝙蝠的活動範圍受到氣候變遷等因素影響，近幾年有逐漸北移的現象，研究人員高度重視此一現象，因為蝙蝠狂犬病迄今仍無有效方法可加以控制。

(三) 臺灣鼬獾狂犬病病例報告 台灣自 1961 年以後即宣告成為狂犬病非疫國，然而，2013 年 7 月，3 例鼬獾被確診為狂犬病陽性使台灣再度淪為疫區。為瞭解台灣狂犬病發生情形，動物防疫機關展開大規模食肉目野生動物流行



病學調查，自 2013 年 7 月迄今，以直接免疫螢光抗體染色法 (FAT) 檢測 3,796 例動物病例，狂犬病陽性率分別為鼬獾 38.27% (403/1,053)、鼬 0.58% (1/171) 及犬 0.05% (1/1,861)，其他物種包括蝙蝠皆為陰性，鼬獾仍為台灣狂犬病主要之發病動物。由特生中心凍存之鼬獾檢體進行之狂犬病回溯性研究，已證實台灣鼬獾攜帶之狂犬病病毒株非新入侵的病毒株，已存在臺灣鼬獾族群中至少 3 年之久。依狂犬病病毒核蛋白核酸序列分析，台灣的病毒株可明顯區分為兩個分群：(1)中部-南部分群(TW-MS)：包括南投縣、台中市、雲林縣、嘉義縣、台南市及高雄市等 6 縣市；(2)東部分群(TW-E)：花蓮縣、台東縣及屏東縣等 3 縣市。另蒐集 48 例鼬獾狂犬病病例進行組織病理學檢查，在非化膿性腦炎病變中，判定病變為嚴重者佔 31%( 15/48)，中度佔 31%( 15/48)，無病變至輕微佔 38% ( 18/48)。利用免疫組織化學染色探討大腦、小腦、海馬角及腦幹之狂犬病抗原分佈，抗原量最多的部位為腦幹，依序為海馬角、大腦及小腦。所有病例皆可在神經元發現 Negri 包涵體，並均可在唾液腺偵測到狂犬病抗原。本研究顯示台灣鼬獾狂犬病病毒株已成一獨立演化分支，所有狂犬病陽性鼬獾病例皆可由唾液腺排毒。鼬獾狂犬病起源、入侵台灣的確切時間及致病機轉等，有待進一步研究。

### 三、狂犬病最新研究現況

(一) 評估利用 blocking ELISA 偵測口服疫苗效力 在 Slovakia 只有紅狐為狂犬病病毒的保毒者，因此口服疫苗被作為排除野生動物狂犬病的主要工具。

Tetracycline 與 blocking ELISA 被利用來針對調查對象的病毒或抗體情形的工具之一，並用來評估是否達到 seroprevalence effective cut-off，其表是口服疫苗是可行的並可應用在野生動物是有效的。疫苗投與單位依各區流行狀況而定，且在每次投與後皆需評估後調整下次投與單位。結果發現 seroprevalences 與 tetracycline levels 的關係成正比，而與病毒檢出率成反比，而只要達到 seroprevalence cut-off 的百分之三十及對於排除病毒有效，且對於病毒在入侵非疫區時對於清除病毒也有效，在波瀾的狂犬病再入侵的情況及可見。雖然 ELISA 的敏感性只有 FAVN 的 95% 以上，所以觀察到的 seroprevalence effective cut-off 值也較低，但有其方便性的優點，該實驗室目前研發 blocking ELISA 並將其製成商品化的診斷套組(BioPro Rabies ELISA Ab kit)，對於應用在野生動物或寵物以評估疫苗效力時仍為方便選擇。

(二) 開發 lyssavirus 診斷之免疫組織化學檢測之進展 直接螢光抗體染色法(direct fluorescent antibody test ,FAT)是世界動物衛生組織(OIE)與世界衛生組織(WHO)對於診斷 lyssavirus 的建議方式，也是其診斷敏感性的黃金標準。對於全球排除狂犬病計畫，其他的診斷方法變得更為需要，以因應被狂犬病感染動物抓咬的暴露後評估或是評估計畫執行效果，direct rapid immunohistochemical test (DRIT)以被用作替代的診斷方方，DRIT 仍需要腦壓片組織，然而其較 FAT 方便的是只要有依般的顯微鏡以及 biotin 標示抗體即可，且結果在 1 小時內即可判讀，此方法在 2008 年後已在美國、歐洲、飛周、亞洲各地被使用，不論在各地區，不同物種的檢體、不同樣本品質、不同病毒型別或拭布類背景狀況下，此方法的敏感性特異性皆達 100%。目前

所有型別的 lyssavirus 皆曾以 DRIFT 診斷出，且在美國於試驗室中也曾以 DRIFT 確認使用 FAT 檢測但結果模稜兩可的檢體，對於田野調查也加強其調查能力，例如在北美口服疫苗計畫中，DRIFT 就如同 FAT 一樣被例行性的用在診斷多達 60,000 多件的檢體。依據實驗室間的比對，使用不同的單株或多株抗體，以抗體應用於不同的病毒與哺乳動物之檢測數據，在在都顯示 DRIFT 可作為狂犬病診斷的替代方法。

### (三) 於經狗或吸血蝙蝠感染之人類狂犬病其中樞神經之促炎性細胞因子輪廓

在狂犬病病毒的抗原刺激下，中樞神經的 glia cell 如 astrocytes and microglia 可分泌一些免疫刺激物，例如促炎性細胞因子(IL-1 beta, IL-6 and TNF-alpha)，這些因子會造成血腦障壁(blood-brain barrier, BBB)的通透性，使白血球進入中樞神經組織進而分泌 cytokines，因此瞭解這些感染後宿主免疫因子的組成有助於對抗感染的傷害以及減少對神經組織的損傷。作者研究 IL-1 beta, IL-6 與 TNF-alpha 在 3 個受感染人類病患神經組織中的表現，使用方法為免疫組織化學法，在每個神經組織切片中評估 40 個視野並定量，每個視野面積為 0.0625 mm<sup>2</sup>。結果在兩個蝙蝠傳染患者的 IL-1 beta and TNF-alpha 有明顯上升，而由狗感染的患者其 IL-6 則有較明顯上升。在蝙蝠傳染的病患中其 IL-1 beta, IL-6 與 TNF-alpha 的出現伴隨著明顯的炎症浸潤現象，但在狗的病患中則只有 IL-6 的明顯表現與較少的炎症浸潤，可能的原因是 IL-6 與其他的免疫反應較有關，例如於 Th1 與 Th2 細胞在清除病毒失敗後調控 Th17 參與反應。

### (四) 使用重組 baculovirus 表現狂犬病病毒的醣蛋白 G

狂犬病是重要的人畜共通疾病，每年超過 55,000 人死於本病，其主要發生於非洲與亞洲，雖然疫苗以及暴露後處理可用於防止本病，然而這些處置對於某些地區人民仍負擔過高，因此開發較便宜的疫苗變成重要的課題，狂犬病病毒的醣蛋白 G(RV-G)是病

毒主要引起宿主保護性免疫反應的部分，且可誘使產生具中核病毒能力的抗體，因此其基因被應用在導入 baculovirus, E. coli 或植物等生物體中，以產生大量的醣蛋白 G，本實驗將蛋白質的穿膜蛋白基因部分去除並嵌入 baculovirus 中再感染細胞以產生水溶性的 RV-G，蛋白質表現則由螢光顯微鏡或 western blot 分析，結果在細胞或培養液中皆可測到水溶性的醣蛋白 G，而未來將繼續評估此水溶性醣蛋白 G 應用作為液名的可行性。

(五) **新型狂犬病疫苗研發概況** 預防狂犬病需要有效、安全及方便投與的疫苗，而溫度穩定型的狂犬病疫苗在熱帶地區會較有優勢，且口服劑型的疫苗應用在廣泛種類的肉食動物會有較好的效果，此研究目的在於開發穩定的口服狂犬病疫苗，減毒活病毒 ERAg333 毒株以蒸發後乾燥且穩定的型態(PBV)保存，而在之前的研究級證明 RV PBV 是溫度穩定的，且即使在經射線照射不活化後，仍和商品化疫苗一樣具有相同的抗原水平。在小鼠單次肌肉注射 RV PBV 後，採血觀察其免疫反應，並進行流浪狗狂犬病毒株攻毒試驗，減毒活病毒或是不活化的 RV PBV 皆可產生足夠的中和抗體，並可保護動物免於病毒感染，同樣的病毒以口服形式是給與紅狐，也可產生中和抗體。節果顯示不論減毒活病毒或是不活化的 RV PBV 可作為後續開發溫度穩定型狂犬病疫苗的毒株。

## 肆、心得及建議

### (一) 美洲研討會提供狂犬病研究人員表現舞台：

本次參加美洲研討會的世界各國的研究人員約 300 餘人，其中約有三分之一的與會者有進行口頭論文或是壁報論文的發表，研討會亦提供" George Baer Latin American Investigator Award " 及" RITA / WAP Award"等兩個獎項給拉丁美洲以及其他國家優秀的研究人員，對從事狂犬病防治及研究的年輕學者而言，本研討會是一個可以讓他們發光發熱的舞台，可以讓他們在此盡情的發揮。研究的路是漫長而孤獨的，藉由參與研討會結識志同道合的夥伴，與其他領域專家討論研究議題摩擦出更閃亮的火花，這也許是為何 RITA 研討會一年舉辦的比一年更盛大的原因。

### (二) 野生動物狂犬病疫情在部分國家被低估：

我國已超過半世紀未曾發生狂犬病，2013 年突然發現鼬獾狂犬病，並在短短的 1 年時間檢出 400 餘例陽性病例，甚至還檢出犬及鼬獾的偶發感染病例。這樣的監測結果讓其他國家非常驚訝，紛紛開始檢討自己國家的狂犬病監測體系，例如許多拉丁美洲國家僅針對蝙蝠做監測，卻完全忽視狂犬病可能入侵其他野生動物的風險，藉由本次研討會進行我國鼬獾狂犬病病例報告，分享我國疾病監測及防治成果，與國際學者進行交流，期能拋磚引玉，使各國的野生動物監測體系更趨完備。

### (三) 野生動物狂犬病是可以撲滅的—使用口服狂犬病疫苗：

野生動物狂犬病的防疫較犬源狂犬病防疫複雜許多，但歐洲許多國家都已成功清除野生動物狂犬病，除了政府的努力外，另一個重要原因就是當地的保毒動物（Reservoir）只有狐狸一種。欲撲滅台灣鼬獾狂犬病，唯一有效的方法就是在鼬獾活動區域有計劃性、大規模投與狂犬病口服疫苗，並且須在政府各部門傾全力投入資源的情況下至少持續 3 年。台灣有很大的機會能

成功清除狂犬病，因為台灣是一個島國且目前只有鼬獾單一物種會攜帶病毒，毋須像美國面臨狂犬病已擴散到多種保毒動物且病毒可能隨鄰近國家野生動物遷徙而入侵的困境。

## 伍、誌謝

感謝防檢局及家畜衛生試驗所長官給予此次出國機會，有幸見識國際研討會舉行之盛況並瞭解國際間最新狂犬病控制現況及技術。此次出國經費由農委會科技處支持提供，始得順利成行，在此特表誠摯謝意。

## 陸、附錄

- 一、附圖。
- 二、美洲狂犬病研討會報告摘要。

### 附圖



圖 1、於 RITA 研討會發表「臺灣鼬獾狂犬病病例報告」。



圖 2、與 RITA 科學審查委員會主席 Dr. Rupprecht 合影。



圖 3、美洲狂犬病研討會開幕式。



圖 4、美洲狂犬病研討會會場。



# Resúmenes

## Abstracts

### Resumos



**Reunión Internacional de  
Rabia en las Américas**  
26-30 de octubre 2014/Cancún, México

**Premio George Baer al Investigador Latinoamericano  
George Baer Latin American Investigator Award  
Prêmio George Baer ao Pesquisador Latino-americano**

***First evaluation of DNA vaccine against rabies, based in G5 epitope linked to molecular adjuvant P28.***

Guillermo Gálvez Romero, Mónica Salas Rojas, Ericka N Pompa Mera & Álvaro Aguilar Setién.

The rabies virus glycoprotein is the main component used for the development of efficient DNA vaccines. It has two conformational immunodominant sites that have been difficult to emulate in vitro. It has been previously reported that a unique linear antigenic site (G5) can generate neutralizing antibodies and induce protection in laboratory animals. To enhance the antibody production, the molecular adjuvant P28 has been employed, which is part of the C3d protein from the complement system and the minimum binding site to the CR2 receptor in B cells. It can generate a 1000-fold increase in the level of specific antibodies to the antigen of interest. Our group has worked in the development of DNA vaccines using the whole glycoprotein gene (pGQH vaccine), however 20ug was required to induce protection in mice. Therefore, we proposed the construction and evaluation of a DNA vaccine that employs the sequence of the linear antigenic site G5 linked to the adjuvant P28. This will allow us to decrease the amount of DNA vaccine needed by directing the immune response to the production of neutralizing antibodies. The DNA vaccine G5-P28 was genetically engineered and subsequently evaluated in a mouse model. Following WHO protocols for vaccine evaluation, 20ug of plasmid was injected by intramuscular route alongside corresponding controls. The animals were challenged with 22 LD<sub>50%</sub> by intracerebral route. Twenty-one days after being challenged, 100% survival was observed in the G5-P28 group without clinical

# Rabia en humanos Human rabies Raiva em humanos

Epidemiología  
Epidemiology  
Epidemiologia

## ***Mordedura por perro y de otros mamíferos: una correlación con los casos confirmados de rabia en humanos y perros en México.***

*Mendoza-Villavicencio Ivonne L, Cabrera-Gaytán David Alejandro & Revuelta-Herrera Martín A.*

Objetivo: Describir la correlación entre diagnósticos de primera vez de mordeduras por perro y de otros mamíferos con respecto a los casos de rabia canina y humana en México.

Material y métodos: Se obtuvieron los casos de mordeduras por perro (CIE-10 W54) y mordeduras por otros mamíferos (CIE-10 W55), del Sistema Único de Información para la Vigilancia Epidemiológica (SUIVE) de enero de 2007 a marzo 2013 por entidad federativa, así como los casos confirmados de rabia humana y canina que están disponibles en el portal del Programa Nacional de Zoonosis de la Secretaría de Salud. Se calcularon tasas de incidencia (TI) por patología con una mediana poblacional oficial para esos años por 100,000 habitantes. Se calcularon intervalos de confianza para proporciones mediante escala de Wilson y coeficiente de correlación con alfa de 0.05 en OpenEpi. Los casos fueron georeferenciados.

Resultados: Las 32 entidades federativas de país notificaron casos nuevos de mordeduras por perro y de otros mamíferos con 689,893 (636.5 IC95% 635, 638) y 63,736 (58.8 IC95% 58.34, 59.26) respectivamente. El 70% (IC95% 71.72, 71.94,  $p < 0.0000001$ ) de los casos nuevos por mordedura de perro se concentró en 17 entidades federativas, las cuales son coincidentes con aquellas que presentaron casos de rabia canina. La edad promedio de los casos de rabia canina fue 3.7 años (3 meses-10 años), mientras que dos días para conocer el resultado de laboratorio ( $n = 115$ ) y siete de cada 10 perros no estaban vacunados. Para el caso de rabia humana, fueron 19 casos en siete entidades, en seis de ellas presentaron tasa de incidencia de mordedura por otros mamíferos por arriba de la nacional ( $R = 0.346$ ), mientras que para los casos incidentes entre ambos diagnósticos también fue positiva ( $R = 0.356$ ). Por cada agresión de otro mamífero notificada, existen 11 por perro.

Conclusiones: Existe un gran número de casos nuevos por mordedura de perro en relación a otros mamíferos, y gran parte de ellos están en entidades federativas que han presentado casos de rabia canina.

# ***Distribución espacial de las mordeduras de perro como factor de riesgo para la transmisión de la rabia humana en el Estado de México.***

*Medina Torres Imelda, Guadarrama Cruz Luis Octavio, Soto Serrano Gabriela & Reséndiz Tinajero Héctor Hail.*

La rabia es una enfermedad infecto-contagiosa, aguda y mortal, que afecta al sistema nervioso central, es provocada por un virus del género lyssavirus y de la familia Rhabdoviridae, es transmitida por la saliva que contiene el virus de alguna persona, animal enfermo o por material contaminado de laboratorio. A nivel mundial los perros representan el principal riesgo para la salud pública. Objetivo: Determinar la distribución potencial de las mordeduras de perro a nivel espacial y temporal para medir el riesgo de transmisión de la rabia. Metodología: Se georeferenció la ubicación de las mordeduras por perro en los humanos a través de las direcciones de las unidades de salud donde fueron atendidas las agresiones. Como variables de entrada al modelo de distribución se utilizaron variables socioeconómicas (número de habitantes de la localidad, actividad de los agredidos y población canina), obtenidas de las bases de datos de CONAPO 2010, y estructuradas e interpoladas para el mismo año de muestreo. Finalmente se estimó la distribución potencial utilizando el algoritmo de máxima entropía (MaxEnt), interfaz de aplicación dentro del Sistema de Información Geográfica Idrisi Selva. Resultados y discusión: De acuerdo a las tasas de agresión durante 2013, se generaron mapas de riesgo, observando que las agresiones por perro muestran patrones espaciales en el sector nororiental del Estado y municipios conurbados con el Distrito Federal, donde se asume existe un mayor riesgo de presentar transmisión de la rabia y con esto apoyar a la implementación de medidas de prevención y control de esta zoonosis.

# ***Human rabies: Trends and challenges.***

*Cathleen A Hanlon, Michael Niezgoda, Xiaoyue Ma, Pamela Yager, Felix Jackson, Miriam Shiferaw, Ryan Wallace, Jesse Blanton*

An overview of human and animal rabies in the USA from 1960 to present consists of 115 human cases and approximately 6,000 – 10,000 animal cases annually. Among human cases, reverse transcription polymerase chain reaction (RT-PCR) assay results were positive in 83 fatal cases thus permitting phylogenetic analysis that identified the source of the disease. Conversely, three survivors were unique in the lack of positive RT-PCR results. Among these 3 cases, two were diagnosed on the basis of a single diagnostic method whereas the first survivor case was confirmed by multiple tests conducted sequentially on sera and cerebrospinal fluid. Natural and iatrogenic routes of transmission, such as through tissue or organ transplantation, occurred. A recent rabies exposure of a 47-year-old female with an immunosuppressive medical intervention in the mid-Atlantic region of the US is a relevant example of current challenges in human rabies prevention. There was a lack of neutralizing antibody in the patient's sera until 5 weeks after the bite of the proven rabid gray fox. Patient follow-up is continuing. The phylogenetics of rabies viruses in human patients and characterization of specific rabies virus variants in reservoir species and "spillover" cases describe regional transmission patterns, translocation of humans and animals, and novel emergences. The surveillance and diagnostic infrastructure in place in developed countries provide detailed insights into the changing patterns in rabies epizootiology. These observations may be increasingly relevant to other less-developed nations. Looking at the last 55 years of rabies transmission patterns, challenges are clear. In North America, an extensive but economically pressured public health infrastructure continues to provide protection from an overwhelmingly-universally lethal disease for which there is limited and, with recent economic developments, diminishing support for diagnosis, surveillance, and public and professional education essential for disease prevention.

# ***National Rabies Information System (NaRIS).***

Raffy A. Deray.

An overview of human and animal rabies in the USA from 1960 to present consists of 115 human cases and approximately 6,000 – 10,000 animal cases annually. Among human cases, reverse transcription polymerase chain reaction (RT-PCR) assay results were positive in 83 fatal cases thus permitting phylogenetic analysis that identified the source of the disease. Conversely, three survivors were unique in the lack of positive RT-PCR results. Among these 3 cases, two were diagnosed on the basis of a single diagnostic method whereas the first survivor case was confirmed by multiple tests conducted sequentially on sera and cerebrospinal fluid. Natural and iatrogenic routes of transmission, such as through tissue or organ transplantation, occurred. A recent rabies exposure of a 47-year-old female with an immunosuppressive medical intervention in the mid-Atlantic region of the US is a relevant example of current challenges in human rabies prevention. There was a lack of neutralizing antibody in the patient's sera until 5 weeks after the bite of the proven rabid gray fox. Patient follow-up is continuing. The phylogenetics of rabies viruses in human patients and characterization of specific rabies virus variants in reservoir species and "spillover" cases describe regional transmission patterns, translocation of humans and animals, and novel emergences. The surveillance and diagnostic infrastructure in place in developed countries provide detailed insights into the changing patterns in rabies epizootiology. These observations may be increasingly relevant to other less-developed nations. Looking at the last 55 years of rabies transmission patterns, challenges are clear. In North America, an extensive but economically pressured public health infrastructure continues to provide protection from an overwhelmingly-universally lethal disease for which there is limited and, with recent economic developments, diminishing support for diagnosis, surveillance, and public and professional education essential for disease prevention.

***A new paradigm for rabies pre-exposure prophylaxis?  
Results of a non-inferiority randomized, controlled,  
observer-blind study with Rabipur® following a new,  
accelerated, one-week, intramuscular PrEP regimen.***

*Jelinek T, Burchard GD, Dieckmann S, Bühler S, Kollaritsch H, Nothdurft HD, Reisinger E, Costantini M, Giesen A, Bosse D, Lattanzi M, Pellegrini M.*

Rabies pre-exposure prophylaxis (PrEP) is recommended for anyone who is at continual, frequent or increased risk of exposure to the rabies virus, as a result of their residence, travel or occupation (WHO 2013). PrEP consists of 3 intramuscular (IM) doses of modern rabies vaccines, with doses on days 0, 7 and 21 (or 28). Individuals requiring PrEP at short notice may not be able to complete this regimen, resulting in suboptimal or no rabies immunization before potential exposure. Therefore, an accelerated, one-week Rabies PrEP would be advantageous. To address this unmet medical need, a phase III, randomized, controlled, observer-blind, non-inferiority immunogenicity study of Rabipur®, inactivated Rabies vaccine (Novartis Vaccines), was conducted in 661 adults, aged 18 to 65 years, in Austria, Germany and Switzerland. The non-inferiority of Rabipur accelerated regimen (3 IM doses, each on days 0, 3, 7), administered concomitantly with Japanese encephalitis (JE) vaccine (Ixiaro®, Valneva; 2 IM doses, each on days 0 and 7) was demonstrated compared with the conventional PrEP Rabies regimen (vaccinations on days 0, 7 and 28) as early as 7 days after last vaccine administration: 99.5% of subjects achieved Rabies virus neutralizing antibody concentrations >0.5 IU/mL, irrespective of the regimen. Day 14 geometric mean concentrations (GMCs) were 26, 21 and 24 IU/mL after accelerated Rabies/JE, conventional Rabies/JE or conventional Rabies regimens, respectively. At the 1-year follow-up, GMCs were above the cut-off for adequate immune responses (>0.5 IU/mL) in all study groups; percentages of subjects with adequate GMCs ranged from 68% to 80%. No impact of concomitant JE vaccination was observed on immune responses or on the safety profile of Rabipur. An accelerated, one-week regimen for Rabipur, if licensed, could potentially be adopted as an alternative to the currently recommended regimen, especially for individuals requiring PrEP at short notice.

# ***Rabies post-exposure prophylaxis compliance of animal bite patients in San Lazaro Hospital, Manila, Philippines: The San Lazaro Hospital Animal B.I.T.E. (Bite Injury Treatment Experience) study.***

*Don B. Maldo, Jason T. Suquila, Efren M. Dimaano, & Ferdinand S. De Guzman.*

Background: Adequate rabies post-exposure prophylaxis (PEP) denotes completion of a series of vaccination and, if indicated, immediate administration of rabies immune globulin (RIG). To ensure compliance, different strategies were developed. However, many bite victims still fail to adhere to their regimen schedule. This may put their health in danger and also could contribute to vaccine wastage.

Objective and Methodology: This is an analytical cross-sectional study to determine the factors that contribute to non-compliance to PEP regimens among animal bite patients. Records of animal bite patients seen at San Lazaro Hospital (SLH) from June 2012 to June 2013 were randomly selected. The demographic data of the bite patients and the profile of the injury-inflicting animal were identified. The PEP compliance rates were determined. Chi-square test for independence was done to determine if there is an association and binary logistic regression analysis was done to distinguish the factors that affect PEP compliance. Epi Info™ 7 and IBM SPSS™ 20 were used for statistical analyses.

Results: 667 records of SLH were randomly selected. The exposures sustained by the patients were mostly Category III (76.91% [95% CI 73.92% - 80.62%]). Male to female ratio was 1.04:1. Majority of the bite victims were children and young adults (mean age = 24.43 years, SD = 19.08). The biting animals were frequently dogs (79.16% [95% CI 75.84% - 82.14%]) and only a few were known to have anti-rabies vaccine (15.29% [95% CI 12.69% - 18.30%]). The chances of completing the active immunization were more likely to be observed from patients who were exposed to an unvaccinated animal (OR = 1.66, 95% CI 1.10 - 2.50, p = 0.015). Likewise, the chances of receiving passive immunization were higher if the injury was sustained on the head and neck regions (OR = 8.64, 95% CI 2.65 - 28.13, p < 0.001). Overall compliance to the 2-site ID PEP was 70.21% and 40.70% for Categories II and III exposures, respectively. There were no factors that could affect PEP compliance among patients with Category II exposures. For Category III exposures, patients with bite-inflicted wounds also have better compliance (OR = 3.48, 95% CI 1.05 - 11.61, p = 0.042). Compliance was lower among patients exposed to healthy animals (OR = 0.37, 95% CI 0.16 - 0.86, p = 0.021). Exposure to an unvaccinated animal favors PEP compliance (OR 1.87, 95% CI 1.13 - 3.10, p = 0.015). PEP compliance among previously vaccinated individuals was high (90.48% [95% CI 77.38% - 97.34%]).

Conclusion: Despite efforts to lower down PEP costs, many bite victims are unable to adhere to their vaccination regimens. Programs to eradicate rabies in the country are already established; however, implementation may need further boosting to control the infection both in humans and in the animal reservoir if the goal of a rabies-free Philippines is intended to be achieved.



# ***Human rabies survivors: From scientific curiosity to scientific reality.***

*Madhusudana SN, Reeta Mani, Satish Chandra P, Netravati M, Veeranna MG, Aaron De Souza, Vrajesh Udani, Ashwin YB, & Ashwini Shetty.*

Traditionally, human rabies is considered a 100% fatal disease despite well documented survivals in animals from the days of Pasteur and also few human survivors from time to time. Based on the data available from NIMHANS, in the past 2 years more than 50 paralytic rabies cases have been treated, majority of them from western and southern parts of India. All these cases have been confirmed to be rabies by laboratory tests. There are 4 survivors among these, who are alive now for more than 4 years. Diagnosis in all these cases have been confirmed by clinical, radiological and virological investigations carried out in the department of Neurovirology, NIMHANS including detection of viral RNA by real time PCR in skin biopsy of one patient. This is the only reported case in world where virus has been demonstrated in a rabies survivor while all other reported cases are based on demonstration of high titers of virus neutralizing antibodies in serum and CSF. All these Indian survivors had very high titers of neutralizing antibodies and in one case we could detect significant Th1 (IFN gamma) response even after 4 years. This could be due to persistence of virus in this patient. The partial N gene sequencing in this case revealed 99% homology with canine rabies strains prevalent in India. In contrast to survivors from rabies in USA who recovered completely with out any residual neurological complications, all the four cases from India have profound neurological deficits and are currently in a vegetative state.

In conclusion, keeping in view the increased survivals from rabies in recent times, particularly the paralytic type, treatment and survivals from rabies can become a scientific reality in near future. New modalities of treatment including immunomodulators and antivirals needs to be further explored.

## ***Joining forces: Our global commitment to human rabies elimination.***

*Michael Attlan & Joanne Maki.*

Collaboration between physicians and veterinarians proved to be essential to the development of the first rabies vaccine by Louis Pasteur in 1885. Along the same lines of this joint effort, Sanofi Pasteur and Merial, two entities of the Sanofi group with a long-standing history of fighting against rabies, continue Pasteur's legacy in joining their expertise and know-how in rabies prevention. The very nature of rabies itself, a human disease whose reservoir is found in animals, shows how much such collaboration is crucial. Preventing rabies deaths requires access to post-exposure prophylaxis as well as reducing exposure risk to infected animals. Proactive vaccination of domestic animals and control of outbreaks in reservoir wildlife species are necessary to eliminate rabies from defined geographies. Essential components of rabies prevention and control also include pre-exposure immunization of people living in or visiting high-risk areas as well as a true understanding of the rabies burden obtained through proper surveillance and reliable reporting of cases. Veterinary and wildlife aspects of rabies control complement human health measures in a holistic or "One-Health" manner. This worldwide vision may differ by region and culture but the necessary actions to prevent rabies are supported by four pillars: i) awareness, education and information; ii) diagnosis, surveillance and reporting; iii) infrastructure, decision- and policy-making; and iv) vaccination. Manufacturers producing and delivering rabies vaccines at the global level honor the unique diversity of rabies prevention. As an illustration of Sanofi's integrated approach against rabies, we commit to working closely with the medical and veterinary community, the general public, government and non-government agencies, laboratories and wildlife professionals to reach our common goals. Examples will be given of transdisciplinary projects, initiatives, and partnerships that foster sector collaboration and are finding ways to adapt rabies prevention measures to regional and global programs.

# ***Epidemiology of Rabies-Related Emergency Department Visits in the United States, 2006 – 2010.***

*Shiferaw, M & Blanton, J.*

The Nationwide Emergency Department Sample (NEDS) is a hospital-based emergency department (ED) survey developed by the Healthcare Cost and Utilization Project (HCUP). The NEDS represents approximately 20% of US hospital-based EDs, and is used to estimate all-cause national and regional ED visits. International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) and Current Procedural Terminology, Fourth Edition (CPT-4) codes along with patient demographic, total charges, and payment source data can be analyzed to describe the epidemiology and cost-effectiveness of ED services. Several studies have shown that rabies postexposure prophylaxis (PEP) is expensive and over utilized. In addition, there is scant data regarding national estimates of PEP utilization, cost, and sources of payment. We analyzed NEDS data with ICD-9-CM and CPT-4 codes related to rabies exposure, need for prophylactic vaccination against rabies, and procedure codes related to PEP administration from 2006 to 2010. Rabies-related ED visits were disaggregated into three groups; ‘exposure evaluation’, ‘possible PEP administration’, and ‘PEP administered, based on ICD-9-CM and CPT-4 codes. Nationally, there were an estimated 345,769 rabies-related ED visits during the study period. Visits were highest among those 0-9 years of age. The northeast region had the highest rate of ‘rabies PEP administration highly likely’ visits. The mean age of those highly likely to have received rabies PEP was 33 years and the most likely form of payment was private insurance (62.88%) followed by Medicaid (11.54%). The overall mean charge per visit for rabies-related ED visits was also analyzed. Our analysis reveals regional differences in rates of ED visits for rabies PEP administration and potentially a disparity between different socioeconomic statuses based on payment source data. Regional differences in PEP administration and potential disparities in PEP administration based on payment source should be further explored.

# ***Development of human monoclonal antibodies for rabies post-exposure prophylaxis.***

*CE Rupprecht, IV Kuzmin & WE Marissen.*

Despite the availability of pure, potent, safe, and efficacious modern biologics, human rabies cases continue to occur in both developed and developing countries. After exposure to a rabid animal, current recommendations for human rabies postexposure prophylaxis (PEP) include prompt wound care, multiple administrations of rabies vaccine and infiltration of rabies immune globulin (RIG). Despite its critical utility for human PEP, RIG is often in limited supply on a global basis. In this work, we provide an update on the application of CL184, a novel monoclonal antibody (mab) combination for use in rabies virus (RABV)-exposed subjects, as a replacement for conventional RIG. Use of such mabs provide significant advantages over polyclonal blood products and could minimize the chronic shortages of RIG. We utilized female Syrian hamsters as an experimental surrogate model to test the efficacy of CL184 in experimental PEP. Animals were inoculated with a bat (*Tadarida brasiliensis*) RABV isolate in the gastrocnemius muscle. Thereafter, hamsters were administered PBS only (controls), a single administration of RIG or mabs in the inoculated limb, combined with commercial human rabies vaccine in the opposite limb, or vaccine only, on days 0, 3, 7 and 14. Hamsters were observed over the next 45 days and were euthanized when clinical signs suggestive of rabies occurred, or at the end of the study. Brain tissue was removed from all euthanized animals and tested for RABV antigens by the direct fluorescent antibody test. Indeterminate or negative results were confirmed by nested RT-PCR. Over the period of observation, all controls succumbed, as did hamsters receiving vaccine only. Significant survivorship ( $p < 0.05$ ) occurred in the animals receiving mabs compared to the RIG group. These preliminary data offer promising additional support towards the application of CL184 in human rabies PEP.

**Premio RITA / WAP  
RITA / WAP Award  
Prêmio RITA / WAP**

***Skin - an alternate sample for rabies diagnosis in dogs.***

Gowri Yale, Reeta Mani PI, Ganesan SN, Madhusudana S, Shankar, Anita Mahadevan, M Vijayabharathi, K Sampada Sanyal & Shaheen Taj.

Rabies is endemic to India and is the most feared disease of all times. Dogs are the primary reservoir host of rabies in India contributing to 90% of human rabies cases. Presently, confirmatory diagnosis of rabies in dogs is by fluorescent antibody test (FAT) of brain tissue from a dead dog, which poses several challenges. Therefore this study evaluated the utility of nuchal skin as an alternate sample for rabies diagnosis in dogs. Brain and skin samples were collected at necropsy from 35 dogs that succumbed to an illness clinically compatible with rabies during quarantine in rabies observation ward. Brain and skin samples were also collected from 10 dogs that died due to gastrointestinal infections as negative controls. All brain samples were tested by FAT, Direct Rapid Immuno-histochemistry test (dRIT) and Rabies Tissue Culture Inoculation Test (RTCIT) and all skin samples were tested by Modified Direct Rapid Immuno-histochemistry test (MdRIT) for detection of rabies virus antigen and Real-time TaqMan PCR (RT-PCR) for detection of viral RNA. Rabies was confirmed in the brain tissue of 34/35 dogs that died following clinically suspected rabies. Skin samples from 28/34 (82.3%) rabid dogs were positive by RT-PCR and 22/34 (64.7%) skin samples were positive by MdRIT. All 10 skin samples obtained from lab-confirmed non-rabid dogs were negative by RT-PCR and MdRIT demonstrating 100% specificity for both assays. RT-PCR demonstrated a higher sensitivity (82.3%) compared to MdRIT; however MdRIT can be used in laboratories which lack molecular diagnostic facilities. We conclude that nuchal skin, a less invasive alternative which obviates the risks involved in obtaining and testing brain tissue, can be used for rabies diagnosis in dogs. Further research should evaluate skin biopsy for antemortem rabies diagnosis in dogs.

# **Rabia animal Animal rabies Raiva animal**

Rabia urbana  
Urban rabies  
Raiva urbana

## ***Control de la población canina en municipios de la jurisdicción 7 de Izúcar de Matamoros, Puebla, México, mediante la esterilización temprana en perros y gatos.***

*Alfredo Mejía Solís.*

Con el objetivo de sostener la ausencia de defunciones por rabia humana, los 23 municipios pertenecientes a esta jurisdicción sanitaria, promueven y coadyuvan en acciones de vigilancia epizootiológica, vacunación antirrábica, estabilización de la población canina y felina; así como con el fomento en sus comunidades sobre la tenencia responsable y bienestar de las mascotas, priorizando la estrategia de la esterilización temprana de perros y gatos.

Dichas acciones se cumplen en coordinación con el programa de prevención y control de la rabia de los servicios de salud del estado, así como con la participación de grupos no gubernamentales e instituciones de gobierno y privadas, procurando brindar una atención integral a la comunidad respecto a esta zoonosis.

En más de 10 años de aplicar la estrategia de esterilización temprana en comunidades de esta jurisdicción, los resultados empiezan a mostrar su impacto en indicadores como: disminución en agresiones de perros a personas, disminución en la indicación de tratamientos antirrábicos humanos, disminución en la cantidad de perros deambulando en la vía pública y lo más importante en mantener la ausencia de casos de rabia en animales y por lo tanto en personas, lo cual eleva la calidad de vida de las personas y sus mascotas.

Por lo anterior se continuará gestionando con las autoridades de los ayuntamientos para que las esterilizaciones se sigan otorgando a la población de manera gratuita y en mayor cantidad, en beneficio de la salud de su población.

# ***Molecular epidemiology of recent rabies “Hot-Spots” in the US.***

*Ashutosh Wadhwa, Yoshinori Nakazawa, William C. Carson, Lauren Greenberg, Rene E Condori-Condori, Cathleen A. Hanlon & Andres Velasco-Villa.*

Rabies is preventable and eradicable from some of its major reservoir hosts. Rabies control in wildlife is primarily addressed with oral rabies vaccination (ORV) along the east coast of the United States. However, intense surveillance is required to monitor emerging epizootics, to detect cross species transmission events, and to assess the impact of ORV in the containment and potential elimination of raccoon rabies. Results of 3 rabies outbreaks from California, Arizona and Alabama are reported here. Brain tissues from 4 California bats, 32 Arizona skunks and 5 Alabama raccoons were studied. Total RNA was extracted and complete N gene sequences were generated. Phylogenetic re-constructions were generated for each independent outbreak by comparing full and partial RV N sequences from GenBank that represented historical and ongoing rabies epizootics in the respective regions studied. The RABV found in California’s *Myotis californicus* bat specimens corresponded to that enzootic in *Tadarida brasiliensis mexicana* bats, which has been either rarely reported in *Myotis* bats or causing massive die-offs. Meanwhile, the RABVs causing the skunk rabies outbreak in AZ samples corresponded to an autochthonous South Central skunk lineage circulating in the region for decades. Potential underlying ecological or demographic reasons that may explain the unexpected increase of rabies cases in skunks should be studied more in depth, since rabies introductions from neighboring states were not detected. Conversely, RABVs found in rabid raccoons beyond the ORV barrier in AL, all corresponded to raccoon variant circulating within AL, GA and FL for decades. These segregated in two sub-lineages, one encompassing RABV from AL only and the other with RABV circulating along a larger geographical range within AL, GA and FL. Evidence of historical long distance translocation of lineages from FL and GA to AL or natural rabies wave dispersal beyond the barrier was observed for both sub-lineages.

# ***Molecular characterization of rabies virus isolated from canids in northern and northeastern region of Brazil.***

*Débora Nunes de Souza, Pedro Carnieli Junior, Carla Isabel Macedo, Rafael de Novaes Oliveira, Helena Beatriz de Carvalho Ruthner Batista, Samira Maria Achkar, & Juliana Galera Castilho.*

In Brazil, with the increase of dogs' vaccination there was a large reduction in the number of cases of rabies in this specie, especially in the South and Southeast regions. However, in the northern and northeastern regions of Brazil still occurring cases of rabies in dogs and along with the expansion of urban area and reduction of native areas, there was a greater reporting of rabies in wild canids. The aim of this study was to make molecular characterization of isolated rabies virus from dogs and wild canids to identify lineages of the virus on the studied regions in the period of 2006 to 2012 and compare with the lineages characterized previously. This study analyzed 34 samples of rabies virus (RABV) from dogs (n=23) and wild canids (n=11) by sequencing of 1318 nucleotides of N gene. The samples were isolated from several states of north and northeastern regions of Brazil. The sequences were analyzed by Neighbor-Joining algorithm and evolutionary model Kimura 2-parameter, and phylogenetic trees were obtained using MEGA 5 program. Phylogenetic analysis revealed the existence of two large clusters previously described: one consisting of dogs and other one with wild canids. Each cluster was subdivided according to geographical origin of the samples. It was determined that there are three different lineages of RABV circulating in wild canids in Pernambuco and there was found two dog infected by RABV lineage of wild canid. In northern region, there was identified the formation of a new phylogenetic clustering among the isolated of the dogs in the states of Pará, Rondônia and Maranhão. Our results show that no great changes in the lineages of RABV circulating in the northeastern were identified after years. In conclusion the molecular epidemiology in constant execution enables the comprehension of the circulation of RABV in distinct geographical regions.



# ***Rabia en San Luis Potosí: 23 años de experiencia. 1991-2013.***

*Raúl Estrada Torres, Marcelo Aranda Álvarez, Juan Carlos Camacho Martínez & José Luis Saldaña Izeta.*

El objetivo del presente es revisar y analizar la información del programa de rabia en el periodo de 1991 al 2013. Como subproductos, esperamos generar estándares de un programa estatal de rabia. Ponderar la importancia de un laboratorio para el diagnóstico de certeza, tipificación de virus con paneles preestablecidos, establecer criterios para toma de decisiones en brotes. Se revisaron las bases de datos en el Estado de los últimos 23 años. El Laboratorio para diagnóstico de rabia cuenta con personal capacitado, aunque la tecnología es limitada, los procesos son de alta calidad, hecho evidente por el control de calidad medido por el InDRE. La información ha sido sistematizada y concentrada en bases de datos, que han evolucionado paulatinamente y que permiten observar un panorama epidemiológico preciso de la evolución de la rabia en el estado. A 23 años de distancia el ordenamiento y sistematización de la información se obtuvo de las mismas fuentes, y se plasma en cuadros y gráficas uniformes que permiten el análisis por años y tipo de variantes y con cifras comparativas. Derivado del fortalecimiento de acciones de control y prevención de esta enfermedad como son concientización de la población en factores de riesgo de contraer rabia, capacitación constante al personal médico, disponibilidad de biológicos de uso humano en cantidad suficiente y oportuna, consolidación de campañas de vacunación antirrábica canina y felina tanto intensivas como permanentes, se ha logrado abatir los casos de rabia humana transmitida por perro desde 1996 y de rabia canina desde 1997. Sin embargo y como consecuencia de una adecuada vigilancia epidemiológica del virus rábico, se determina que en el Estado se presentó un cambio epizootiológico de la enfermedad, destacando el ciclo aéreo (quirópteros). Como lo muestra el caso de rabia humana que se registro en el año 2005 cuyo trasmisor fue un murciélago.

# ***Dog population management in rabies control in India.***

Sira Abdul Rahman.

Rabies is endemic through out India except for the islands of Andaman and Nicobar and Lakshadweep. Data on animal rabies is very scarce and there is no organized system of surveillance to assess the disease burden annually. Molecular epidemiological studies on Indian isolates need to be undertaken.

The dog (97%) is the principal vector followed by cats (2 %) and others such as cattle, sheep, goats, horses, pigs, camels and monkeys. The pet dog population is about 28 million and an equal number are estimated to be stray.

The role, approaches to and effectiveness of rabies control are not well defined. Though it is the mandate of the Government Veterinary Services it is often animal welfare organizations supported by Animal Welfare Board of India, through the Municipal corporations, which are involved in vaccination of dogs as a part of catch neutering release programmes. In some cities dog population has been stabilised by neutering of dogs and vaccination coverage done which has resulted in control of both canine and human rabies. The main obstacle in preventing canine and human rabies in India is the lack of sustainable centralized effort and the fact that rabies by law is not a notifiable disease.

The affliction of domestic animals of economic importance by rabies in rural areas is an important public health concern. People in rural areas are largely ignorant of the disease. The myths and traditional practices further compound this problem. The curriculum in the medical, veterinary and other health sciences colleges does not emphasise the importance of this deadly disease.

An effective and economical tool to combat rabies would be the oral immunization of stray dogs as a complementary to parenteral immunisation. Community empowerment projects for Rabies control have been highly successful.

# ***Toward canine rabies elimination: A health economics assessment of three project sites.***

*Stephanie Shwiff, Aaron Anderson, Louis H. Nel & Brody Hatch.*

Rabies remains a significant problem throughout much of the developing world. An estimated 69,000 people die annually from exposure to rabies. Most of these deaths are the result of being bitten by a rabid dog. Mass vaccination campaigns targeting dogs have been implemented around the world in an attempt to control or eliminate canine rabies. We analyzed the vaccination and cost data for three campaigns in South Africa, Tanzania, and the Philippines. We found the cost per dog vaccinated to range from \$1.18 to over \$10 between these project sites, while the cost of PEP ranged from \$54.87 to \$97.64 for a full series. The cost per life saved ranged from \$427 to \$6,974. We found that as dog vaccination efforts intensify, PEP administration increases. Understanding cost variations across project sites can improve elimination strategy planning for canine rabies. This study suggests possible reasons for such variations across these three sites.

# ***Free-roaming domestic dog population dynamics and rabies vaccination in rural villages near Serengeti national park, Tanzania.***

*Anna M. Czupryna, Lisa J. Faust, Machunde Bigambo & Joel Brown.*

Free-roaming domestic dogs, (*Canis familiaris*), are a public health and conservation concern in Tanzania because they are a reservoir for rabies. In the villages immediately bordering Serengeti National Park, domestic dogs have been vaccinated annually for rabies, canine distemper, and parvovirus since 2003. As a result, rabies incidence has declined in human, dog, and wildlife populations. However, the full impact of the vaccination program on domestic dog population dynamics remains unclear. In 2010, we initiated a four-year longitudinal study to determine the effects of the dog vaccination campaign on domestic dog population dynamics by characterizing and comparing dog demography, welfare, and ownership practices in villages within the current vaccination zone (n=2) and outside the vaccination zone (n=2). We collected demographic data by following the life histories of individually marked and photographed dogs, assessed welfare through body condition scores, and collected ownership practice data with questionnaires for households owning study dogs. Preliminary results of data collected 2010-2012 revealed differences in dog survival between individual villages, but no clear differences in survival between vaccination and non-vaccination zones. Puppies (<6.5months old) had lower survival rates than adults. Adult male dogs had higher survival than females. Body condition scores of adult dogs strongly anticipated survival into the following year. Our data suggest factors other than vaccination may influence dog population dynamics. Understanding dog population ecology in the context of the Serengeti ecosystem will provide vital information for rabies vaccination programs in Tanzania and other areas where domestic dogs are a public health and conservation concern.

# ***La Raj Du Chien: Establishing a surveillance system for canine rabies in Haiti.***

*Ryan M. Wallace, R. Franka, K. Crowdis, S. Salyer, JD Blanton, P. Dilius, A. Destine & M. Millien.*

Background: Over the past decade Haiti has reported one-third of all canine-transmitted human rabies deaths in the Western hemisphere. Although rabies is an important public health concern in Haiti, inadequate surveillance and laboratory capacity has precluded efforts to quantify the burden of rabies in canines and humans. Without laboratory based surveillance and rabies control strategies in Haiti, the Pan American Health Organization goal to eliminate canine-transmitted human rabies by 2015 in the Western Hemisphere, remains elusive. A one-health modeled surveillance system was designed in which animal bites and rabies suspect animals are reported for assessment and diagnosis.

Results: From January 2013 – May 2014, 290 reports of rabies suspect animals were investigated in the West Department, Haiti. A total of 194 animals were assessed for clinical signs of rabies, of which rabies was laboratory confirmed in 46 (24%). An additional 17 (9%) probable cases were identified. Of the 63 confirmed and probable rabid animals, 52 (83%) were dogs, 4 were cats, 3 were goats and 1 was a pig. A total of 278 people reported potential rabies exposures through the surveillance system. Thirty-five persons (12.5%) were confirmed to have been exposed to a rabid animal, 120 (43%) were ruled out for rabies exposure through testing or observation of the animal, and an exposure status could not be confirmed for 123 (44%) people.

Conclusions: Historically, Haiti has reported an average of 5 rabid dogs and 6 human rabies cases annually; a gross underestimate of the true burden of disease. This has resulted in decreased awareness of the problem and a deficient domestic and international response for prevention. Extrapolation of the surveillance findings that 12.5% of reported animal bites were found to be from confirmed rabid animals suggests that more than 25,000 human rabies exposures likely occur each year. In a country where rabies vaccine supply is inconsistent and unevenly distributed, many of these exposures are likely to go unreported and untreated. Further engagement with medical professionals, community members and international organizations will be paramount for the establishment of a successful rabies control program in Haiti.

Rabia transmitida por murciélagos  
Rabies transmitted by bats  
Raiva transmitida por morcegos

## *Qualitative risk model of bovine rabies in Brazil.*

*Elaine Fatima de Sena, Carlos Henrique Pizarro Borges, Ellen Elizabeth Laurindo, Guilherme Basseto Braga, Mariana Figueira.*

In rural areas, rabies has singular importance both for being a drastic and fatal zoonosis, as the high social and economic costs due to the death of thousands of cattle and the indirect costs of herds' vaccination and treatment of people exposed to virus.

In Brazil, herbivorous rabies is endemic, with an annual average of two thousand cases, and the hematophagous bat (HB) *Desmodus rotundus* is the main vector of rural rabies. Since 1966, control measures related to herbivorous rabies were established, currently: epidemiological surveillance; vaccination of domestic herbivores in focus/perifocus areas; registration and monitoring of bat roosts; health education.

Aiming a predictive tool occurrence of rabies in cattle, a qualitative risk model (Braga et al., 2014) was elaborated considering the biology of *Desmodus rotundus* and environmental variables related to rabies transmission by HB in rural areas.

For this risk classification concepts of "Technical Manual for the control of herbivorous rabies" (Brazil, 2009) were used, being:

- Receptivity: variables in maintaining populations of HB in the region;
- Vulnerability: variables in the occurrence of new focus in cattle and the spread of the disease.

This model results in five risk categories: undetermined, negligible, low, medium and high, on maps allowing spatial visualization of risk by municipality. Due to the dynamism of the variables, the model should be updated periodically.

Targeting of control measures for higher risk areas, identified by the model, would significantly reduce the potential for new outbreaks of rabies, which could be in the order of 80% (Dias et al., 2011).

Despite the dependence of data systematically collected by the official veterinary service, the tool is applicable to areas where rabies surveillance is structured. Identifying of areas of attention would optimize applying of staff and financial resources, allowing strategic planning actions.

# ***Rabies studies in *Diphylla ecaudata* colony in periurban area from São Paulo city, Brazil.***

*Rosa AR, Martorelli LFA, Almeida MF, Trezza Netto J, Kataoka, APAG, Uieda W.*

In the world there are a wide variety of bat species involved in the rabies transmission. While in the United States the insectivorous bats have been responsible for the last and rare human rabies cases, in Latin America the hematophagous bat *Desmodus rotundus* is the only species involved in outbreaks of human rabies. *D. rotundus* is the hematophagous species more studied to rabies although there were more two other hematophagous species, the *Diphylla ecaudata* and *Diaemus youngi*. *D. ecaudata* and *D. youngii* have specialized habits, preferring to feed on the blood of birds, so these species do not cause economic damage. In 2011, July, the Zoonosis Control Center from São Paulo City (CCZ/SP), Brazil located the biggest colony of hematophagous bat *D. ecaudata* (68 bats) found in building of periurban area living in cohabitation with another hematophagous bat *D. rotundus* (seven). All bats were captured with entomological net and after collected biological data, part of the *D. ecaudata* was relocated to a preserved area near the place where they were caught. As rabies control measure some animals was sent to rabies diagnosis at CCZ laboratory, including all *D. rotundus* individuals. The bats were submitted to rabies diagnosis by FA (Fluorescent antibody) and MIT (Mouse Inoculation Test) techniques. Blood samples were collected from 24 animals and the sera were analyzed to rabies antibodies presence using the SFIMT (Simplified Fluorescent Inhibition Microtest). None bats were positive for rabies. The prevalence of rabies antibodies was 61% among *D. ecaudata* and 83% among *D. rotundus*, using 0.5IU/ml as a cut-off. The expressive levels of rabies antibodies verified in these bats indicate that rabies virus circulates actively among bats of these species, otherwise, in Brazil, there are only two reports of rabies in *D. ecaudata* and there is no serological rabies studies in this species.

# ***Investigating genetic demography of the common vampire bat at the edge of their range.***

*Antoinette Piaggio, Ignacio Amezcua Osorio, Alejandro Jiménez Ramírez, Jennifer L. Neuwald, Annie E. Tibbels, Amy L. Russell & Luis Lecuona.*

Understanding demography and evolutionary history of a species at the edge of its range can provide insight into factors influencing the distribution of a species. Obtaining information from the genome of a species and applying a Bayesian statistical framework allows inferences about demography and evolutionary history. In a long term study from 2003-2010 we sampled common vampire bats (*Desmodus rotundus*) across part of the north-easternmost portion of their range in the states of San Luis Potosí and Tamaulipas, Mexico. We collected over 600 samples and obtained mitochondrial DNA sequences and nuclear DNA genotypes from 12 microsatellites from each sample. One goal was to test for population expansion as field work suggests vampire bats from this region are being found farther north and at higher elevations than expected. Another goal was to estimate a date for the beginning of population expansion to infer if expansion was potentially due to human and livestock availability or if it pre-dated the appearance of those resources. From the mitochondrial DNA sequences we generated haplotype networks, Bayesian skyline plots, and calculated mismatch distribution, Tajima's D and Fu's FS to infer recent and historical demography. Further we used an approximate Bayesian computation with our total genetic data to test various models of demographic signals and to test whether signals of demographic expansion detected in mitochondrial markers were consistent across the genome. If vampire bat populations are expanding in this region it may mean that the U.S./Mexico border region should be considered in monitoring and surveillance efforts for vampire bats and their associated rabies virus in livestock and wildlife populations.



# ***Is the thermography a possible tool for detecting rabies in vampire bats in field?***

*Méndez-Ojeda ML, Loza-Rubio E, Escartín SLU, López GA, Galindo G, Amezcua MI & González FJ.*

Vampire bats (*Desmodus rotundus*), are the most important transmitters of rabies to cattle. The use of thermography has been proposed as a quick and inexpensive noninvasive method to demonstrate inflammatory processes, nerve dysfunction and even detect infected with influenza. The aim of this study was to conduct a kinetic thermal imaging in *D. rotundus* in captivity, inoculated with rabies virus strain CASS-88. Nine vampire bats were inoculated subcutaneously with 10<sup>6</sup> MLD50%. Two animals served as negative control. Vampires kept in captivity with relative humidity and temperature controlled. These digital images were taken using a FLIR T400 infrared (FLIR Systems) which has 320x240 focal plane array of microbolometer with a range of range of 7.5-13 microns and a temperature sensitivity of 50 mK at 30 ° C. The thermograms were taken on face, ventral and dorsal regions for 30 days, all regions at the same time. The results of the thermal images show that the temperature in the eyes of bats controls averaged 35.44 ± 0.84 ° C during the thirty days of the study. It was determined that temperature decreased according to the rabies evolution in inoculated animals. A decrease of 2.5 ° C was observed at day 13 post- inoculation when the animals became ill or died by themselves. In the ventral and dorsal regions, temperature ranged between 29.8 and 32.2 ° C in infected animals which was not statistically significant compared to controls. The presence of virus in the brain was demonstrated by direct immunofluorescence. We conclude that this tool could help with the identification of animals infected with rabies, although they may also be infected with another pathogen. This study is the first approach for using this tool for further studies related to this subject.

# ***Modeling and mapping the distribution of common vampire bat (*Desmodus rotundus*) in North America, and potential influence of a changing climate.***

*Mark A. Hayes & Antoinette J. Piaggio.*

Common vampire bats (*Desmodus rotundus*) occur throughout much of South America to northern Mexico. These bats feed regularly on the blood of mammals and transmit rabies to livestock, causing substantial economic impacts to agricultural economies. Vampire bats have not yet been documented in the United States, but have been documented within about 50 km of the U.S. state of Texas. This talk describes a collaboration between U.S. Department of Agriculture's National Wildlife Research Center and the U.S. Geological Survey aimed at using species distribution modeling (SDM) to model and map the potential distribution of Vampire bats in North American. We used >5,000 occurrence records of Vampire bats from North America, and analyzed the potential distribution of this species using 5 approaches to species distribution modeling: logistic regression, multivariate adaptive regression splines, boosted regression trees, random forest, and maximum entropy. We compared performance among SDM approaches and used these models to develop maps of potential North American distribution. We then extrapolated these models into future climate scenarios to generate hypotheses about potential future distribution in North America. We discuss these results in light of previous projects aimed at modeling and mapping the distribution of Vampire bats in North and South America, including under future climate scenarios. Some of our SDM models support the hypothesis that suitable habitat for Vampire bats may currently exist in parts of the Mexico-US borderlands, including extreme southern portions of Texas. We view these SDM models and maps as representing hypotheses about current and future Vampire bat distributions, and emphasize that field studies would help assess the validity of these distribution models and maps. Our results also demonstrate how species distribution models and maps can help generate well-justified hypotheses about animal distributions within the context of wildlife disease ecology.

# ***Distribución actualizada del murciélago hematófago en el estado de San Luis Potosí, México.***

*Amezcuca OI, Pérez TE, Rodríguez MC, Climaco FR, Plascencia FI, Valerio SJ & Gómez MM.*

Los desplazamientos de las colonias se dan en pequeños grupos, que se instalan en nuevos lugares adaptándose a las nuevas condiciones de clima, que en el caso de las zonas áridas del estado son extremas, ya que las temperaturas durante el día son de entre 18° C y 36 ° C y por la noche bajan a los 6° C, condiciones a las que en sus habitas anteriores no se tenían y el factor humedad es casi nulo ya que la época de lluvia se ha modificado y es escasa o nula en esta nueva área, sin embargo dentro de los nuevos refugios (cuevas, minas abandonadas, norias o pozos) encuentran las condiciones de temperatura y humedad, similares a las de sus anteriores refugios ya que la humedad es de 80 a 90 % y la temperatura es de 20° C a 35° C, estas condiciones les permiten estar dentro del refugio, sin ser perturbados por los cambios de clima exteriores al refugio, lo que le permite estar adaptándose a esta nueva zona; y en cuanto al alimento este lo encontraran sin ningún problema ya que en el área localizan en su mayoría ganado caprino, equino y en menor cantidad bovino.

El desconocimiento de los productores sobre los murciélagos hematófagos y la rabia parálitica bovina, la cual transmiten al ganado al momento de morder para alimentarse, pone en riesgo a otras poblaciones de murciélagos considerados como benéficos, ya que por la desesperación de ver agredido a sus animales los productores matan a otros murciélagos no hematófagos causando daños ecológicos, el desconocimiento de la rabia parálitica bovina sus síntomas y su prevención, causa una pérdida económica por la muerte de los animales infectados; problema que actualmente se está presentando en el estado y aunado a esto el riesgo de una zoonosis.

# ***Seasonal variation of Lagos bat virus neutralising antibodies in the Egyptian fruit bat, Rousettus aegyptiacus in South Africa.***

*SD McCulloch, JT Paweska, LH Nel, A Coetzer, J Coertse, M Geldenhuys, A Kemp, M Mortlock, TP Scott, N Storm, P Jansen van Vuren, J Weyer & W Markotter.*

Lagos bat virus, a rabies related lyssavirus unique to Africa has been isolated from the Epauletted fruit bat (*Epomophorus wahlbergii*), a mongoose and a dog in South Africa. In other parts of Africa this virus has also been isolated from the Egyptian fruit bat (*Rousettus aegyptiacus*). This fruit bat is widely distributed in Africa including South Africa. However, the true extent of the presence of Lagos bat virus in South African bat populations is unknown. In August 2012 we started sampling *R. aegyptiacus* monthly in the Limpopo province of South Africa. The bats were anaesthetized and blood was collected by cardiac puncture. The bats were then tattooed with a unique sequence onto the propatagium of the wing membrane before release, in order to facilitate the identification of recaptured individuals. Serum was separated by means of centrifugation in serum separator tubes and tested by means of the miniaturized Rapid Fluorescent Focus Inhibition Test (RFFIT). Each sample was screened in triplicate in a 5 fold dilution series from 1:5 to 1:625. The results indicate an annual mean sero-prevalence of ~40 %. This sero-prevalence increases to an excess of 60 % during the birthing pulse - and continues whilst young pups are suckling - yet falls dramatically as maternal antibodies wane and the population reaches its maximum annual size. We also recaptured tattooed bats on several occasions and could indicate seroconversion in individuals that were seronegative during previous capturing. This study not only provides the first sero-surveillance of a South African *R. aegyptiacus* population for Lagos bat virus but also demonstrates the seasonal fluctuation of LBV within this population. This information can assist in identifying high risk period for spillover to other animals and humans.

# ***Determinación del ecotipo de muestras positivas al virus de la rabia mediante secuenciación de las remitidas al Centro Nacional de Servicios de Diagnóstico en Salud Animal correspondientes a enero – agosto de 2014.***

*Robles- Pesina MAG, Ramírez-Torres J, Nieves- García Edwin, Venegas-Cureño Emilio, Ortiz-Rico A, Delgadillo-Álvarez JB & Montaña-Hirose JA.*

El virus de la rabia afecta a varias especies domésticas, silvestres y al humano, siendo la infección de este último un problema de salud pública, los animales portadores pueden agredir a especies domesticas de producción, lo cual produce pérdidas económicas, siendo el más afectado el sector ganadero. Con la finalidad de brindar mayor información sobre los ecotipos presentes en el país para una mejor aplicación de las medidas de control aplicadas en la campaña zoonosanitaria para el control de la rabia paralítica bovina, así como la generación de una base de datos de los virus de la rabia presentes en México y su distribución geográfica se realizo la secuenciación de muestras positivas al virus rábico por la prueba de PCR e inmunofluorescencia obtenidas durante los primeros seis meses del año 2014. Con este fin, las muestras positivas por inmunofluorescencia fueron confirmadas por RT-PCR para la detección del gen de la nucleoproteína empleando los oligonucleótidos JW12 y JW6. Las muestras confirmadas fueron amplificadas con los oligonucleótidos 550 y 304 con los cuales se obtiene un producto aproximado 886 pares de bases, este fragmento corresponde a la posición 647 a 1533, posteriormente, los productos obtenidos fueron secuenciados con el kit BigDye 3.1 en el analizador genético 3130XL. Las secuencias obtenidas fueron editadas y se tomo como referencia la región que va de la base 750 a 1250 para ser comparadas con secuencias reportadas en el banco de datos del NCBI (National Center for Biotechnology Information). Con los datos obtenidos se pudo observar que los ecotipos de los casos positivos a rabia trabajados en el CENASA corresponden principalmente a vampiro y en menor proporción a otras especies silvestres y domesticas, las cuales presentan una distribución geográfica variable.

Rabia en pequeños mamíferos silvestres  
Rabies in small wild carnivores  
Raiva en pequenos mamíferos silvestres

## ***Development of vaccine baits for the small Indian mongoose against rabies: Laboratory and field studies.***

*Steffen Ortmann, Antje Kretzschmar, Ivan Budinski, Ivana Selanec, Ivana Lojkic, & Ad Vos.*

The small Indian mongoose (*Herpestes auropunctatus*) is considered one of the world's most invasive species; not only in terms of predation on endemic endangered species but also a potential transmitter of infectious diseases like rabies. This species has been introduced among others on some islands off the coast of Croatia in the early 20th century. It has also invaded the coastline of the mainland and presently extends its range southwards. Hence, the potential of a rabies spill-over event from foxes, the major rabies reservoir species in this area, to mongooses cannot be ruled out. Oral vaccination of mongooses against rabies has been suggested as the only method capable of controlling the disease in the species. Unfortunately, no suitable bait for this small animal is available. Therefore a specific bait for mongooses in terms of acceptability and vaccine delivery efficiency was developed. Furthermore, bait disappearance and bait uptake by mongooses and non-target species was assessed under field conditions (Island Mljet, Croatia). The results of these experimental and field studies will be presented.

# ***Rabies virus variants that prevail in Guatemala and their relationship with rabies viruses circulating in other parts of Central America.***

*Andres Velasco-Villa, Ashutosh Wadhwa, Rene E Condori-Condori, William C. Carson, Lauren Greenberg, Victor H de Paz, Leticia del Carmen Castillo-Signor, Yolanda Mencos, Luis R Sandoval-Cambara, Luis E Escobar, & Cathleen Hanlon.*

Rabies has been reduced dramatically in Latin America due to concerted control and prevention efforts over the last three decades. Guatemala has reported a significant reduction of rabies cases in dogs and consequently in humans during the last decade. However, current disease surveillance systems in country are not consistent in reporting, which RABV variants are still prevailing in association with recent rabies cases in domestic animals and humans. The genetic characterization of 51 RABV specimens from dogs, livestock and one human pertaining to 13 Guatemalan departments and collected from 2012 to 2014, was conducted to investigate prevalent rabies reservoir hosts, special distribution of rabies foci and potential dissemination patterns with neighboring countries. We found dog and vampire bat-associated rabies virus variants involved with all these cases. Dogs and livestock were affected by both variants with a human case from 2014 confirmed with dog-specific RABV variant. The dog variant segregated in at least three distinctive sub-lineages consistent with geographic location indicating the same number of independent rabies foci. These rabies foci were distinctive from several other RABV sub-lineages associated with dogs across Mexico, Honduras and El Salvador, but close related with a couple of historic sub-lineages reported in Chiapas Mexico. This observation indicates historic dissemination events of dog rabies between Guatemala and Mexico, with unknown net directionality given the reduced number of sample analyzed in this study. As for the vampire bat-associated RABV, these also segregated in at least three sub-lineages with a less clear geographic compartmentalization pattern. Two sub-lineages were found circulating in Guatemala only, and the other shared similarity with RABV circulating in vampire bats of Chiapas Mexico and El Salvador. This study stressed the importance of conducting laboratory based surveillance to support and assess efforts for rabies control and elimination.

# ***Use of RFFIT for anti-rabies antibodies detection in Capuchin monkeys (Cebus apella) and coatis (Nasua nasua) in a Brazilian native rainforest.***

*Danielle Bastos Araujo, Luzia de Fátima Alves Martorelli, Ana Paula Kataoka, Luiz Francisco Sanfilippo, Edison Luis Durigon, Silvana Regina Favoretto.*

Although the number of rabies cases in domestic animals (dogs and cats) is decreasing, wildlife rabies remains an important issue in Brazil. Four independent rabies virus variants, directly responsible for reported human cases, have been identified in Brazil. One in dogs (*Canis lupus familiaris*) and three in wild species, namely: common vampire bat (*Desmodus rotundus*), marmosets (*Callithrix jacchus*) and crab-eating foxes (*Cerdocyon thous*). The aim of this work was the detection of anti-rabies specific antibodies (RVNAs) in capuchin monkeys (*Cebus apella*) and coatis (*Nasua nasua*) from a native rainforest area in Sao Paulo State, Southeast of Brazil. Sera samples were submitted to the rapid fluorescent focus inhibition test (RFFIT). Of 15 capuchin monkey samples analysed, one – 7,0% - was positive (Titer 0,33 IU/mL); and the two coati samples presented positive results (Titers 0,20 IU/mL and 0,12 IU/mL). Primates are the third highest animal order that transmits rabies to the humans in Brazil. Capuchin monkeys were diagnosed with rabies and the increasing presence of these animals in urban areas associated with their great capacity for adaptation demonstrate the importance of constant surveillance in all non-human primate species. Coatis present omnivorous feeding habits and are considered curious animals with an easy socialization with humans. These animals, and other species from the Procyonidae family, were diagnosed with rabies in Brazil. The detection of RVNAs in this study suggests that the rabies virus circulates between these species in the area. This finding, the possibility of transmission of rabies virus among different animal species and the increasing human interventions in native forest areas demonstrate the importance of studies regarding the role of wild species in the circulation and transmission of rabies virus; especially when considering the great and unique variety of wildlife in Brazil.



# ***A recent epizootic of skunk rabies and associated spillover in Northern Colorado.***

*Amy Gilbert, Tara Rigg, Samantha Eaton, Justin Fischer, Dennis Kohler, Terry Spraker, Richard Bowen, Karen Fox, & Kurt Vercauteren.*

Rabies is a fatal viral zoonosis transmitted by bite contact with an infected animal. In the US, human deaths are rare, but each year over 5000 rabid animals are reported and over 90% of cases are in wildlife. Carnivores and bats are the primary sylvatic reservoirs in the US, and several variants of rabies circulate independently among these two taxonomic orders. Rabies prevention efforts led by USDA Wildlife Services using live recombinant vaccine baiting began in 1995 and primarily target raccoons and canids, but the territory of the south-central skunk rabies virus variant has been expanding in recent years, leading to incursions into areas previously free of carnivore rabies. A limited number of skunk rabies cases were detected in Colorado between 1990 and 2006. Three probable south-central skunk variant cases were detected in 2007, and by 2012 the number of rabid skunks exceeded rabid bats in Colorado. Larimer County and Weld County, in Northern Colorado, experienced severe skunk rabies epizootics in 2012 and 2013, with Larimer County reporting 35 rabid skunks in both years. Spillover events into raccoons, red foxes, domestic animals and livestock occurred at a rate of 4% of non-bat submissions during 2012 and 2013 in both counties, indicating limited involvement of other species in circulation of the virus. However, rabies virus was isolated from 60% of 40 rabid skunk salivary glands tested and from 60% of ten rabid spillover host salivary glands, including one raccoon, two red foxes and one domestic cat, suggesting the possibility of secondary transmission of south-central skunk variant from carnivore spillover hosts. Age profiles of rabid skunks indicate broad age-class involvement in the seasonality of cases. Case incidence and environmental data are being used for predictive modeling to understand the spread of this skunk rabies epizootic along the Front Range of Colorado.

# ***Genetic population structure of two rabies hosts in Alaska.***

*Karsten Hueffer, Elizabeth W. Goldsmith, Elizabeth Himschoot, Christopher J. Clements, Ben Renshaw, & Kris J. Hundermark.*

Rabies is absent from Interior Alaska but occurs across the range of its reservoir host, Arctic fox (*Vulpes lagopus*), in northern and western Alaska. The virus comprises three distinct genetic strains, one of which is endemic to Alaska, that are segregated geographically. This is unusual because there is no evidence for population structure in Arctic foxes over small geographic scales, and structure would not be expected due to habits of the species to wander long distances across sea ice in search of food. Moreover, there is evidence that Arctic foxes are being displaced by red foxes (*Vulpes vulpes*), a spillover host for rabies, across their range in Alaska, likely due to climate-change induced range expansion. We describe genetic population structure of these two species to gain insight into the geographic segregation of rabies strains and potential for red fox to spread rabies to Interior Alaska. Mitochondrial DNA and standard assignment tests of microsatellite variation assign Arctic foxes to two groups, North Slope and western Alaska. However, a method that accounts for spatial genetic variation of microsatellites divides foxes into 3 populations: North Slope, Seward Peninsula, and southwestern Alaska, which matches the distribution of the three genetic strains of rabies. This is the first evidence of fine-scale population structure in Arctic foxes. Red foxes show a higher degree of structure in mitochondrial DNA but not in microsatellites. We hypothesize that lack of movement of infected Arctic foxes among populations serves to keep rabies strains geographically restricted and represents a long-term equilibrium. Replacement of Arctic foxes by red foxes throughout their mainland range in Alaska, however, may change host-pathogen dynamics and potentially could change the geographic distribution of the virus.

# **Urban ecology of striped skunks in Flagstaff, Arizona, USA, and implications for skunk-to-skunk and bat-to-skunk rabies transmission.**

*Tad Theimer, Jesse Maestas, Anthony Clayton, Alexa Martinez, & David Bergman.*

We placed proximity-sensing radio collars on 50 striped skunks in Flagstaff, Arizona, USA to determine social contacts between October and February, 2012-2013. Rates of contact were highest in fall and spring, but we also documented potentially important movements of >1km during winter that linked at least 14 skunks in a social network. Juvenile females had the greatest number of contacts in fall and mature males in the spring. In 2012 and 2014, we used video surveillance to document skunk use of bird seed spilled below bird feeders and then added cat food to quantify how visitation and behavior changed in the presence of these two important anthropogenic food sources. Skunks used 18 of 20 feeders monitored across both years. Number of visits, number of skunks and number of aggressive interactions all increased in the presence of cat food. Finally, we investigated the potential for rabies to pass from bats to skunks via scavenging of dead bats by placing dead, rabies-negative *Tadarida brasiliensis* at 51 locations around Flagstaff. Mesocarnivores approached bats 53 times (raccoons (5), cats (14), skunks (27), gray fox (6), coyote (1)) and chewed or removed them in 28 (25 striped skunks, 2 raccoons and 1 cat). These data suggest that 1) rabies could be transferred from bats to skunks via scavenging, 2) once contracted, social contacts at bird feeders or at sites where cat food is available could be foci for rabies transmission among skunks and 3) TVR programs for skunks, at least in the Flagstaff area, could focus on periods before increased seasonal contacts (early fall and winter) and target juvenile females in the fall and mature males in the spring.

# ***Environmental niche of rabies virus distribution in a changing Alaska.***

*Emily Magnuson, Falk Huettmann, & Karsten Hueffer.*

Many rural communities across the state of Alaska do not have access to adequate veterinary care and may not get basic immunizations for their domestic animals. Additionally, due to remote location and frequency of subsistence hunting, many people also live and work in close proximity to wildlife. Knowing the distribution pattern of rabies in Alaska and how it may change in the future is important for human health. The virus is continuously maintained among wildlife in northern and western Alaska in endemic areas, while in the central and southern regions, rabies only occurs in temporally separated outbreaks. Little is known about the drivers of this distribution pattern, other than anecdotal evidence. In this study we used GIS ArcMAP to model environmental and anthropogenic predictors (such as elevation (m), temperature (°C), precipitation (mm) and distance to human infrastructure (km)) in order to analyze their correlation with rabies distribution in Alaska. Because foxes are the primary vector of the rabies virus in Alaska, the habitat ranges of red fox (*Vulpes vulpes*) and Arctic fox (*Vulpes lagopus*) were additionally included as environmental predictors. Our analysis shows the most important drivers of rabies distribution are low elevation, low distance to coastlines, and low distance to human infrastructure. These predictors likely reflect the mostly coastal distribution of villages, hunting camps, or roads in rural Alaska where rabid animals would be more easily seen. Secondly, locations with high temperatures and low precipitation compared to regular averages in March and April and those within Arctic fox habitat ranges were more likely to have rabies cases reported. Additionally, we also investigated how climate change will effect rabies distribution based on these different environmental driving factors of the rabies distribution.

# ***Informe de un caso positivo a rabia proveniente de un puma (Puma concolor) identificado con la variante 7 – zorro de Arizona y su seguimiento epidemiológico.***

*Orduña Sumarán José Alberto, Gutiérrez Reyes José Alfredo, Aréchiga Ceballos Nidia, Padilla Medina Irma, Iguala Vidales Miguel, Melo Munguía Martín, Chavez Crisostomo Diana, Rodríguez Mendoza Mario Arturo.*

En 11 y 12 marzo del año 2014 se presentó una agresión por un puma (*Puma concolor*), el cual agredió primero a un equino del predio Lienzo charro, y posteriormente a un equino y dos bovinos del predio Rancho Santa Delfina todos localizados en el municipio de Caborca Sonora, en esas mismas fechas este animal también agredió a 3 personas, en total estuvieron en contacto 43 persona y 32 animales (28 caninos 2 equinos y 2 bovinos), se remitieron muestras del puma y de 4 caninos, al laboratorio estatal de salud pública de Hermosillo Sonora donde por medio de la técnica de inmunofluorescencia directa se obtuvo resultado positivo al puma y negativo a los 4 encéfalos de canino, la muestra se mandó al Indre en donde se realizó la confirmación del resultado y se llevó a cabo la identificación de la variante génica obteniendo como resultado V7- Zorro de Arizona, a esta muestra además se realizaron pruebas de reacción en cadena de la polimerasa (PCR) y secuenciación de ácidos nucleicos, confirmado el resultado obtenido y el árbol filogenético. Todos las personas y animales que estuvieron en exposición fueron tratados de acuerdo a los protocolos de inmunización ya establecidos, se establecieron las zonas perifocales en donde se presentaron las agresiones, en total se vacunaron a otros 260 animales. A principios del mes de mayo se observaron cambios en el comportamiento del caballo proveniente del Rancho Santa Delfina y el 14 mayo de 2014, se remitió el encéfalo al laboratorio estatal de salud pública de Hermosillo Sonora donde por medio de la técnica de inmunofluorescencia directa se confirmó como positivo, después de todas las actividades de profilaxis y tratamiento hasta el 30 de agosto de 2014 no se han presentado más casos.

## ***Ferret-badger rabies in Taiwan.***

*WC Hsu, SH Lee, YC Tu, MS Lee, KR Tsai, JC Chang, SC Hu, WC Li, LJ Ting, YL Lin & HJ Tsai.*

Taiwan has been considered a rabies-free country since 1961. However, three Formosan ferret-badger (*Melogale moschata subaurantiaca*) rabies cases were identified in July, 2013. Following the first case, authorities of veterinary services intensified surveillance on dogs, cats, bats, ferret-badgers and other wildlife. According to the surveillance data at present, the disease has been still contained among the wild ferret-badgers (390 positive cases) in the mountain regions, except a house shrew (*Suncus murinus*) and a puppy bitten by a rabies-infected ferret-badger. Phylogenetic analysis of rabies virus isolates and retrospective study using frozen ferret-badgers samples have proved that this rabies strain was not a newly invading strain. Instead, it has already existed among wild ferret-badgers population in Taiwan far earlier than the first found case in July, 2013. Phylogenetic tree based on nucleoprotein of Taiwan rabies virus showed that these viruses were clearly divided into two subgroups namely: (1) the Middle and Southern group (TW-MS): Nantou, Taichung, Yunlin, Chiayi, Tainan, Kaohsiung; and (2) the Eastern group (TW-E): Hualien, Taitung, Pingtung. In pathology study of 48 rabid ferret-badgers, the lesion severity defined by histopathological examination demonstrated that 38% (18/48) showed none to mild, 31% (15/48) moderate, and 31% (15/48) severe nonsuppurative meningoencephalitis. Examined by immunohistochemistry, the viral antigens distribute most prominently in the brainstem, followed by the hippocampus, cerebrum, and cerebellum. All the examined cases present intracytoplasmic Negri bodies in neuron and viral antigen in the salivary glands. Our study revealed that the ferret-badger-associated rabies virus in Taiwan has already evolved into a distinct strain. Ferret-badger rabies is characterized by variable degrees of viral encephalitis and all the cases could shed rabies virus via saliva. The timeline of existence, possible invasive route and pathogenicity to animals are still needed to be further studied.

# ***Rabies epizootics in the Ethiopian wolf (*Canis simensis*).***

*Nicholas Johnson, Chris Gordon, Claudio Sillero-Zubiri & Anthony R. Fooks.*

Introduction of infectious diseases such as rabies and canine distemper virus can lead to dramatic population reduction and threaten the long-term survival of rare canid species. Infectious diseases have played a devastating role in the decline in populations of the African wild dog (*Lycaon pictus*) across its natural range. The Ethiopian wolf (*Canis simensis*) is one of the rarest canid species in the world and is restricted to small populations in the Ethiopian highlands. As a result, intense conservation effort is applied to protect the species and intervene following events such as disease outbreak. There have been three well documented outbreaks of rabies within the Ethiopian wolf populations within the Bale Mountains National Park in 1991/2, 2003/4 and 2008/9. A recent detection of rabies in an Ethiopian wolf carcass suggests that a fourth outbreak has occurred in 2014. Phylogenetic analysis of the viruses detected in each of these epizootics indicates that the source is likely to be from a local reservoir, probably domestic dogs, which are present around the national park. A range of intervention strategies have been applied including trap-vaccinate release of Ethiopian wolves, parenteral vaccination of the local dog population and oral vaccination targeting canids within known wolf habitats. This study reviews previous rabies outbreaks within wolf populations of the Bale Mountains and considers the impact of the current case and options for disease control.

# ***Raccoon (Procyon lotor) habitat suitability and rabies spread risk: Information-theoretic, maximum entropy, and circuit theory modeling.***

*Timothy P. Algeo, Michael W. Palace, Dennis Slate, Rosemary M. Caron, Todd Atwood, Sergio Recuenco, Richard B. Chipman & Mark J. Ducey.*

The USDA, APHIS, Wildlife Services National Rabies Management Program (NRMP) has led a cooperative oral rabies vaccination (ORV) campaign to prevent the spread of the raccoon (*Procyon lotor*) variant of the rabies virus into mid-western states and Canada since 1997, with the ultimate goal of elimination. Given the overlap of the enzootic range of raccoon rabies with the heavily populated eastern United States, ORV decisions by the NRMP and cooperators have potentially profound implications in terms of public health. NRMP raccoon density index (RDI) information is critical to ORV planning and RDI for pine- (*Pinus* sp.) dominated landscapes have been among the lowest observed. An Information-theoretic model selection process was used to gain an understanding of the factors that likely influence raccoon density on eastern pine-dominated landscapes through the Akaike Information Criteria procedure for small sample sizes (AICc). Maximum Entropy (Maxent) and circuit-theory (Circuitscape) modeling provided habitat suitability assessments and risk visualization tools for an increased understanding of raccoon habitat use and to assist in delineating potential habitat corridors for raccoon rabies spread. RDI assessments (15 from 11 pine-dominated sites in 6 eastern states) made during 2007-2012 produced RDI ranging from <1-3.3 raccoons/km<sup>2</sup>. Of seven candidate models comprised of landscape and other variables, the model that included only the habitat edge variable (Akaike  $w_i = 0.58$ ) ranked the best. Influential variables in terms of raccoon habitat suitability from the Maxent procedure were annual mean precipitation (Massachusetts) annual mean temperature (Florida) and soil type (Alabama). Maxent probability surface maps ingested into Circuitscape provided conductance visualizations which identified potential areas of connectivity for raccoons, and hence, risk for the movement of rabies. Incorporating these results into ORV planning should result in substantial cost-savings, and suggests potential applications to other rabies variants, and management programs.



# ***Spatial patterns of neutral and functional genetic variations reveal patterns of local adaptation in raccoon (*Procyon lotor*) populations exposed to raccoon rabies.***

*Kyle CJ, Rico Y, Castillo S, Srithayakumar V, Cullingham CI, White BN & Pond BA.*

Local adaptation is necessary for population survival and depends on the interplay between responses to selective forces and demographic processes that introduce or retain adaptive and maladaptive attributes. Host-parasite systems are dynamic, varying in space and time, where both host and parasites must adapt to their ever-changing environment in order to survive. We investigated patterns of local adaptation in raccoon populations with varying temporal exposure to the raccoon rabies virus (RRV). RRV infects a large proportion of the population when epizootic and has been presumed to be completely lethal once contracted; however, disease challenge experiments and varying spatial patterns of RRV spread suggest some level of immunity may exist. We first assessed patterns of local adaptation in raccoon populations along the eastern seaboard of North America by contrasting spatial patterns of neutral (microsatellite loci) and functional (major histocompatibility complex (MHC)) genetic diversity and structure. We explored variation of MHC allele frequencies in the light of temporal population exposure to RRV (~0-60years) and specific RRV strains in infected raccoons. Our results revealed high levels of MHC variation and pronounced genetic structure relative to neutral microsatellite loci, indicative of local adaptation. We found a positive association linking MHC genetic diversity and temporal RRV exposure, but no association with susceptibility and resistance to RRV strains. These results have implications for landscape epidemiology studies seeking to predict the spread of RRV and present an example of how population demographics influence the degree to which populations adapt to local selective pressures.

# Estado actual de la investigación en rabia

## Current status of rabies research

### Raiva en pequenos mamíferos silvestres

Patogénesis  
Pathogenesis  
Patogenia

## *Mitochondrial dysfunction in rabies virus infection and implications for new therapeutic approaches to rabies.*

*Alan C. Jackson, Wafa Kammouni, Heidi Wood, Ali Saleh, Camila M. Appolinario, & Paul Fernyhough.*

Our previous studies in an experimental model of rabies showed neuronal process (dendrites and axons) degeneration in association with severe clinical disease. Cultured adult rat dorsal root ganglion neurons infected with the challenge virus standard-11 strain of rabies virus (RABV) showed axonal swellings and reduced axonal growth with evidence of oxidative stress. We have shown that CVS infection alters a variety of mitochondrial parameters and increases reactive oxygen species (ROS) production and mitochondrial Complex I activity. We have hypothesized that a RABV protein targets mitochondria and triggers dysfunction. Mitochondrial extracts of mouse neuroblastoma (MNA) cells analyzed with a proteomics approach showed that extracts were highly enriched with the RABV phosphoprotein (P). P was also detected by immunoblotting in RABV-infected purified mitochondrial extracts and in Complex I immunoprecipitates from the extracts. A plasmid expressing P in cells increased Complex I activity and increased ROS generation, whereas expression of other RABV proteins did not. Expression of a peptide from amino acid 139-172 of the P increased Complex I activity and ROS generation similar to expression of the entire P protein, whereas peptides that did not contain this region did not. These results indicate that a region of the RABV P interacts with Complex I in mitochondria causing mitochondrial dysfunction, increased generation of ROS, and oxidative stress. Hence, the RABV P plays a key role in the induction of mitochondrial dysfunction and generation of ROS resulting in oxidative stress in rabies virus infection through a direct interaction with mitochondrial Complex I. The resulting mitochondrial dysfunction produces oxidative stress in neurons that causes acute degenerative changes affecting neuronal processes and plays an important role in producing severe clinical disease with a fatal outcome. Combination therapy for rabies should include therapy directed at reducing mitochondrial dysfunction, antiviral therapy, and immunotherapy.

# ***Natural and experimental infection of big brown bats with rabies virus.***

*James Ellison, Steven Taylor, Shylo Johnson, William Carson, Lauren Greenberg, Richard Franka & Amy Gilbert.*

Big brown bats (*Eptesicus fuscus*) are a natural reservoir of rabies virus in North America. They are a peridomestic species and can roost in dense aggregations, but populations can vary in genetic structure and ecology across their geographic range. This species has also been implicated in spillover events of rabies virus into wild carnivores in the United States, notably in Flagstaff, Arizona. Outbreaks of natural infection have been shown to occasionally occur within captive colonies of this species. Prior captive experimental rabies infections have demonstrated that big brown bats can develop immunity to repeated rabies virus infection. In this experiment, bats were taken into captivity and housed in two cages. Two cases of natural rabies infection were observed in one cage during the quarantine period, separated in time by 76 days. Seroconversion of cagemates was observed during the outbreak, suggesting nonlethal exposure of bats to rabies virus within a cage. However, the colony was observed for over one year without any additional rabies cases. During the outbreak, rabies virus was isolated from the salivary glands of one rabid bat, with a titer of 103.8 TCID<sub>50</sub>/ml. The surviving colony was experimentally challenged with the salivary gland isolate 509 days after the last case of natural rabies infection in the colony. The mean incubation period of bats developing rabies from experimental infection was 25 days (range: 18-40 days). Seropositive bats were more resistant to experimental infection compared to bats that were seronegative throughout the captive period (17% versus 73% mortality, respectively). Infrared thermographic images were also taken of bats prior to and daily until day 60 following experimental infection, and changes in facial temperatures correlated with clinical outcomes of individual bats will be discussed.

# ***Altered in situ expression of claudin-5 tight-junction protein in human rabies cases transmitted by vampires bats and dogs.***

*Fernanda Guedes, Maria Irma Seixas Duarte, & Elaine Raniero Fernandes.*

Claudins are proteins and presents in brain, which have an essential role in the formation and capacity of tight junctions in the blood-brain barrier (BBB) integrity. The objectives of the study were to demonstrate the claudin-5 expression in central nervous system (CNS) lesions of the human rabies transmitted by dogs and vampires bats and compare it to a control group of normal brain. Two fragments of central nervous system were selected (cerebellum and brainstem) of each one of the groups three: human rabies cases transmitted by dogs (2), vampires bats (2) and normal brain (2). We performed a quantitative immunohistochemical study with antibody for claudin-5. For each specimen, vessels with expression for claudin-5 were quantified in forty fields. For normalizing, a x10 ocular lens was used with a square grid in a x40 objective marking an area field of 0.0625 mm<sup>2</sup>. Statistical analysis was performed by Graph Pad Prism version 6.0 using the nonparametric Kruskal-Wallis test. Samples were considering different at the 95% ( $p \leq 0.05$ ) level of significance. The number of claudin-5-positive vessels in human rabies transmitted by vampires bats was significantly lower ( $p=0.0010$ ) compared with control group. No significant difference in expression of the claudin-5 in vessels was observed between the human rabies cases transmitted by dogs and bats. The expression of claudin-5 was less on rabies transmitted by dogs compared with control group, although no significant statistic difference. Therefore, the lower expression of claudin-5 in human rabies cases, particularly for transmitted vampires bats, may represent one of the changes linked to the pathogenesis probably by interferes with the permeability of the BBB. However, further studies are necessary to investigative the role of pro-inflammatory cytokines (IL-1-beta, TNF-alpha and IL-17) in enhancement of BBB permeability during the course of infection in the CNS.

# ***Pathogenesis of homologous rabies virus in hematophagous bats (*Desmodus rotundus*).***

Méndez-Ojeda ML, Loza-Rubio E, Rojas-Anaya E, Suzán AG, Medellín MR, Rupprecht CE.

Vampire bat attacks on cattle are a major concern in cattle raising areas. Blood loss and paralytic rabies due to bat bites can cause severe losses in this livestock industry. Likewise, across Latin America human rabies cases transmitted by these reservoirs have increased. Nevertheless, there have been few studies carried out in hematophagous bats (*D. rotundus*), and therefore transmission and maintenance of the rabies virus in natural and captive populations remains poorly understood, thus the objective was to elucidate the pathogenesis of the homologous rabies virus in this species while captive. Twenty two adults bats was seronegative to rabies by RFFIT and then were inoculated intramuscularly in gluteus with 10<sup>6</sup> LD<sub>50</sub>% homologous virus (CASS-88). The negative control group was formed by 11 animals, that were inoculated with an non-infected mouse brain supernatant. Animals died of rabies at 8, 9, 10, 11, 13, 14, 15, 17 and 19 days post-inoculation (pi). Moreover, at the days 4, 12 and 20 the bats were sacrificed to identify rabies genome, as the established calendar. The bats were bled in order to detect neutralizing antibodies by RFFIT at the same days. The virus was detected in brain, salivary gland and tongue tissue by RT-PCR and nested RT-PCR. It was also found in organs not related to the nervous system such as lung, liver, stomach, spleen and kidney. The virus was not detected in saliva, perhaps because the antibodies neutralized the virus. Furthermore, the humoral response was intermittent. Nevertheless, it is necessary to continue other research studies to help elucidate the role that the immunity in this species. Our study contributes to the knowledge on rabies pathogenesis in the main transmitter of the virus in Latin America, since it is one of the few studies carried out on *D. rotundus*.

# ***Immune control of rabies virus spread through the spinal cord.***

*D. Craig Hooper, Darryll A. Barkhouse, Carla Portocarrero, Jianwei Li, & Rhonda B. Kean.*

Rabies post-exposure prophylaxis (PEP) with virus neutralizing antibody (VNA) and vaccination is highly effective in the first days after virus entry but fails when the infection spreads to central nervous system (CNS) and clinical signs of rabies appear. This is at least in part due to the ability of wild-type RABV to evade immune clearance from brain tissues, a feature that is not shared by laboratory attenuated RABV. While it is clear that wild-type RABV are vulnerable to immune mechanisms in the periphery, the juncture in their trans-axonal passage to the CNS that immune control initially fails is unknown. To resolve this issue we have comparatively assessed virus replication and the appearance of different aspects of innate and adaptive immunity as wild-type and attenuated RABV spread from the periphery through the spinal cord to the brain. To assess the importance of the timely induction of these processes, we performed parallel studies in normal and mice lacking TLR7, known to exhibit a delay in the development of RABV-specific immunity. We conclude that anti-viral immune mechanisms acting in spinal cord tissues can interfere with the spread of neurotropic virus to the CNS. As confirmed by RABV engineered to express interferon-gamma (IFN $\gamma$ ), the rapid onset of IFN $\gamma$  production in neural tissues is critical in controlling RABV replication, at least partly through the induction of high levels of type I interferons. Clearance is associated with the production of antibody in spinal cord tissues by infiltrating cells. The rapid induction of both aspects of the immune response is dependent upon the recognition by TLR7 of virus in lymph nodes draining the site of infection. Infection with wild-type RABV fails to rapidly induce TLR7-dependent responses in the draining lymph node as well as immune effector infiltration into the spinal cord and brain.

# ***BBB alteration is required for intravenously administered VNA to clear an established rabies virus infection from the CNS and prevent the development of rabies in mice.***

*Chien-Tsun Huang, Zhenguang Li, Ying Huang, Guoqing Zhang, Ming Zhou, Qingqing Chai, Hua Wu, & Zhen F. Fu.*

Rabies virus (RABV) is a neurotropic virus that causes fatal disease in humans and animals. Currently there is no cure for rabies once clinical signs appear. It is believed that once RABV enters the CNS, virus neutralizing antibodies (VNAs) in the periphery cannot cross BBB and into the CNS. Furthermore, it has also been hypothesized that VNAs produced in the CNS by invading B cells, rather than those produced in the periphery and then transported into the CNS, are important in clearing RABV from the CNS. In the present study, mouse serum containing VNA was administered intravenously into mice after infection with wild-type RABV. Mice were infected with wt RABV and five days later treated with VNA by the tail vein. At the same time, the BBB permeability was enhanced by giving MCP-1 by ic. Our studies demonstrate that intravenous administration of VNAs is capable of clearing RABV from the CNS and preventing the development of rabies in both immunocompetent and immunocompromised mice as long as the BBB permeability remains enhanced. This present study therefore provides a foundation for the possibility of developing VNA therapy for clinical rabies in humans.

## ***Comparison of biotinylated monoclonal and polyclonal antibodies in an evaluation of a direct rapid immunohistochemical test for the routine diagnosis of rabies in Southern Africa.***

*André Coetzer, Claude T Sabeta, Wanda Markotter, Charles E Rupprecht & Louis H Nel.*

The major etiological agent of rabies, rabies virus (RABV), accounts for tens of thousands of human deaths per annum. The majority of these deaths are associated with rabies cycles in dogs in resource-limited countries of Africa and Asia. Although routine rabies diagnosis plays an integral role in disease surveillance and management, the application of the currently recommended direct fluorescent antibody (DFA) test in countries on the African and Asian continents remains quite limited. A recently developed novel diagnostic assay, the direct rapid immunohistochemical test (dRIT), has been reported to have a diagnostic sensitivity and specificity equal to that of the DFA test while offering advantages in cost, time and interpretation. Prior studies using the dRIT utilized monoclonal antibody (MAb) cocktails. The objective of this study was to test the hypothesis that a biotinylated polyclonal antibody (PAb) preparation, applied in the dRIT protocol, would yield equal or improved results compared to the use of dRIT with MAbs. We also wanted to compare the PAb dRIT with the DFA test, utilizing the same PAb preparation with a fluorescent label. The PAb dRIT had a diagnostic sensitivity and specificity of 100%, which was shown to be marginally higher than the diagnostic efficacy observed for the PAb DFA test. The classical dRIT, relying on two-biotinylated MAbs, was applied to the same panel of samples and a reduced diagnostic sensitivity (83,50% and 90,78% respectively) was observed. Antigenic typing of the false negative samples indicated all of these to be mongoose RABV variants. Our results provided evidence that a dRIT with alternative antibody preparations, conjugated to a biotin moiety, has a diagnostic efficacy equal to that of a DFA relying on the same antibody and that the antibody preparation should be optimized for virus variants specific to the area where the test is implemented.



# ***PCR anidado para la detección del virus rábico en saliva.***

*Huesca SAD, Loza-Rubio E, Méndez OML & Rojas-Anaya E.*

La rabia se manifiesta de forma furiosa o paralítica y es una zoonosis mortal. Para el diagnóstico se realiza de manera post-mortem la prueba estándar que es la Inmunofluorescencia Directa (IFD). Por lo que es conveniente implementar metodologías sensibles y rápidas que detecten al genoma del virus “in vivo”, para de esta manera contar con alternativas diagnósticas. El objetivo de este estudio fue estandarizar una RT-PCR-anidada (PCRa), para detectar al genoma del virus de la rabia en saliva de humano y así aumentar la sensibilidad en la detección del virus. Para implementar la PCRa, se realizaron diluciones decuples (10-1 a 10-6) en saliva de humano sano, se hicieron tres réplicas utilizando el mismo origen de la saliva agregando virus de rabia proveniente de murciélago-hematófago (CASS-88). A cada dilución se le extrajo el ARN, se sintetizó el ADN complementario (ADNc) usando el kit M-MVL, se procedió a realizar la RT-PCR utilizando los iniciadores previamente notificados (Loza-Rubio et al., 2005) y posteriormente se llevó a cabo la estandarización de la PCRa utilizando los iniciadores For 2: (+): 5'GCCGCRATGCAGTTGTTTGA 3', Rev 2: (-): 5'ACAGTRGGGTCCCTTGTC A 3' utilizando las mismas condiciones que en el PCR reportado por Loza-Rubio (2005). Al analizar el control positivo (virus sin diluir) y cada una de las diluciones, se obtuvo la amplificación de un fragmento de 761 pb para la RT-PCR en el control positivo, así como en la dilución 10-1; mientras que para la PCR-a, se detectó un amplicón de 100 pb además del virus concentrado, en los productos obtenidos de las diluciones 10-1, 10-2 y 10-3. Se concluye que la PCRa estandarizada resultó más sensible, lo que podría ser útil para realizar la detección del genoma del virus de la rabia en saliva tanto de forma confirmatoria, como ante mortem.

# ***Cross-platform evaluation of commercial real-time SYBR Green RT-PCR kits for sensitive and rapid detection of rabies virus.***

*Evelyne Picard-Meyer, Carine Peytavin de Garam, Jean Luc Schereffer, Clotilde Marchal, Emmanuelle Robardet & Florence Cliquet.*

We evaluate the performance of five two-step SYBR Green RT-qPCR kits and five one-step SYBR Green qRT-PCR kits using real-time PCR assays. Two real-time thermocyclers showing different throughput capacities were used. We found that the optimised one-step PCR assays had a higher detection sensitivity than the optimised two-step assays regardless of the machine used, while no difference was detected in reaction efficiency, R2 values, and intra- and inter-reproducibility between the two methods. The limit of detection at the 95% confidence level varied between 15 to 981 copies/ $\mu$ L and 41 to 171 for one-step kits and two-step kits, respectively. The study demonstrated the pivotal influence of the thermocycler on PCR performance for the detection of rabies RNA, as well as that of the master mixes. PCR protocols should be systematically optimised and validated for different instruments following the recommendations of the World Organization for Animal Health

## ***Panel ampliado de 20 anticuerpos monoclonales anti-nucleocápside como herramienta en la diferenciación de las variantes antigénicas atípicas del virus de la rabia dentro del territorio mexicano.***

*Mauricio Gómez, David-Martínez, Irma Leticia-Padilla, Susana-Chavez, Martin-Melo, Nidia-Arechiga, Albert-Sandoval, Israel Animas, Alfredo-Gudiño, Beatriz Escamilla, Miguel Iguale, Charles E Rupprecht, Liliian A Orciari, Andrés Velasco.*

En 1994 científicos latinoamericanos y bajo la coordinación de Jean Smith desarrollan un panel simplificado de 20 anticuerpos monoclonales, el cual identifica 11 variantes del virus de la rabia asociadas a los reservorios de la enfermedad con mayor impacto epidemiológico en América Latina. En el 2002 por primera vez se describe la diversidad antigénica del virus de la rabia en el territorio mexicano usando un panel reducido de 8 anticuerpos monoclonales que permitió distinguir variantes del virus asociadas de forma específica a: zorros grises *Urocyon cinereoargenteus* que mantienen la variante 7, dos subespecies de zorrillos el *Spilogale putorius leucoparia* V8 y *Spilogale putorius lucasana* V10, *Desmodus rotundus* como reservorio de la variante 11 la tipificación del virus de la rabia en bovinos reveló la circulación de V3 y V5 asociadas con vampiros y para *Tadarida brasiliensis mexicana* reservorio de la variante V9. Además, la V4 asociada con *Tadarida brasiliensis brasiliensis* en Sudamérica y por primera vez se reporta la presencia de variantes atípicas. En la última década y media el InDRE ha incrementado la detección de variantes atípicas las cuales en su mayoría han sido encontradas en quirópteros. Durante el 2011 se emprendió la caracterización de todas las variantes circulantes en México (atípicas y comunes) con el panel ampliado de 20 AbMo descrito por Smith y colaboradores. En este trabajo se incluyeron 25 muestras de encéfalos positivos a rabia de reservorios, especies de importancia económica y seres humanos con VAg atípicas, además de, 32 aislamientos de los virus de la rabia con VAg comunes. Todos los virus estudiados fueron previamente amplificados en ratones o cultivos celulares y algunos aislados en ambos sistemas. Después de amplificados, todos los virus fueron tipificados con el panel reducido de 8 AbMo para corroborar el patrón antigénico anteriormente obtenido. Los virus se sometieron a tipificación usando el panel ampliado de 20 AbMo (C1, C2, C3, C4, C5, C6, C17, C8, C9, C10, C11, C12, C13, CR54, C15, C16, C17, C18, C19 y C20). Se determinaron los patrones de reacción para cada aislamiento por Inmunofluorescencia indirecta y se obtuvieron patrones específicos que permitieron distinguir variantes geográficas del virus asociadas al mismo reservorio. Tal fue el caso de los virus de la rabia asociados a murciélagos hematófagos en los cuales se pudo definir *Desmodus rotundus* de Puebla, Jalisco, Guerrero, San Luis Potosí y la península de Yucatán y el *Desmodus rotundus* de la región de la huasteca que abarca los estados de Puebla Hidalgo y SLP. Así mismo se identificaron variantes del virus de la rabia asociadas a murciélagos no hematófagos como los de *Lasiurus ega* de Nuevo León, *Myotis/Lasiurus borealis* del estado de Jalisco, *Tadarida brasiliensis* de Oaxaca, *Tadarida brasiliensis-Coati* (*Nasua narica*) de Quintana Roo, *E. fuscus/L. cinereus* de Jalisco, *E. fuscus II*-del estado de Tabasco y *E. fuscus I* del estado de Baja California Sur. Así mismo se lograron identificar patrones únicos para virus asociados a reservorios terrestres como el *Urocyon cinereoargenteus-mexicano* presente en Sonora y Sinaloa que se pudo distinguir del virus que circula en la misma especie en Arizona EEUU. La variante del zorrillo moteado de BCS *Spilogale putorius lucasana* También se logró discriminar la variante asociada a zorrillos moteados *Spilogale putorius leucoparia* que circula de forma endémica en el centro norte de México en los estados de San Luis Potosí, Zacatecas, Aguas Calientes, Nayarit y Jalisco, de otras variantes que circulan en zorrillos rayados de Sinaloa, Jalisco y Durango. Asimismo, se pudo discriminar otro virus que circula en el estado de Chihuahua en zorrillos, el cual, es muy cercano al que circula en la región centro sur de los EEUU en zorrillos rayados de otro que también circula en zorrillos pero que es más cercano a los virus que circularon en perros de esa región. Podemos concluir que el panel ampliado de 20 AbMo incrementó el poder resolutivo en la identificación del virus de la rabia en México y contribuyó con la distinción de aquellos que circulan en los EEUU. Es necesario que la capacidad diagnóstica y el poder resolutivo de las técnicas de tipificación viral se vayan ajustando de acuerdo a la evolución de los patógenos estudiados. Este trabajo demuestra el dinamismo que debe tener el laboratorio de referencia nacional para ajustarse a la creciente demanda que nace a consecuencia de la evolución continua del virus de la rabia y de la implementación de sistemas mejorados de vigilancia epidemiológica para un mejor control y prevención de esta enfermedad.

# ***Evaluation of blocking ELISA as tool for control oral vaccination effectiveness.***

*Miroslav Mojzis, Slavomir Jerg & Korytar Peter.*

It was proved that in Slovakia red foxes were only species with the potential to be reservoir of rabies virus. Therefore oral vaccination of foxes was used as main tool to eradicate disease from wildlife. From the year 2000 for the purposes of vaccination effectiveness control the all samples were investigated for the presence of virus, antibodies and tetracycline. For detection of the antibodies the same Blocking ELISA test was used for whole country during entire investigated period. Aim of this study was evaluation of Blocking ELISA in term to estimate the “seroprevalence effective cut-off”, at which the eradication of rabies was feasible and therefore oral vaccination should be considered as effective. For the purpose of analyses, vaccination area was divided based on epidemiological situation and vaccine used to smaller units, where each unit was evaluated independently after each vaccination campaign. It was found that seroprevalences correlated very well with tetracycline levels and adversely with presence of virus and based on independent evaluations of different vaccination units it was concluded that obtained seroprevalence cut-off more than 30 percent was enough to eradicate the disease in any conditions. This proposed cut-off has been also proved to be effective to stop virus spreading after the virus reintroduction to Rabies free regions, which was clearly shown after last virus reintroduction from Poland in 2013. Nevertheless the sensitivity of ELISA test was above 95% (vs. FAVN), investigated “seroprevalence effective cut-off” was lower than previously known based on FAVN and RIFFT. Evaluated Blocking ELISA was developed in our laboratory and during following years was commercialized as diagnostic kit (BioPro Rabies ELISA Ab kit), and its suitability for detection rabies antibodies in wildlife and pets was proved by the other authors. Concluding our results we suppose that using ELISA as tool for control of oral vaccination effectiveness would be very simple and robust approach.

# ***Identificación específica y distribución de las variantes genéticas del virus de la rabia en México.***

*Escobar-Escamilla Noé, Trejo-Rodríguez Rosalba, Morales-López Zoila, Meléndez-Félix Alejandra & Rosa María Ribas-Aparicio.*

La rabia representa un problema de salud pública y pérdidas económicas en el área pecuaria. En caso de brotes, el uso conjunto de herramientas inmunológicas y moleculares para identificar las variantes antigénicas y genéticas del virus de la rabia (RABV), asociadas con animales reservorios específicos, incrementa el tiempo de respuesta epidemiológica. Técnicas innovadoras como la PCR acoplada a la transcripción reversa en tiempo real (RT-qPCR), podrían reducir este tiempo. Los objetivos de este trabajo fueron: 1) Determinar el comportamiento filogenético y geográfico de las variantes genéticas del RABV circulantes en México y 2) Diseñar y evaluar una RT-qPCR, basada en tecnología TaqMan®-LNA, para detectar específicamente a los diferentes filogrupos del RABV. El análisis filogenético incluyó 143 secuencias parciales del gen N viral, obtenidas entre 1991-2012 a partir de muestras de diferentes carnívoros terrestres y murciélagos de diversas entidades de México. Para la estandarización de la RT-qPCR, se diseñaron iniciadores y sondas específicos. Para la validación de la técnica, se utilizaron 42 muestras de encéfalo colectadas entre 1998-2011 en diferentes estados del país y los resultados se compararon con la prueba de inmunofluorescencia (IFD), así como con la tipificación antigénica y molecular. Se identificaron siete principales patrones filogenéticos, cada uno asociado a un reservorio específico: zorro, perro, zorrillos de tres regiones diferentes, murciélago hematófago y murciélago insectívoro. Estas variantes genéticas se diferenciaron de las circulantes en EUA. Los resultados de la RT-qPCR, correlacionaron con los resultados de IFD, caracterización antigénica, reservorio involucrado y región de aislamiento. Este trabajo brinda un panorama actual sobre el comportamiento geográfico de las variantes RABV en México. Además, por primera vez se desarrolló una RT-qPCR específica para los filogrupos del RABV que circulan en nuestro país. Esta técnica complementará y mejorará el diagnóstico de laboratorio, así como la vigilancia epidemiológica de la rabia en México.

# ***Lateral flow devices as diagnostic tools for rabies – a comparative study with six different tests with surprising results.***

*Conrad Freuling, Jeannette Kliemt, Bernd Hoffmann, Kore Schlottau, Thomas Mettenleiter, Thomas Müller.*

Rabies is a neglected zoonotic disease that causes estimated 60,000 human rabies deaths annually. The main burden suffer developing countries of Asia and Africa, where surveillance and disease detection is hampered by lacking laboratory facilities and/or the difficulties of submitting samples from remote areas to laboratories.

Under these settings, alternative easy-to-use tests such as immunochromatographic assays, i.e. lateral flow devices (LFD) may increase surveillance and improve control efforts. Previously, while specific LFDs had shown their applicability, others showed a poor performance. These contradictory results prompted us to investigate the test performance of available LFDs for the detection of rabies antigen in brain. We purchased LFDs in Germany (N=2), in the US (N=1), in India (N=1), and in China (N=2). All LFDs were used according to the manufacturers' instruction for experimentally infected animals, as well as for field samples. Additionally, a dilution series of positive in negative brain was also tested with various modifications of the procedures and realtime RT-PCR was also used to quantify the viral load in each sample tested.

Although LFDs have been shown to be highly sensitive and specific diagnostic tools for other diseases, surprisingly, we observed a low sensitivity in all tests and none of the tests was fit for purpose. Our results indicate that this may be due to several reasons including differences in batch quality, dilution effects or the general design of the test.

The high number of false negative results reiterates the necessity to perform a proper test validation before being marketed and used in the field. In this respect, marketing authorization and batch release control would grant a sufficient quality for these alternative tests, which could then fulfil their potential.

## ***Rabies vaccine booster decision and rabies serology: how to interpret results. Susan Moore.***

For people at risk of rabies exposure, the protection afforded by pre-exposure rabies vaccination is a significant health assurance measure. Though rabies vaccines are among the safest and most successful vaccines made, the level of immunity induced can wane over time while the level of risk may not. Periodic rabies titer checks are recommended by the two main sources of rabies prevention, the Advisory Committee on Immunization Practices (ACIP) in the United States and the World Health Organization (WHO). While both guidelines provide similar instructions, there are some differences, such as the level of rabies virus neutralizing antibody (RVNA) that represents evidence of seroconversion or adequate response. These different levels--complete neutralization at a 1:5 serum dilution and 0.5 IU/mL--in combination with different rabies serology methods in practice can lead to misinterpretation when decisions about rabies booster vaccination are made.

In our laboratory, the RFFIT reports began referencing both the ACIP and WHO adequate response levels as of May, 2011. Subsequently, Veterinary Schools expressed concerns about an increase in number of students requiring booster vaccination. To determine if this concern was based on the initiation of use of the WHO level for adequate RVNA response (higher than the ACIP level), a retrospective study covering seven years of RVNA titers in veterinary students who had received pre-exposure vaccination two years prior was performed. The study demonstrated that the sero-positive rates were similar to those previously published.

Given the importance of rabies vaccination in the prevention of rabies in at-risk individuals and in people undergoing post-exposure vaccinations, guidelines for booster vaccination decisions should provide an understandable definition of adequate response as related to rabies serology results.

## ***Blue ribbon panel on rabies serology in wild meso-carnivores.***

*Richard B. Chipman, Amy T. Gilbert, Dennis Slate, Kurt C. Vercauteren, Kathy M. Nelson and Charles Trimarchi.*

A blue ribbon panel (BRP) convened in 2014 to provide insight and recommendations on factors that may affect the measurement and interpretation of serologic results in wild meso-carnivores in response to oral rabies vaccination (ORV). Understanding ORV effectiveness relies on enhanced surveillance to delineate the distribution of cases, and monitoring biomarker and rabies virus neutralizing antibody (rVNA) prevalence in target species post-ORV as indices of bait uptake and seroconversion. The BRP included experts from all laboratories conducting rabies serologic testing in the U.S. and Canada and select laboratory representatives from Europe, as well as personnel with expertise in regulatory, research and management aspects of rabies from government agencies, non-government organizations and the private sector. The panel was facilitated by a retired rabies expert from the New York State Department of Health Rabies Laboratory. The facilitator requested panel members respond to questions before the 2-day meeting to ensure pertinent topics would receive attention. Key areas of focus included: 1) requirements to assure comparability and compatibility among laboratories and assays; 2) non-specific inhibitors; 3) seronegative/positive cutoffs; and 4) optimum serum sampling window post-ORV. The draft final report contains the following action items: 1) a sub-committee will reconvene to recommend steps for laboratory standardization; 2) inter-laboratory proficiency testing using seronegative, and low, moderate and high seropositive sera should be instituted; 3) serologic analysis should include regular submission of blind samples; 4) cutoff recommendations will be provided by the standardization sub-committee; 5) conclusions of the BRP and sub-committee reports should be appended to the U.S. National Plan for Wildlife Rabies Management. The advantages and limitations of ELISAs vs. serum neutralization tests and continued use of 0.06 IU to index wildlife population immune responses, with reporting in groupings of  $\geq 0.06 - 0.10$ ,  $> 0.10 - 0.5$ , and  $> 0.5$  IUs, remain under consideration as a result the BRP.



# ***Progress in the development of a direct rapid immunohistochemical test for lyssavirus diagnosis.***

*C.E. Rupprecht, F. Cliquet, H. Ertl, C. Fehlner-Gardiner, A.R. Fooks, T. Mueller, C. Sabeta & D. Slate.*

The direct fluorescent antibody test (FAT) is an OIE-prescribed and WHO-recommended technique and is the 'gold standard' for sensitive and specific rabies diagnosis. The FAT provides consistent results on fresh brain specimens in more than 95% of cases. As plans for the global elimination of canine rabies progress, the need for additional relevant diagnostic testing is crucial to improve de-centralized laboratory-based surveillance, improve risk assessments after exposures to suspected rabid animals and to monitor program success, especially in developing countries. A direct rapid immunohistochemical test (DRIT) for rabies diagnosis has been developed as one alternative to meet this need. Similar to the FAT, the DRIT involves the examination of brain impressions but, rather than using immunofluorescence, employs standard light microscopy and biotin-labelled antibodies. Results are obtained typically in less than 1 hour. Using the DRIT, thousands of suspect brains have been examined since 2008 by diagnosticians and researchers in the Americas, Africa, Asia and Europe. Despite dramatically varying conditions by locale, species tested, sample quality, viral type, and background conditions, test sensitivity and specificity have approached 100%. All currently recognised lyssavirus species have been identified in naturally or experimentally infected mammals using the DRIT. In the USA, the DRIT has been applied to laboratory confirmatory testing of equivocal FAT results and to enhance surveillance under field conditions. For example, in support of oral rabies vaccination programs, more than 60,000 specimens have been tested within North America and, as with the FAT, the DRIT is included in routine proficiency testing among laboratories. Moreover, preliminary inter-laboratory comparisons, using different monoclonal and polyclonal conjugates, with antibodies tested against a wide variety of viral variants and mammalian taxa, support the basic premise of the DRIT as a relevant method for future validation and OIE/WHO consideration as a prescribed test for point of care rabies diagnosis.

## ***A Th1-type immune response is required for efficient clearance of RABV from CNS tissue.***

*Aurore Lebrun, Carla Portocarrero, Rhonda B. Kean, Milosz Faber, D. Craig Hooper.*

Little is known about immune cell infiltration into the central nervous system (CNS) in the absence of pathological inflammation. Attenuated rabies viruses (RABV) are unique tools to study this process, as they spread from the site of inoculation to the CNS trans-axonally, without compromising the blood-brain barrier (BBB). Infection with live-attenuated RABV elicits virus-specific IgG2a antibodies, indicating a bias towards Th1 immunity. Moreover IFN $\gamma$ , a key Th1 product, has been associated with elevated BBB permeability in the response to attenuated RABV. To further investigate the role of the Th1-type immune response to RABV infection, we performed a temporal comparison of the development of antiviral immunity in C57BL/6 and T-Bet knock-out (T-Bet $^{-/-}$ ) mice which cannot generate a Th1 immune response. We first observed that T-Bet $^{-/-}$  mice fail to develop a protective immune response after RABV immunization and consequently do not survive a challenge with a lethal virus. Despite equivalent spread of attenuated RABV through the CNS of both mouse strains, immune cell infiltration into the CNS and antibody production, both in the periphery and CNS tissues, differs. Even though immunized T-Bet $^{-/-}$  mice produce a substantial amount of virus neutralizing antibody in the periphery, the predominant IgG antibody isotype is IgG1 (Th2) as opposed to the IgG2a (Th1) produced by C57BL/6 mice. In the CNS tissues of wild type mice, a peak of immune cell infiltration was observed 8 days after immunization, followed by extensive antibody production concomitant with the clearance of RABV. B but not CD4 $^{+}$  T cells accumulate in the CNS tissues of T-Bet $^{-/-}$  mice infected with attenuated RABV resulting in poor local antibody production and impaired RABV clearance from the CNS. These findings indicate that Th1 cells play a critical role in the clearance of RABV from CNS tissues despite the absence of inflammatory pathology.

# ***Comparative evaluation of the proinflammatory cytokines profile in central nervous system from human rabies cases transmitted by dogs and vampire bats.***

*Elaine Raniero Fernandes, Fernanda Guedes, Samira Maria Achkar & Maria Irma Seixas Duarte.*

Upon antigenic stimulation, such as infection by rabies virus, glial cells of the central nervous system (CNS), especially astrocytes and microglia are capable of secreting soluble factors, including proinflammatory cytokines (IL-1 beta, IL-6 and TNF-alpha). These factors can increase the permeability of the blood-brain barrier (BBB), occurring the entry of leukocytes from the periphery, which secrete other cytokines, thereby helping to define the profile of immune response in attempting to combat the infection and cause less neuronal damage possible. We evaluated and compared the expression of this cytokines in CNS fragments from human rabies cases transmitted by dogs and vampire bats. Thus, three human rabies cases transmitted by dogs and three transmitted by vampire bats were compared in relation to the expression of IL-1 beta, IL-6 and TNF-alpha. CNS fragments were analyzed by immunohistochemical reaction. All immunostained cells were quantified in 40 fields in every CNS fragment with the aid of the graticule of 1 cm<sup>2</sup> coupled in light microscope with 10x eyepiece and 40 x objective, resulting in an area field of 0.0625 mm<sup>2</sup>. There was a predominance of IL-1 beta and TNF-alpha in cases of human rabies transmitted by vampire bats, while in cases of rabies transmitted by dogs, there was a predominance of IL-6. In cases of human rabies transmitted by vampire bats was possible characterize a proinflammatory profile of immune response outlined by the presence of IL-1 beta, IL-6 and TNF-alpha, concomitant with increased inflammatory infiltrate, however, in cases of rabies transmitted by dogs, was seen an increased expression of IL-6 in relation to other cytokines studied and less inflammatory infiltrate, possibly because the IL-6 could be contributing to the definition of other immunological profile, such as Th17 in an attempt to overcome the infection after failure of Th1 and Th2 profiles in the viral clearance.

# ***Glycosylation of the rabies virus G protein impacts antibody neutralizing activity in vitro.***

*Ferreira JS, Katz ISS, Caporale GMM & Silva SR.*

Rabies virus glycoprotein (RVG) is important in the biology and pathogenesis of virus infection, in addition to be the main target of neutralizing antibodies. The extracellular domain of this glycoprotein has N-glycosylation sequons at Asn37, Asn247, and Asn31. It is known that appropriate glycosylation of these sequons is necessary for the cell surface expression and function of the glycoprotein. For example, nonglycosylated RVG is not expressed at the cell surface and is not effective as vaccine. Several studies have been demonstrated the role G protein glycosylation in pathogenicity, infectivity and rabies virus production, however, little is known about how differential glycosylation affects immunogenicity of the viral proteins. In this study, we have tested the hypothesis that glycosylation of RVG contributes to resistance of the virus to neutralizing antibodies. For this, murine neuroblastoma cells (N2A) were inoculated with RABV isolated from canine (M.O.I = 1) and incubated for 24 h in the absence or presence of tunicamycin (TM) at concentration 1 µg/ml. After the TM treatment, were analyzed: the titer viral (FFU/ml) values and the neutralizing antibodies reactivity with RABV by RFFIT test. In this work, we observed that in N2A cells inoculated with RABV in the presence of TM resulted in lower viral titer (FFU= 1,77x10<sup>3</sup> versus FFU=1.34 x 10<sup>5</sup>), however had no influence in its capacity to infect cell compared with the parent virus. On the other hand, TM-treated RABV reduced the ability of the antibody neutralize this virus in RFFIT test compared to RABV native (0.11 UI/ml versus 0.65 UI/ml, reduction in neutralizing antibody activity of 83%). These data suggest that, the interference with the glycosylation process of RVG can affect the antibody neutralizing activity in vitro. These results are encouraging and warrant further examination.

## ***Production of a chromatographically purified Vero cell rabies vaccine.***

*María Rosario Tubio, Santiago Chiappini, Analía López Díaz, Analía De Nichilo, Andrés Hernando Insúa, Carlos Palacios, Oscar P. Larghi & Alejandro Daniel Parola.*

The present work summarizes the development of a new chromatographically purified Vero cell rabies vaccine. This approach involved the elaboration and characterization of a Vero cell bank (ATCC-CCL81), accordingly to international guidelines. In addition, two viral banks of Pasteur Virus (PV) 2061 and Pitman Moore (PM) 1503 strains were prepared and titrated in Vero cells (PV =  $3.3 \times 10^9$  ID<sub>50</sub>/mL and PM =  $1.19 \times 10^8$  ID<sub>50</sub>/mL). Molecular identity was confirmed by sequencing the viral glycoprotein. The main results shown here were obtained with PV virus. For PV infections cells were grown in spinner flasks on 3 g/L Cytodex 1, and infected when cell density reached  $2 \times 10^6$  cells/mL at different multiplicities of infection (0.1 to 1 ID<sub>50</sub>/mL), cell culture medium was replaced every 48 h, during 14 days post infection. The highest titer obtained was  $1.5 \times 10^{10}$  ID<sub>50</sub>/mL, with a viral glycoprotein content of 19 IU/mL, with a volumetric efficiency of 53 IU/mL from the initial working volume. Harvests were clarified by filtration, concentrated and diafiltered by tangential flow filtration membranes (TFF). Final concentrates were purified by cation exchange chromatography followed by gel permeation chromatography, inactivated with beta Propiolactone, diafiltered by TFF and filtered finally. This purification strategy was able to produce a vaccine lot that satisfied the NIH test. Furthermore purified virus showed lower contaminant levels than the established by the British Pharmacopoeia: BSA: <50 ng/dose and DNA <10 ng/dose. To our knowledge this is the first report of a human rabies vaccine produced under international standards, entirely developed by a private company, in Latin America

# ***Expression of rabies virus glycoprotein G by using recombinant baculovirus.***

*Chang-Kweng Lim, Wouter van den Braak, Madoka Horiya, Mutsuyo Ito-Takayama, Yukie Yamaguchi, Satsuki Kakiuchi & Masayuki Saijo.*

Rabies is a disease of the central nervous system of major importance to human and veterinary medicine. Every year over 55,000 people die due to complications caused by the Rabies virus (RV), most of which occur in Africa and Asia. Although disease preventing vaccines and post-exposure prophylaxis are available, these treatments are too expensive for many locals. To prevent more RV deaths a cheaper and more immunogenic vaccine need to be generate. RV glycoprotein (G) is the major antigen to trigger a protective immune response and the only protein capable of generating virus neutralizing antibodies. Rabies glycoprotein was tough to express at sufficient level in soluble form in baculovirus, E. coli and plant. In this study we describe the development of a recombinant baculovirus expressing the RV-G in soluble form. Two different constructs were created featuring the RV-G gene of HEP-flury strain in a transfer vector pBacPAK9. In one construct the transmembrane domain (TMD) was deleted in order to produce soluble G (Gs). A control construct was also created containing the complete G gene (G1). Protein expression was analyzed by fluorescence microscopy and western blot. The G gene containing vectors were cotransfected with linear viral DNA BacPAK6 in Sf9 cells. This resulted in production of recombinant baculoviruses expressing the G protein, as detected on western blot. RV-Gs was detected in both culture supernatant in soluble form and cell-fraction. However RV-G1 was only detected in cell-fractions. It appears that RV-Gs was transported out of the cells in soluble form. It indicated that RV-Gs has advantage of protein purification. Further study needs to characterize RV-Gs. It needs to be compared to the native RV-G1 protein in order to determine if its conformational state corresponds to the natural protein.

# ***VerorabVax™: A Step Forward in Human Rabies Vaccines.***

*Françoise Guinet-Morlot, Sylvie Pichon & Nicolas Sève.*

As part of its commitment to the continuous improvement of its vaccine production process, and as a major step forward in the field of human rabies vaccines, Sanofi Pasteur has developed VerorabVax™, a next-generation Vero-cell rabies vaccine. This vaccine benefits from Sanofi Pasteur's long-standing history of fighting against rabies. VerorabVax is based on the company's current reference vaccine Verorab™, a WHO-qualified purified Vero-cell rabies vaccine (PVRV) whose safety, immunogenicity and efficacy have been established and demonstrated by successful clinical use over nearly 30 years. VerorabVax was developed with an innovative vaccine manufacturing technology encompassing human and animal origin components free processes, while still making use of qualified Vero bank cell substrate and established Pitman Moore rabies virus seed strain further inactivated with beta-propiolactone. Extensive characterization studies, including state-of-the-art analytical methods, demonstrated the biocomparability of the resulting serum-free vaccine drug substance to Verorab, achieving a high level of purity and thorough consistency in molecular and structural properties. The manufacturing of VerorabVax relies on high standards, robust processes and cutting-edge technology such as disposable bioreactors allowing for good scale-up performance and process reproducibility, and high viral productivity at industrial scale. This amplification bioprocess provides consistent and homogeneous performance throughout the cell passages. The purification process and the stabilizer formulation, both patented, lead to a highly-purified and stable vaccine. Phase II and Phase III clinical evaluations conducted so far show that VerorabVax is at least as immunogenic as Verorab and well tolerated in pre- and post-exposure settings. Building on the legacy of innovation and using technological and industrial improvements in rabies prevention biologicals, Sanofi Pasteur has developed a modern effective highly-purified Vero-cell derived rabies vaccine as an evolution of its currently marketed PVRV for the prophylaxis of rabies in humans.

# ***Safety and immunogenicity study of rabies G protein vaccine administered to healthy volunteers: A preliminary report.***

*Rajiv I Modi, Khamar B, Sawhney D, Talpada M, Madhusudana SN, Greg G & Fries L.*

**Background :** Rabies is a fatal encephalitis which can be prevented by effective post exposure vaccination. A vaccine which can simplify the dosing schedule and possibly bring down the overall cost of treatment is highly desirable as an advancement over the currently available vaccines. We have developed a novel rabies vaccine based on purified G protein and evaluated its safety and immunogenicity in healthy volunteers

**Study design and Methods:** This study is designed to be conducted in two stages: A preliminary study to assess the immunogenicity and safety of G vaccine administered in different doses and schedules (stage I) and a final dose confirmation study and its evaluation ( stage II). The doses selected for stage I were 5, 10, 20 and 50 mcg of Rabies G protein per dose given as 0.5 ml injected intramuscularly. All doses were given in four schedules (0,3 days; 0,3,7 days, 0,7 days; 0,7,10 days). There was a control group of a marketed vaccine given as the recommended 5 dose intramuscular injections regimen. Serum samples on day 0, 14, 21, 42, 90, 135 and 180 were analysed by Rapid Fluorescent Focus Inhibition Test (RFFFIT). Solicited and unsolicited adverse events were recorded.

**Results:** A total of 170 healthy adult male volunteers were dosed in Stage I of the trial. Baseline antibodies above 0.5 IU were found in 5 volunteers and these were excluded from the overall analysis. All volunteers in all groups achieved seroprotection levels at day 14 and remained seroprotected at day 135. The GMTs at day 14 in different groups ranged from 5.2 to 17.42 IU/ml. The vaccines were found to be safe and there were no serious or unexpected vaccine emergent adverse events noted.

**Conclusion:** This is the first ever human study with a purified Rabies G protein vaccine. Both two and three dose regimens were found to be highly immunogenic. These regimens will be further evaluated in stage II study.



# ***A recombinant rabies vaccine expressing the trimeric form of the glycoprotein confers enhanced immunogenicity and protection in outbred mice.***

*Penelope Koraka, Berend-Jan Bosch, Manon Cox, Rick Chubet Geert van Amerongen, Karen Lövgren Bengtsson, Byron E.E. Martina, Jouke Roose, Peter J.M. Rottier and Albert D.M.E. Osterhaus.*

Rabies, an invariably lethal encephalitis, can be controlled by preventive vaccination programs of wildlife, domestic animals and humans in areas with a high risk of exposure. Currently available vaccines are expensive, cumbersome to produce and require intensive immunization and booster schemes to induce and maintain protective immunity. In the present study, we describe the development of candidate recombinant subunit rabies vaccines based on the glycoprotein G of the prototype rabies virus (RABV-G) expressed either as a monomer (RABV-mG) or in its native trimeric configuration (RABV-tG), with or without Matrix-MTM adjuvant. Immunogenicity and protective efficacy of the respective candidate vaccines were tested in outbred NIH Swiss albino mice. The RABV-tG candidate vaccine proved to be superior to the RABV-mG vaccine candidate both in terms of immunogenicity and efficacy. The relatively poor immunogenicity of the RABV-mG vaccine candidate was greatly improved by the addition of the adjuvant. A single, low dose of RABV-tG in combination with Matrix-MTM induced high levels of high avidity neutralizing antibodies and protected all mice against challenge with a lethal dose of RABV. Consequently RABV-tG used in combination with Matrix-MTM is a promising vaccine candidate that overcomes the limitations of currently used vaccines.

# ***Evaluation of anti-rabies virus immune response in rabbits immunized with different plasmid DNA vectors encoding the rabies virus glycoprotein genes.***

*DV Volokhov, A Fry, CE Lewis, V Furtak, A Dabrazhynetskaya, JR Hermann & VE Chizhikov.*

Genetic immunization of different animal models has shown to be an efficient approach to induce protective immunity to infectious diseases. The advantages of DNA vaccines are the simplicity of their construction, the low production cost, the high stability, and the ability to elicit a full spectrum of immune responses to a target pathogen. Previously, DNA-based immunization has successfully been used to study the cross-reactivity of virus neutralizing antibodies (VNA) and to explore the induction of protection against a wide range of different Lyssaviruses. The goal of this study was (i) to assess the rabbit humoral immune response to the rabies virus glycoprotein (gpG) after a course of intramuscular DNA injections with either wild-type (WT) or human codon-optimized (OPT) gpG genes, and (ii) to prepare a substantial amount of polyclonal antibodies against gpG that can be used as in-house reference reagents for development of rabies virus ELISA, single radial immunodiffusion assay, and the rapid fluorescent focus inhibition test. To prepare required antibodies, rabbits were immunized with commercially available mammalian expression vectors pTarget-gpG and pVAC2-gpG containing the chemically synthesized gpG genes under control of the CMV enhancer/promoter or the SV40 enhancer/rhEF1 promoter, respectively. The immunization of rabbits was conducted using two doses of either 200 or 500 µg of plasmid DNAs with or without adjuvants (Emulsigen-D and AddaVax). Overall, our results demonstrated that DNA-based immunization of rabbits with the WT and OPT-gpG genes elicited high titers of VNA. No differences in the titers of VNA were observed in rabbits immunized with WT or OPT variants of the gpG gene. No difference in the titers of VNA was observed in rabbits immunized with either 200 or 500 µg of DNA. The higher titers of VNA were observed in rabbits immunized with the pVAC2-gpG than with pTarget-gpG. The strongest humoral response was obtained when a combination of the pVAC2-gpG with Emulsigen-D was used. The results of the study demonstrated that the DNA-based immunization with the WT or OPT gpG could be a promising approach to induce VNA to the Lyssaviruses in animals and for development of alternative methods for production of polyclonal reagents to proteins of the Lyssaviruses.

# ***Rabies vaccine preserved by vaporization is immunogenic and effective pre-exposure vaccine.***

*Todd G. Smith, Marina Siirin, Cathleen Hanlon & Victor Bronshtein.*

To prevent rabies, safe, easy-to-administer, potent vaccines are required. A thermostable rabies vaccine would be very advantageous for tropical regions where canine rabies is endemic. In addition, oral immunization of free-ranging carnivores plays an important role in rabies control. The goal of our studies was the development of stable formulations of rabies virus (RV) vaccines, suitable for oral delivery. Live attenuated RV strain ERAg333 was preserved by vaporization (PBV) in a dry, stable foam. Previously reported studies demonstrated RV PBV was thermostable. After RV PBV was completely inactivated by -irradiation, studies using an antigen capture assay showed that inactivated RV PBV contained similar levels of antigen as a commercial vaccine. To confirm the efficacy of the RV PBV to act as a live attenuated or inactivated vaccine, mice were given a single intramuscular dose, bled for detection of an immune response, and challenged peripherally with a street canine RV. Live attenuated and inactivated RV PBV both effectively produced RV neutralizing antibodies (rVNA) and protected animals from rabies virus challenge. RV PBV and alginate encapsulated RV PBV were delivered to the intestinal mucosa in gray foxes (*Urocyon cinereoargenteus*) and rVNA was measured. These results demonstrate the efficacy of live attenuated and inactivated RV PBV and support the continued development of a thermostable rabies vaccine.

# ***Evaluation of herbal extract for its anti-viral activity against rabies virus.***

*Ashwin YB, Sampada Sudarshan, Reeta Mani & Madhusudana SN.*

**Objective:** Rabies is fatal viral encephalitis and till today no effective anti-viral agent has been developed. Through agents such as Ribavarin, ketamine and interferon- $\alpha$  were found to be effective in experimental animals, they have been ineffective in human rabies

**Methods:** Crude extract and different fractions of a herbal extract were evaluated by both in-vitro and in-vivo studies. In vitro studies were done by checking the anti-viral activity of the extract against CVS and street viruses using BHK-21 cells and murine neuroblastoma cell lines at various time points post infection. In vivo studies were done on Swiss albino mice by checking the anti-viral activity of the extract against CVS by Intramuscular, intra-peritoneal and oral routes of administration.

**Results:** Of the 26 fractions that were studied, 3 of them showed complete inhibition of viral replication of the both the CVS and the street virus in both BHK 21 and Neuro2 a cells up to 8 hours post infection. The crude extract or its fractions did not show promising results in in-vivo experiments in Swiss albino mice where they failed to demonstrate any anti-viral activity against CVS.

**Conclusions:** For the first time an herbal extract has been evaluated for its anti-viral activity against rabies virus. In-vitro experiments have shown promising results. The anti viral property by in-vivo experiments needs be further studied taking into consideration the bio-availability of the active component and its pharmacokinetics in larger animal models.

# ***A comparison of the anamnestic response to rabies vaccination in currently vaccinated and out-of-date dogs and cats: Rationale for a re-evaluation of recommendations for previously vaccinated animals exposed to rabies.***

*Mike Moore, Rolan Davis, Christopher Vahl, Ryan Wallace & Cathleen Hanlon.*

The purpose of our study was to compare the anamnestic antibody responses of dogs and cats with current rabies vaccination status with those that have an out-of-date vaccination status to provide a framework for serological standards which may one day be used by public health officials to determine the fate of rabies exposed domestic animals. Animals in this study were supplied by veterinarians whose clients' pets had been exposed to rabies or clients seeking routine rabies boosters for their pets. A serum sample was harvested upon initial presentation (day 0) and then again 5-7 days later. At day 0 a rabies vaccine of the veterinarian's choice was administered to the animal according to label recommendations. The paired serum samples were analyzed for antibodies against rabies by Rapid Fluorescent Focus Inhibition Test at Kansas State University. Out- of-date dogs as a group had a greater average change in titer, fold rise, and end point titers compared to currently vaccinated dogs. Out-of-date dogs were non-inferior to their current counterparts ( $p=.0287$ ). This data suggests that rabies exposure for out-of-date animals can be managed the same as those considered to be current on vaccinations. The results of this study warrant consideration for a policy change by the Compendium of Animal Rabies Prevention and Control for healthy out-of-date vaccine status animals that are exposed to rabies.

# POSTERS

## **Rabia en humanos Rabies in humans Raiva em humanos**

### ***P01 Cross-neutralizing activity of antibodies induced by vaccination with Purified Chick Embryo Cell Rabies Vaccine (PCECV) against different Lyssavirus species.***

*Alexandra Giesen, Conrad Freuling, Claudius Malerczyk, Thomas Selhorst & Thomas Müller.*

Rabies is a zoonotic disease caused by lyssaviruses of the rhabdoviridae family. An estimated 60,000 human deaths occur worldwide each year from rabies, the majority in endemic countries of Asia and Africa. Human transmission occurs primarily through exposure to the classical rabies virus (RABV) via infected animals, mostly dogs. Currently, there are 15 identified virus species belonging to up to proposed four phylogroups (I-IV). For phylogroup I cross-protection with classical rabies virus vaccines is suggested. Previously, we showed evidence that suggests Purified Chick Embryo Cell Rabies Vaccine (PCECV) elicits immune response not only against classical RABV, but also cross-neutralizing antibodies against Australian Bat Lyssavirus (ABLV), and European Bat Lyssaviruses Type 1 and 2 (EBLV-1, EBLV-2). Using the same serum samples as in our aforementioned study, we extended our investigation by examining PCECV-induced cross-neutralizing antibodies against other selected non-classical lyssavirus species, namely Duvenhage Virus (DUVV), Bokeloh Bat Lyssavirus (BBLV), Mokola Virus (MOKV) and Lagos Bat Virus (LBV), as measured by a modified rapid fluorescent focus inhibition test (RFFIT). Antibodies elicited by PCECV showed cross-neutralizing activity against BBLV and DUVV to the same degree as against ABLV, EBLV-1 and EBLV-2. As expected, no cross-neutralizing activity was detected for the phylogenetically more distant species LBV (phylogroup II). Interestingly, 15 of the 94 serum samples (16%) with positive neutralizing antibodies against RABV also displayed specific cross-neutralizing activity (albeit 65-fold lower than against RABV) against one specific MOKV (phylogroup II) strain (Ethiopia isolate), but not against a different strain (Nigeria isolate). Cross-neutralizing antibodies correlate to some degree with the phylogenetic distance of the virus species. Similar to previous results showing cross-neutralizing antibodies against ABLV, EBLV-1 and EBLV-2, cross-neutralizing antibodies against BBLV and DUVV were demonstrated. Partial cross-neutralizing antibodies against more distant lyssaviruses (e.g. MOKV strains) warrants further research.

## ***P02 Ante-mortem laboratory diagnosis and treatment support in case imported of human rabies in the city of São Paulo, Brazil.***

*Samira Maria Achkar, Luciana Botelho Chaves, Helena Beatriz de Carvalho Ruthner Batista, Juliana Galera Castilho, Karen Miyuke Asano, Andréa de Cássia Rodrigues da Silva.*

This describes the laboratory diagnosis ante-mortem and support for treatment of rabies in São Paulo, of a human attacked in Bolivia by a dog, and the last record of human rabies transmitted by dogs in this city occurred in 1981. Samples of saliva, hair follicle, cerebrospinal fluid (CSF) and serum were sent to Laboratory Diagnosis of Rabies at the Pasteur Institute of São Paulo during the period of hospitalization, with average intervals of three days between collections, being eleven samples of saliva and serum, six of the hair follicle and three of CSF. After the first positive result, the patient was subjected to the treatment by Protocol of Human Rabies. The evaluation of virus neutralizing antibodies (VNA) was performed on serum and CSF by the Rapid Fluorescent Focus Inhibition Test. In CSF the first sample was negative, but the second and third presented title of 1.60 and 1.75 UI/mL, respectively. All serum samples showed high titers of VNA from the first collection (10.45 UI/mL). Virus detection techniques were performed by mice viral isolation (MVI) in saliva samples and semi-nested RT-PCR in all samples received. Viral RNA was detected in the second and fourth follicle samples and in the second sample of saliva, which were submitted to genetic sequencing of the gene N. No sample was isolated by the MVI and, after the fourth collection, no samples resulted positive, suggesting a viral clearance arising from treatment. By phylogenetic analysis was identified the lineage specifics of Bolivia's dogs. The epidemiological investigation associated with the genetic sequencing of the virus allows to say that this case of human rabies was imported from Bolivia, since this genetic lineage is distinct from that circulates commonly in dogs in Brazil.

## ***P03 Proyecto de Plataforma para la sistematización y organización de la vigilancia epidemiológica de la atención del expuesto al virus rábico en Chiapas México.***

*Raúl Betanzos López, Luis Lecuona, Rodolfo de Jesús Sánchez Cruz, Karla Elena Cruz Pérez, Silvia Gloria Nuricumbo Gallegos.*

Introducción: Proyecto de plataforma para la atención de pacientes expuestos al virus rábico será de gran importante para la vigilancia epidemiológica, desde el punto de vista operativo, incluye la recopilación, ordenamiento y el análisis de los daños y riesgos en salud, incluyendo también la vigilancia de indicadores positivos, con el fin de utilizarlos en el diagnóstico poblacional e identificar los problemas de mayor magnitud y otras situaciones de alto riesgo, que permitan la planeación, ejecución y evaluación de los programas de salud.

Objetivo: Elaborar una plataforma de estudio y vigilancia epidemiológica para la prevención de la rabia en el humano.

Material y metodos: Se revisaron diferentes formatos de múltiples programas de salud pública, incluyendo los utilizados en el programa rabia hace más de dos décadas y se adopta la Guía para la atención médica y antirrábica de la persona expuesta al virus de la rabia actual documento oficial nacional.

Resultados: La plataforma cuenta con cinco ítems: A) Datos básicos de la persona accidentada, B) Datos del animal, C) Datos de observación del animal agresor a partir de la fecha de agresión D) Datos de la exposición y E) Datos de tratamiento, así también se incluyen 35 preguntas con respuestas dicotómicas y de selección múltiples.

Conclusiones: Las unidades de salud se dispondrá una clave que permitirá ingresar la información que proporcionara el demandante del servicio, la cual ingresara en los cuatro ítems iniciales para que posteriormente a través de algoritmos preestablecidos se dispondrá de información para tomar decisiones, anteriormente existía una libreta el médico tratante solicitaba y registraba la información que consideraba necesaria en base a su conocimiento, pero esta era escasa y sin ninguna secuencia acorde a lo requerido, posteriormente se diseño el estudio epidemiológico ante demanda de tratamiento antirrábico humano, el siguiente paso es disponer de una plataforma.



## ***P04 Toward canine rabies elimination: Rabies extension of the WHO-CHOICE Model.***

*Brody Hatch, Aaron Anderson, Louis Nel, Christopher Fitzpatrick, Bernadette Abela-Riddler, & Stephanie Shwiff.*

We extended the World Health Organization's CHOICE Model to include rabies. This is the first neglected tropical disease and zoonosis that the model has been extended to cover. Aggregate costs of canine vaccination and post-exposure prophylaxis interventions can be estimated for WHO subregions as well as individual countries on an annual basis or over ten years in the form a discounted present value. The influence of each intervention on mortality reduction is also estimated by the model which allows for the estimation of incremental cost effectiveness ratios. An accurate estimation of costs and benefits of rabies allows for improved strategic elimination efforts. Extending the WHO-CHOICE Model to cover rabies allows for the comparison of rabies with other global diseases and their respective interventions.

***P05 I Traveled the world, and all I got was PEP: Developing a transparent country risk classification system to inform pre-travel rabies vaccination recommendations.***

*Jesse D. Blanton.*

Recommendations for travel related rabies pre-exposure prophylaxis (preEP) is a nuanced process requiring detailed risk assessments. In general, risk assessments include evaluation of a traveler's age, length of stay, planned activities, local rabies epidemiology and availability of biologics at all travel locations. Information can be limited when determining the endemicity of rabies and the availability of biologics in many parts of the world. To make specific recommendations, CDC classifies countries into five categories: Canine rabies present, of concern to travelers; Canine rabies present, not a significant concern to travelers; little or no canine rabies reported; rabies present in other carnivores; and bat lyssaviruses present/likely/unknown. Subject matter experts (SMEs) in rabies and travelers health evaluate existing literature, surveillance sources, and personal communications with regional health experts to determine individual country classifications on a regular basis. While this system provides additional information about rabies endemicity and availability of rabies biologics, it is heavily reliant on limited expert opinion. To improve this process, an open access list of country-specific risks is needed based on standardized questions regarding endemicity of canine, wildlife, and bat lyssaviruses and the availability of biologics. This list would provide an opportunity for input from a broader pool of rabies SMEs and be more responsive to local changes in epidemiology and biologics. In addition, providing individual risk levels for primary reservoir groups (e.g. canine, wildlife, and bats) and availability of biologics will give more detailed information that can be used by travel health providers when counseling patients.

## ***P06 Human rabies in Brazil between 2004-2013.***

*Juliana Galera Castilho, Rafael de Novaes Oliveira, Pedro Carnieli Junior, Carla Isabel Macedo, Samira Maria Achkar, Silene Marinque Rocha, Lúcia Regina Montebello, Eduardo Pacheco de Caldas, Helena Beatriz de Carvalho Ruthner Batista.*

Despite of the canine vaccination has been successful in controlling of rabies actually this disease continues causing thousands of human deaths. In Brazil, rabies virus (RABV) is maintained in variety of reservoirs, including domestic dogs, wild canids (*Cerdocyon thous*), marmosets (*Callithrix jacchus*), haematophagous bats (*Desmodus rotundus*) and numerous species of non haematophagous bats. Human rabies transmitted by all the described reservoirs, except non haematophagous bats were registered in Brazil. The aim of this study was to correlate the number of human rabies and the source of contamination in each case in Brazil, between 2004 and 2013. During the analyzed period were registered a total of 104 cases of human rabies, the most prevalence of this cases was in 2004 and 2005. In these years was registered 74 cases, where 64 (86.5%) were transmitted by haematophagous bats, the other cases of human rabies in these years were transmitted by dogs (8.1%), cat (1.35%), herbivore (1.35%), marmoset (1.35%) and 1.35% with unknown origin. Between 2006 and 2013 only 30 cases of human rabies were registered where the transmitter were: domestic dogs (17 cases- 56.7%), haematophagous bats (6 cases- 20%), marmosets (5 cases- 16.7%), bovine (1 case- 3.3%) and deer (1 case- 3.3%). Except the outbreaks occurred in years 2004 and 2005, 74% of the RABV involved in human rabies in Brazil was submitted to antigenic or genetic characterization. In all samples submitted to characterization was confirmed the typical RABV variant current in these reservoirs. This study show that wild animals were responsible for 77 (74%) of human rabies in the period in Brazil however the majority (80.5%) of this number is related with an specifics outbreak of rabies in North and Northeast of country and domestic dogs remaining an important reservoirs for rabies, responsible for transmission of 23 (22.1%) of described cases.

## ***P07 Situación del programa de rabia durante el año 2013 en el estado de Guerrero, México.***

*Lorena Dorantes Salgado & S. Carrillo P.*

El estado de Guerrero, por su colindancia, principalmente con los estados de Oaxaca y Michoacán, es una zona de riesgo para la transmisión de la rabia (en bovinos, caninos o animales silvestres). En el estado existen experiencias que han permitido reducir el impacto de esta enfermedad a través de intervenciones específicas, cuyos costos de ejecución son razonables. Sin embargo, este mal persiste aún en forma latente en determinadas áreas geográficas, representando un riesgo grave para la población residente de estos lugares. Las acciones del programa para el control de la rabia en el estado han propiciado que en el año 2013, se hayan tenido cero casos de rabia canina y humana, aun cuando en el estado se reportaron durante este año 2,583 agresiones, de las cuales 2,395 fueron por perros sospechosos y 188 por otros animales (murciélagos, zorrillos, etc.). Del total de agresiones registradas se dieron 1,135 tratamientos de rabia a humanos, lo que equivale al 43.64% de las agresiones. Además, estas acciones se reforzaron con la esterilización de 2,124 mascotas como medida de apoyo para la disminución de fauna rural y urbana nociva. Complementadas también estas acciones con el envío de 110 muestras para vigilancia epidemiológica. Finalmente como medida preventiva y siendo la acción más fuerte de este programa, se realizó la aplicación de 472,619 dosis de vacuna antirrábica canina. Para el año 2014 se incrementarán las acciones específicas en cada Jurisdicción Sanitaria, con el objetivo seguir manteniendo el estado libre de esta zoonosis de muy alta letalidad.

## ***P08 Incidencia de personas agredidas por animales susceptibles de transmitir la rabia en el municipio de Morelia.***

*María Beatriz Pichardo Argüello, Liliana Hernández Corona & Melitón Rosales Rosas.*

Introducción: El Municipio de Morelia registra el 30% de las agresiones por animales susceptibles de transmitir la rabia en el estado, con un promedio de 900 incidentes por año, ocupando asimismo y el 1er. lugar en indicación de tratamientos antirrábicos a la población expuesta. Objetivo: Identificar la edad, sexo, lesiones, exposición, circunstancias de la agresión, estado vacunal de animales agresores, colonias de procedencia de las personas expuestas y presupuesto erogado en la atención antirrábica de personas agredidas del municipio de Morelia. Métodos: Revisión retrospectiva de información de personas agredidas en el período 2011-2013 atendidas por el Centro de Salud urbano “Dr. Juan Manuel González Urueña de la ciudad de Morelia, así como costos de biológicos antirrábicos humanos de los años señalados. Resultados Los resultados de la investigación referida, permitirán justificar intervenciones que den cabal respuesta a las necesidades de salud pública identificadas. Conclusiones: La información obtenida, permitirá tomar decisiones que contribuyan a la disminución de agresiones y gastos derivados de la atención antirrábica, en el Municipio de Morelia.

## ***P09 Análisis epidemiológico de la encefalitis rábica humana en el Estado de México. 1979-20014.***

*Pérez-Sotelo LS, Gutiérrez MCA & Soto GG.*

El objetivo es describir el comportamiento epidemiológico de todos los casos de encefalitis rábica humana ocurridos en el Estado de México en el periodo que comprende los años de 1979-2003. Para abordar el análisis epidemiológico de la encefalitis rábica humana, se llevó a cabo un estudio retrospectivo de los casos registrados durante el periodo de tiempo comprendido del año 1979 al año 2014. Este lapso de tiempo a su vez se dividió en cuatro periodos; el primero comprende del año 1979 a 1982; el segundo de 1983 a 1987, el tercero de 1988 a 1997, el cuarto de 1998 al 2003 y por último el periodo 2004-2014. La delimitación de estos cuatro periodos de tiempo se consideró con base en los registros de la fuente de datos (ISEM), lo cual facilitó el manejo y análisis del objeto de estudio. Se elaboraron las estadísticas vitales en salud y se construyeron los instrumentos de vigilancia epidemiológica. Primer periodo que comprende los años 1979 a 1982 con 40 casos. El segundo periodo comprende los años de 1983 a 1987 con 42 casos. El tercer periodo comprende de 1988 a 1997, donde se presentaron 48 casos. El cuarto periodo que comprende de 1998 a 2003 registra 3 casos. El último periodo 2 casos. Los resultados finales de este análisis nos indican que de los 135 casos referidos entre el periodo de 1979 a 2014, el mayor número de agredidos fueron varones que habitaban en el medio semirural, se encontraban en edad escolar y pertenecían a la clase económica I Respecto a los agresores, fueron caninos en el 87.97% se caracterizaron por tener dueño el 45.86%, realizando la transmisión de la rabia por mordedura en el 85.71% de los casos, generalmente en los miembros superiores 27.07% e inferiores 20.30%.

# Rabia animal

## Animal rabies

### Raiva animal

#### ***P10 Estudio histológico descriptivo y comparativo del sistema cardiovascular de los murciélagos hematófagos *Desmodus rotundus* y *Diphylla ecaudata*.***

*Vargas-Soto Luz María, Estrada-García Julieta, Olave-Leyva J Ignacio, García-Reyna Patricia B & Ocampo-López Juan.*

Los murciélagos hematófagos *Desmodus rotundus* y *Diphylla ecaudata*. Son mamíferos clasificados dentro del orden Chiroptera, suborden Microchiroptera, superfamilia Phyllostomidae y familia Desmodontidae. Su importancia radica principalmente en la producción pecuaria y la salud pública, ya que son potencialmente transmisores del virus de la rabia, que afecta al hombre y sus animales domésticos. El objetivo de este trabajo es describir y comparar las características histológicas del sistema cardiovascular de estos mamíferos. Se obtuvieron muestras a partir de 5 murciélagos de cada especie, que fueron capturados en los municipios hidalgüenses de Progreso de Obregón (*D. rotundus*) y Huehuetla (*D. ecaudata*). Se colectaron muestras de los diversos órganos y estructuras del sistema cardiovascular, las cuales fueron fijadas en formalina al 10% y procesadas mediante el método de inclusión en parafina, para obtener cortes de 6  $\mu$ m de espesor, los cuales se tiñeron con los métodos de H-E o Tricrómica de Masson. A partir de las preparaciones histológicas obtenidas se describe y compara la organografía de los órganos y las estructuras del sistema cardiovascular de ambas especies: corazón, arterias elásticas, arterias musculares, arteriolas, capilares (continuos, fenestrados y sinusoides), vénulas y venas pequeñas, medianas y grandes. Se pretende que esta investigación sirva como marco de referencia para estudios posteriores de estas especies, poco estudiadas a nivel de su anatomía microscópica.

## ***P11 First description of rabies virus in a cat after 13 years, in the State of Rio Grande do Sul, southern Brazil.***

*Helena Beatriz de Carvalho Ruthner Batista, Julio Cesar Almeida Rosa, Marcelia Emanuele Sad Fernandes, Patricia Mariano Cruz Pereira, Rafael de Novaes Oliveira, Jose Carlos Ferreira, Juliana Galera Castilho, Laura Lopes Almeida, Pedro Carnieli Junior & Paulo Michel Roehle.*

Epidemiologically, rabies virus (RABV) is maintained in distinct biological cycles, with different natural reservoir species. In the urban cycle, the domestic dog is the main reservoir of RABV. In the sylvatic cycle, in Latin America, the main reservoir of RABV is the vampire bat *Desmodus rotundus*. In the state of Rio Grande do Sul (RS), southern Brazil, urban rabies has not been detected since 1988. Nevertheless, rabies in vampire bats remains endemic. The aim of this study was to determine genetic characteristics of a RABV recovered from a cat (26-V/14) that died of rabies in the city of Capão do Leão, RS, and to infer the most likely source of contamination of the animal. The virus was identified by direct immunofluorescence and mouse intracerebral test. A fragment of the viral genome corresponding to the nucleoprotein (N) gene was submitted RT-PCR and the amplicon obtained was sequenced and compared to other RABV sequences available at GenBank. By alignment the sequenced fragment of 26-V/14 with equivalent RABV sequences available at GenBank, the highest identity was found between 26-V/14 and RABV genetic lineage whose natural reservoirs are the vampire bats *D. rotundus*. Therefore, the most likely source of contamination of the cat was an incidental contact with an infected bat of that species. In view of that, the status of “urban rabies-free” of the area should not be compromised.



## ***P12 Formación de brigadas municipales como estrategia para el control de rabia transmitida por murciélago hematófago en Michoacán, México.***

*Jorge Arturo Arana Sandoval, Edgar Ulises González Solache, Enrique Anaya Rodríguez & Antonio de Jesús Trejo Marin.*

El estado de Michoacán se localiza en el centro occidente del país. Se considera una zona endémica con presencia de murciélago hematófago *Desmodus rotundus*, principal transmisor de virus rábico, que ocasiona pérdidas importantes en la ganadería bovina del estado, con una consecuente disminución del inventario ganadero; además de que en los últimos años (2008-2013), se ha presentado un aumento progresivo en el número de agresiones a personas que normalmente habitan en el medio rural y eventualmente del área urbana, lo que invariablemente constituye un alto riesgo para la salud pública. Ante esta problemática, se ha propuesto la formación de brigadas municipales que mediante la firma de un convenio de colaboración, coordinación y aportación con municipios que presentan esta problemática en Michoacán, se realizan acciones de capacitación a brigadas municipales integradas por personas que, previo tratamiento pre exposición, efectúan operativos tendientes a controlar las poblaciones de murciélago hematófago, para disminuir de manera considerable las agresiones al ganado y personas.

# ***P13 Estudio histológico descriptivo y comparativo del aparato respiratorio de los murciélagos hematófagos Desmodus rotundus y Diphylla ecaudata.***

*Pérez-Aguirre Germán, Hernández-Guzmán J Luis, Acosta-Salinas Rosalinda, García-Reyna Patricia B & Ocampo-López Juan.*

Los murciélagos hematófagos *Desmodus rotundus* y *Diphylla ecaudata* son mamíferos clasificados dentro del Orden Chiroptera, suborden Microchiroptera, superfamilia Phyllostomidae y familia Desmodontidae. Su importancia radica principalmente en la producción pecuaria y la salud pública, ya que son potencialmente transmisores del virus de la rabia, que afecta al hombre y sus animales domésticos. El objetivo de este trabajo es describir y comparar las características histológicas del aparato respiratorio de estos mamíferos. Se obtuvieron muestras a partir de 5 murciélagos de cada especie, que fueron capturados en los municipios hidalguenses de Progreso de Obregón (*D. rotundus*) y Huehuetla (*D. ecaudata*). Se colectaron muestras de los diversos órganos y estructuras del aparato respiratorio, las cuales fueron fijadas en formalina al 10% y procesadas mediante el método de inclusión en parafina, para obtener cortes de 5  $\mu$ m de espesor, los cuales se tiñeron con los métodos de H-E o Tricrómica de Gomori. A partir de las preparaciones histológicas obtenidas se describe y se compara la organografía de los órganos y estructuras del aparato respiratorio de ambas especies: cavidad nasal, senos paranasales, nasofaringe, laringe, tráquea, bronquios extrapulmonares y pulmones. Se pretende que esta investigación sirva como marco de referencia para estudios posteriores de estas especies, poco estudiadas a nivel de su anatomía microscópica

## ***P14 Dueño socialmente responsable.***

*José Ramón Hernández Pérez.*

Actualmente los Servicios de Salud del Estado de Tlaxcala trabajan en cinco estrategias para el control de la rabia: Atención de personas agredidas, Vacunación antirrábica canina y felina, Estabilización de la población canina y felina, Vigilancia epizootiológica y Promoción a la salud.

De esta última se desprende el presente trabajo, teniendo como objetivo que los dueños de perros y gatos conozcan y apliquen normas mínimo necesarias para proveer al animal de los cuidados básicos de bienestar: salud, alimentación adecuada, espacio de descanso protegido de las inclemencias del tiempo, espacio para eliminar sus residuos, recreación y cariño. Lo anterior a través de talleres dinámicos y divertidos, dirigidos a alumnos de nivel preescolar y primaria, estos talleres se realizan en comunidades en donde la población canina presenta una alta densidad, existe un alto número de agresiones a la población y la vacunación antirrábica canina y felina no alcanza el 100% de cobertura, buscando eliminar en el mediano plazo el perro callejero de nuestras comunidades y por ende mantener coberturas de vacunación al 100%, disminuir al mínimo las agresiones de perros a la población y el porcentaje de tratamientos antirrábicos indicados y estabilizar como mínimo el 10% de la población canina y felina.

## ***P15 Macro jornadas de esterilización quirúrgica de perros y gatos: estrategia coadyuvante en la prevención de la rabia.***

*Juan M. Balderas T., Solís-Tapia D., Martínez-Mateos V., Franco-Domínguez V., Doroteo-Cid V., Calderón-Tirado F., Luna-Luna G. & Andrade-Roca J.*

La rabia, tiene en la vacunación oportuna y con biológicos de calidad, una fórmula efectiva de prevención y control, en el corto y mediano plazo, pero las acciones coadyuvantes para la regulación de la cantidad de perros y gatos son indispensables; los Servicios de Salud del Estado de Puebla a partir del año 2013, decidió privilegiar las esterilizaciones quirúrgicas y promover el sacrificio humanitario y profesional de estos animales. En febrero de 2014 se implementan las Macro Jornadas de Esterilización Quirúrgica, Voluntarias y Gratuitas con el lema “La responsabilidad hacia nuestras mascotas se enseña”, en municipios de interés epidemiológico y capacidad organizativa; se instalan módulos en localidades, centros o casas de salud y en un solo día se da respuesta a todos aquellos poseedores responsables que hayan decidido esterilizar a sus animales. Son convocados los más de 60 Médicos Veterinarios del Programa Estatal de Zoonosis, que habitualmente atienden a las 10 jurisdicciones sanitarias y los 217 municipios que conforman al Estado. Es fundamental la colaboración de las autoridades municipales para el desplazamiento del personal Médico Veterinario hacia los módulos asignados. La promoción de estos eventos cuenta se apoya en los medios masivos de comunicación y con el apoyo de las auxiliares y comités de salud para la promoción, y difusión cara a cara. En este año se han efectuado 7 Macro Jornadas, en los municipios de Puebla, San Martín Texmelucan, Atlixco, San Matías Tlalancaleca, Tehuacán, Zacatlán, Acatzingo y Atempán, atendiendo a 7 de las 10 jurisdicciones, permitiendo superar un 40.1% lo logrado en el primer semestre del año 2013. Se han sumado Organizaciones de la Sociedad Civil, Facultades de Medicina Veterinaria y grupos de Médicos Veterinarios locales y se han contemplado a las empresas pecuarias. La gestión administrativa central ha sido clave para garantizar el aporte de los insumos propios.

## ***P16 Foci of bovine rabies and the railroad Carajás in the Maranhão.***

*Roberto Carlos Negreiros de Arruda & Anizio José Rocha.*

Aiming to raise the impact of the construction of the hydroelectric plant (Usina Hidrelétrica de Estreito) and the displacement of *Desmodus rotundus* in rabies cases in the western region of the state of Maranhão, in the period 2007 to 2013 and movement of these vampire bats and the outbreaks of rabies. We used terraView software. In the evaluation it was observed that outbreaks of rabies are in the area, follow the Carajás Railroad (Estrada de Ferro Carajás), at least 45km per year, of which 16 cases observed, 90% of them are in the city of Açailândia. Attention is drawn to the North South Railway (Estrada de Ferro Norte Sul), which does not have surveillance and capture and treatment of *D. rotundus*, are new cases that may occur. We conclude that the construction of the hydroelectric plant shifted vampire bats with rabies virus to other locations, but outbreaks do not spread very limiting to the same municipalities, yet those sick who took shelter in the Carajás Railroad, who were causing a real impact on cattle in the region.

## ***P17 Resultados del censo canino y felino 2013 en la jurisdicción sanitaria Atizapán de Zaragoza.***

*Roberto Castillo Delgado.*

Para la vacunación antirrábica canina y felina principal indicador del programa de prevención y control de la rabia, es necesario conocer en todo nivel operativo el universo de perros y gatos por vacunar hasta la unidad mínima las áreas geográficas estadísticas básicas (AGEBS) de responsabilidad. En el caso particular de la Jurisdicción Sanitaria Atizapán de Zaragoza en principio se estableció la metodología para programar los animales por vacunar cada año tanto por municipio como por unidad aplicativa de salud: por logros históricos de los últimos cinco años; y posteriormente con bases de datos del INEGI en la que se tomó la columna de total viviendas por ageb's para determinar que encada casa existe un perro o gato, y así calcular la relación humano:perro/gato. Lo que ha permitido contar a la fecha con una cobertura útil. Sin embargo, se carece de un estudio actualizado y contundente para complementar la información disponible por otras fuentes oficiales de información o estudios previos. El objetivo del presente trabajo es estimar con la metodología por encuestas el universo de perros y gatos por vacunar en la jurisdicción sanitaria Atizapán de Zaragoza en base a la densidad de población humana de cada área geográfica estadística básica de responsabilidad (AGEB's). Con las estrategias del: Diseño conceptual, diseño muestral, levantamiento, procesamiento y análisis de resultados. Además de mostrar mapas temáticos por municipio y AGEBS con gradiente de colores mostrando las áreas con mayor cantidad de perros y gatos y encima de estos las capas de personas agredidas por animal sospechoso con vacunación antirrábica canina vigente y no vigente desde el año del 2008 al primer semestre de 2014. Lo que ha permitido focalizar acciones de prevención y control en el reservorio.

## ***P18 Intratesticular injections of calcium chloride result in necrosis of the testicles.***

*Ruth Steinberger.*

The technique developed by Wayne Haney, DVM of Oklahoma, US for intratesticular injections of calcium chloride dihydrate in ethyl alcohol is an effective, low cost, non-surgical method to permanently castrate dogs. While castrating male dogs is not documented to have a significant impact on population control, it does reduce behaviors that escalate when a female is in estrus. The U.S. Centers for Disease Control and Prevention (US CDC) notes that 76 percent of dog bites involve intact males. A 1976 study concluded that the male behavioral patterns of roaming, fighting with other males, and mounting of other dogs or people are amenable to alteration by castration in adulthood. Castration of male dogs alters libido, roaming and behavior that may lead to bites; papers have existed since 1978 on the use of calcium chloride in alcohol for non-surgical castration of animals, and the formulation, dosage and technique developed by W. Haney, DVM provides an easy, effective technique for non-surgical castration of male dogs. A 1978 brief in *Modern Veterinary Practice* by Koger, DVM, described the use of calcium chloride for castration of dogs. 2007 and 2011 publications by Kuladip Jana and Prabhat Kumar Samanta documented that intratesticular injections of calcium chloride result in necrosis of the testicles, permanently and significantly reducing testosterone without surgery. The compound is easy to prepare and the ingredients are widely available across the globe. In 2013, Wayne Haney, in partnership with Spay FIRST created an experimental three dog pilot study in order to discover if the use of this compound may simplify canine castrations. 28 days after injection the dogs received 50 mg/kg ml HCG. Blood was drawn 120 minutes following injection of HCG and testosterone samples were analyzed at Colorado State Endocrine Lab. Two of the three dogs had testosterone levels below that of a castrated dog, one large dog had a slightly higher testosterone level. Haney has modified the techniques described in earlier publications and developed a dosing chart and technique that has resulted in testosterone levels at or below that of a castrated dog when used on dogs under 55 pounds.

## ***P19 Statistical survey of unconventional species submitted for rabies diagnosis at Pasteur Institute of São Paulo.***

*Samira Maria Achkar, Helena Beatriz de Carvalho Ruthner Batista, Rafael de Novaes Oliveira, Elaine Raniero Fernandes; Fernanda Guedes; Carla Isabel Macedo, & Juliana Galera Castilho.*

Rabies can manifest in several mammalian species, however, some species are more frequently affected by the disease and therefore can be considered as conventional species when sent for laboratory diagnosis. In Brazil, the main representatives of these species are dogs, cats, cattle, horses and bats. However, unconventional species may also be affected by rabies and should be sent to laboratory diagnosis when was suspected. For the present study, unconventional species were classified according to the Order and Family or Subfamily. In the period 2009-2013, the Rabies Diagnostic Laboratory of the Pasteur Institute of São Paulo received 40.950 samples of which 451 (1.1%) were from unconventional species, which were subjected to direct immunofluorescence test and viral isolation in cell culture and in mice. Only one sheep sample (Order Artiodactyla, Subfamily Caprinae) was positive in the three techniques. The species belonging to the Primates Order were the most abundant, representing 31.7% of the total, followed by Didelphimorpha 21%; Carnivora and Artiodactyla, with 15.8% each; Rodentia with 9.3%; Lagomorpha with 2.9%; Pilosa 2.7%; Cingulata with 0.6% and Erinaceomorpha with 0.2%. The main families were the Callitrichidae, represented by marmosets; Didelphidae, represented by opossums; Canidae, represented by the wild dogs; Suidae, represented by the pigs; Muridae, represented by rats; Leporidae, represented by rabbits and Myrmecophagidae, represented by anteaters. Even with the low positivity in the samples studied, the routing of unconventional samples for laboratory diagnosis is extremely important for that epidemiological surveillance measures to be adopted and, in cases where there is a history of attack on humans, rapid measures are taken regarding the medical conduct in relation to the individual exposed.



## ***P20 First report of rabies in crab-eating fox (Cerdocyon thous) due to the virus variant maintained by marmoset (Callithrix jacchus) in northeast Brazil.***

*Naylê Francelino Holanda Duarte, Keila Iamamoto Nogi, Danielle Bastos Araujo, Rossana de Aguiar Cordeiro, Adriana Candido Rodrigues, José Júlio Costa Sidrim, Rogério Azevedo, José Airton de Negreiros, Benedito Neilson Rolim, & Silvana Regina Favoretto.*

A crab-eating fox (*Cerdocyon thous*) found dead (probably road-kill) in Guaraciaba do Norte in Ceara State, Northeast Brazil, was diagnosed as positive for rabies virus through FAT and MIT. The sample was also submitted to the identification of virus variant using a panel of monoclonal antibodies (CDC, Atlanta, USA), and the pre established variant maintained by marmosets (*Callithrix jacchus*) was identified. This is the first report of the marmoset variant identified in a crab-eating fox. In Brazil, there are four animal species responsible for maintain independent variants of rabies virus with direct transmission to humans: dogs (*Canis familiaris*), common vampire bat (*Desmodus rotundus*), marmosets and crab-eating fox. To date, the marmoset and the crab-eating fox variants are restricted to the Northeast region. The city of Guaraciaba do Norte is located 299Km from the State capital, Fortaleza, and the animal was found in a rural area. The habitants report the presence of other dead crab-eating foxes (without diagnosis), the finding of live foxes in the residences and the occurrence of human aggressions caused by marmosets bites (material sent for diagnosis). After the positive diagnosis, the Ceara Secretary of Health made a vaccine blocking in dogs and cats. The unique case of a rabid crab-eating fox, where the isolated variant did not correspond to the maintained by this species, was identified in Sao Paulo State, Southeast of Brazil, was due to the *Desmodus rotundus* variant. The presence of different virus variants in the area, the occurrence of contamination between these two host species and reports of interaction with wild animals and humans are important issues to be observed in rabies surveillance and control. The findings of the present study confirm the importance of epidemiologic studies regarding rabies in terrestrial wild mammals in the region, combined with the actions for rabies control already adopted in the State.

## ***P21 Control de murciélago hematófago utilizando vampiricida sistémico en época de lluvias.***

*José Luis Hernández Guzmán.*

El objetivo del presente trabajo de campo en la sierra Otomí-Tepehua Estado de Hidalgo es reducir las agresiones ocasionadas a los bovinos por el murciélago hematófago al alimentarse de sangre durante la noche; geográficamente Hidalgo se ubica en la parte central del país. Incrustada al Noroeste del Estado, la Región Otomí-Tepehua, se localiza en la sierra madre oriental, con una orografía muy irregular, donde predominan montañas y cerros con presencia abundante de vegetación; se encuentra en el paralelo 20° 20' y 20° 40' de latitud norte y los meridianos 98° 00' y 98° 20' de latitud oeste, con una superficie de 1,105.3 Km<sup>2</sup>. Cubre 4 municipios (Agua Blanca de Iturbide, Tenango de Doria, San Bartolo Tutotepec y Huehuetla), temperatura promedio anual entre los 16° y 22°C, clima semicálido-humedo (lluvias todo el año), precipitación pluvial de 2000-2500 mm promedio anual, que duran de julio a febrero regularmente. En temporada de lluvias es muy difícil realizar trampeos con la técnica de red para mantener un control de este quiróptero, se programan reuniones con productores de ganado bovino de cuatro comunidades en tres Municipios: Mpio. Tenango de Doria, Comunidad San Pablo el Grande cuenta con 25 productores y 220 cabezas de ganado; Comunidad La Concepción cuenta con 10 productores y 173 cabezas de ganado; Mpio. Huehuetla, Comunidad Chicontla cuenta con 9 productores y 110 cabezas de ganado; Mpio. San Bartolo Tutotepec, Comunidad Los Manantiales cuenta con 12 productores y 88 cabezas de ganado; sumando un total de 56 productores y 591 cabezas; se les da a conocer los beneficios de usar esta técnica durante la temporada de lluvias y estando de acuerdo se revisa el ganado observando lo siguiente: Comunidad San Pablo el Grande 50 bovinos mordidos, 22.7% agresiones; Comunidad La Concepción 45 bovinos mordidos, 26.0% agresión; Comunidad Chicontla 47 bovinos mordidos, 42.7% agresión; Comunidad Los Manantiales 32 bovinos mordidos, 36.3% agresión; dando un total de 174 bovinos mordidos y 29.4% de agresión antes del tratamiento (AT); por ello se optó por utilizar un vampiricida inyectable elaborado con un anticoagulante (warfarina) a una concentración de 5 mg/kg; se aplica el vampiricida sistémico solo a los animales que se observan agredidos; diez días posteriores a su aplicación regresamos a revisar, cuantos bovinos siguen siendo agredidos, encontrando: Comunidad San Pablo el Grande 3 bovinos mordidos, 1.3% agresión; Comunidad La Concepción 1 bovino mordido, 0.5% agresión; Comunidad Chicontla 0 bovinos mordidos, 0.0% agresión; Comunidad Los Manantiales 2 bovinos mordidos, 2.2% agresión; dando un total de 6 bovinos mordidos y 0.7% de agresión después del tratamiento (DT); reduciendo la presencia de mordeduras de 174 bovinos y 29.4% de agresión (AT) a 6 bovinos y 0.7% de agresión (DT); el % de reducción de mordeduras fue del 99.3%.

## ***P22 Evaluation of a novel bait matrix for delivery of oral rabies vaccine.***

*Kurt VerCauteren, Shylo Johnson, Nikki Crider, Christine Ellis, & Amy Gilbert.*

The goal of the Rabies Project at the National Wildlife Research Center is to provide research support to the National Rabies Management Program of USDA/APHIS/Wildlife Services to aid in the control of wildlife rabies. Previous efforts have demonstrated that uptake of oral rabies vaccine baits by wild reservoir species is marginal and thus one primary focus is to develop baits that are more attractive and have better ballistic potential for delivery of oral rabies vaccine. To this end, in cooperation with SmartVet, Inc., we have conducted trials to evaluate handling, consumption, and flavor preference of prototype placebo baits with captive and wild raccoons and skunks. In captive trials, raccoons consumed 82% of baits placed in their pens within 4 hours and 100% within 24 hours, and they preferred fish-flavored baits to sweet. Skunks consumed 44% of baits placed in their pens within 4 hours and 91% within 24 hours; results of flavor preference are pending. We have also completed a preliminary trial to assess the uptake of the placebo baits by target and non-target species in rural and urban areas. Conclusions and products from this effort will lead to improved bait delivery and consumption by target species.

## ***P23 Intervención de los municipios en la vacunación antirrábica canina y felina.***

*Verónica Ivett Franco Domínguez & Juan Manuel Lagunes Lara.*

El objetivo del presente trabajo es dar a conocer la importancia de la intervención de las autoridades municipales en coordinación con la Jurisdicción Sanitaria 2 Chignahuapan, Puebla en la ejecución de las campañas masivas de vacunación antirrábica canina y felina (SNVACyF) acción indispensable para el control del virus rábico urbano (perro y gato) circulante en el territorio de responsabilidad de la Jurisdicción antes mencionada. Se desarrolla en los 14 municipios que conforman la Jurisdicción Sanitaria No 2 Chignahuapan de la siguiente forma: 1. Antecedentes de la vacunación canina, 2. Justificación de la intervención de los municipios, 3. Transición de la ejecución para la vacunación en la región, 4. Coordinación de las actividades de la vacunación con Jurisdicción Sanitaria No. 2, 5. Estrategias para realizar las acciones para el logro de la vacunación, 6. Capacitación teórico-práctico del personal municipal asignado para la ejecución de la actividad 7. Resultados obtenidos 8. Entrega de resultados. 9. Análisis de resultados para reorientar acciones enfocadas para el cumplimiento de la meta. Este material contiene la organización y coordinación con las autoridades municipales para realizar las jornadas masivas de vacunación en fases intensivas como lo es en Semana Nacional y de Reforzamiento de la vacunación antirrábica canina, donde se refleja que en el trabajo conjunto y coordinado, brinda resultados satisfactorios para asegurar la vacunación de todos los perros y gatos de responsabilidad en el territorio de cada municipio y como consecuencia de la Jurisdicción, así como la hacer partícipe a diferentes instancias municipales para ejecutar dicha acción y creando conciencia a las autoridades locales y municipales de la importancia de su participación para mantener en control la enfermedad de la rabia que es un padecimiento mortal. Esta estrategia ha permitido mantener a la Jurisdicción sin casos de rabia canina desde 2001 que fue el último registro de rabia en perros en la Jurisdicción Sanitaria No. 2 Chignahuapan con una cobertura desde mayor a 90% con la sensibilización de las autoridades y la población sobre la importancia de mantener vacunados a sus mascotas haciéndose responsables de ellas.

## ***P24 Sistema de vigilancia epizootiológica de la rabia basado en riesgo, en el estado de Puebla, México.***

*Verónica Ortega-Chávez, Balderas-Torres J.M, Franco-Domínguez V, Mejía-Solís A, Calderón F, Sánchez-Barrera E, Pérez-Ramírez A, Arroyo-Vázquez G & Martínez-Vázquez V.*

Un sistema de vigilancia epizootiológica confiable es la clave para la alerta temprana de un cambio en el estado de salud de cualquier población animal; en el caso específico de la rabia canina en el estado de Puebla, México, a pesar de su control en el año 2008, es indispensable mantener las acciones exitosas e innovar con estrategias específicas, integrales y locales para evitar la emergencia o reemergencia de la enfermedad. Por tanto, en el 2010 se implementó el Sistema de Vigilancia Epizootiológica de la Rabia Basado en Riesgo (SVERBR), que permite analizar 16 factores de riesgo que influyen en la probabilidad y consecuencia de transmisión y difusión del padecimiento, cabe señalar que esta evaluación permite una mejor toma de decisiones para la prevención y control. Con este Sistema de Vigilancia se busca fortalecer la regionalización, para lograr la descentralización y desconcentración del personal para obtener un diagnóstico integral con mayor sensibilidad, una mejor administración del recurso humano y operación más efectiva. Este análisis se realiza mediante el llenado de las cédulas de caracterización de riesgo mediante un sistema de calificación de 0 a 5 puntos por cada factor de riesgo, obteniendo una puntuación máxima de 80 puntos, evaluando cada unidad médica y sus áreas de influencia de las zonas SESA e IMSS-Oportunidades. Este SVERBR en los años de evaluación (2010, 2011 y 2014) ha permitido visualizar la transición o mantenimiento del riesgo y ha permitido redirigir y priorizar las zonas a trabajar de acuerdo a los determinantes identificados y a los resultados obtenidos de la evaluación, para minimizar el riesgo de la circulación del virus rábico, aumentando la sensibilidad en el monitoreo y en el mantenimiento del estatus epizootiológico de la rabia en el Estado.

## ***P25 Control de la rabia paralítica bovina.***

Hernández Guzmán JL

El objetivo del presente trabajo es la capacitación a través de la información del control de la rabia bovina y del murciélago hematófago, de una manera didáctica y comprensible para que se lleven a cabo actividades referentes a la prevención y control de esta enfermedad que además de ser mortal representa un problema de salud pública.

Material didáctico para capacitación en el control y prevención de la rabia paralítica bovina.

Se desarrolla en los siguientes temas:

1. Situación actual de rabia.
2. Etiología de rabia.
3. Enfermedad rabia bovina.
4. Vacunación en el ganado.
5. Toma y envío de muestras.
6. Diagnóstico de rabia.
7. Biología de los murciélagos.
8. Captura de vampiros.
9. Calendario lunar.
10. Control del vampiro.

Este material contiene una interesante recopilación de fotografías y está diseñado de una manera didáctica y ágil. Con esta capacitación se espera un incremento en la implementación de medidas de control y prevención, aunado a un mayor reporte de animales con signos nerviosos; se pretende formar brigadas de captura de vampiro integrada por los mismos productores de ganado. Con la capacitación lograr dominar la técnica de red, tanto en el tendido como retiro de redes y no sólo identificarlos, sino manejar los murciélagos y aprender a diferenciarlos, para mantener un control en la población de los hematófagos y no dañar a los murciélagos con un hábito alimenticio diferente. Es muy importante saber que se requiere realizar un buen diagnóstico y esto empieza desde la toma de muestra, consideramos sea importante conocer qué órganos debemos conservar y qué tipo de prueba de laboratorio pedir. Recordando que la fauna silvestre es susceptible de enfermar de rabia y ser transmisor de esta enfermedad a los animales domésticos y al hombre. Después de la capacitación los productores serán conscientes de las causas y consecuencias de la rabia, y las ventajas de la medicina preventiva; logrando así un impacto favorable en la producción nacional.

## ***P26 Constatación de productos biológicos contra la rabia en México.***

*Robles-Pesina G, Lima-Borja LM, Rivera-Zárraga A & Montaña-Hirose JA.*

En México la constatación es parte del registro de productos biológicos veterinarios realizada para asegurar que estos tienen la trazabilidad, rastreabilidad, características de calidad, seguridad, inocuidad y eficacia.

La constatación se realiza con base a la NORMA Oficial Mexicana NOM-063-ZOO-1999, Especificaciones que deben cumplir los biológicos empleados en la prevención y control de enfermedades que afectan a los animales domésticos, y se realiza a los biológicos veterinarios empleados en México, de fabricación nacional o extranjera y deben concordar con los requisitos establecidos a nivel nacional e internacional.

La DGSA es la responsable de coordinar las acciones de registro y constatación, esta última se realiza en el Centro Nacional de Servicios de Diagnóstico en Salud Animal (CENASA), en los últimos tres años, se han constatado 8 productos de rabia para uso en caninos y felinos, 7 casos para vacunas inactivadas 4 cepa CVS, 2 con Pasteur y una con PV; el otro caso se realizó la constatación de un fluido viral activo.

Cuadro de productos para caninos y felinos

Muestra	Cepa	Año
Vacuna	CVS (ATCC VR-959)	2012
Fluido viral activo	CVS	2012
Vacuna	CVS	2013
Vacuna	CVS	2013
Vacuna	CVS	2013
Vacuna	Pasteur	2013
Vacuna	PV	2013
Vacuna	Pasteur	2014

Las pruebas que se realizan para la constatación consisten en: Esterilidad, Pureza, Seguridad o inocuidad, Prueba de titulación, Prueba de inactivación de un biológico de origen viral, Prueba "in vivo", Prueba de identidad, Prueba de potencia, las cuales deben obtener resultados satisfactorios en su totalidad para su liberación, dado que la vacunación representa una estrategia vital que permite reducir la incidencia de las enfermedades que merman la riqueza pecuaria del país.

Lo anterior se complementa con base a la legislación mexicana, que en el caso de rabia tenemos:

NOM-063-ZOO-1999, Especificaciones que deben cumplir los biológicos empleados en la prevención y control de enfermedades que afectan a los animales domésticos.

NOM-035-ZOO-1996, Requisitos mínimos para las vacunas, antígenos y reactivos empleados en la prevención y control de la rabia en las especies domésticas.

NOM-067-ZOO-2007, Campaña nacional para la prevención y control de la rabia en bovinos y especies ganaderas.

NOM-011-SSA2-1993, "Para la Prevención y Control de la Rabia" "For the Prevention and Rabies Control".

Su cumplimiento a esta normativa está dividido en entre el sector salud y salud Animal, por lo que es necesario definir y homologar criterios con base a una Salud.

## ***P27 Proyecto formativo integrador para el fomento de la cultura de tenencia responsable de animales de compañía y prevención de la rabia dirigido a estudiantes de nivel bachillerato y la comunidad de San Buenaventura en Ixtapaluca, Estado de México.***

*Barrera HMA, Hernández VF, González MS, Ojeda DJC, Ramírez GP, Miranda RLC, Reyes MT de J, Ortega MA, Montes de Oca CAE & Mendizábal GDG.*

El objetivo del trabajo fue realizar un proyecto formativo, considerando la dinámica de la Reforma Integral de Educación Media Superior en la que los estudiantes deben incluir el uso de la transversalidad entre las asignaturas para resolver problemáticas de la vida cotidiana. Para ello se propuso una campaña sobre la tenencia responsable de animales de compañía y la prevención de la rabia. Metodología: 1. Aplicación de un cuestionario sobre cultura de tenencia responsable de mascotas de compañía en la Unidad Habitacional San Buenaventura. 2. Selección de información bibliográfica de la problemática planteada. 3. Diseño de material de difusión sobre la importancia de ser un dueño responsable de animales de compañía. 4. Organización y realización de una Jornada Informativa, campaña de vacunación antirrábica y esterilización canina y felina en las instalaciones del CobaEM con el apoyo de las autoridades de la Jurisdicción Sanitaria de Amecameca, Estado de México. Resultados: Impartición de 3 conferencias a los alumnos y al público asistente sobre “La rabia y su diagnóstico”, “Atención médica antirrábica del paciente expuesto” y “El vínculo entre humanos y animales”. Exhibición de carteles informativos, entrega de folletos y trípticos. Instalación de un puesto de vacunación antirrábica y esterilización canina y felina. De acuerdo a los datos estimados en la encuesta realizada se tenía contemplado la vacunación de aproximadamente 348 animales y la esterilización de 80 mascotas, rebasando la expectativa al atender 560 animales para vacuna y 118 para esterilización. En el presente proyecto se destaca la participación de los estudiantes a nivel bachillerato en la prevención de enfermedades transmitidas por los animales de compañía, lo que fortalece el desarrollo de las competencias genéricas al participar de manera propositiva frente a fenómenos de interés general en la sociedad como es la prevención de la rabia y la tenencia responsable de sus mascotas.



## ***P28 Challenge of frugivorous Artibeus intermedius bats captured in the wild, with rabies virus (vampire bat origin V3 variant).***

*Obregón Morales Cirani, Aréchiga Ceballos Nidia, Perea Martínez Leonardo & Aguilar Setián Álvaro.*

Artibeus spp. bats are abundant in tropical and sub-tropical regions of Mexico and other Neotropical countries. Rabies virus (RABV) variants circulating in Artibeus spp. in Mexico are closely related to vampire bat variants. The objective of this study was to investigate the susceptibility of Artibeus intermedius adult bats seronegatives against RABV captured in the wild, to V3 variant, assessed through observations of incubation period, clinical signs, serologic response and distribution of RABV. We performed an experimental infection of Artibeus spp. with vampire bat isolates (V3). High ( $1 \times 10^5.34$  FFU) and low ( $1 \times 10^3$  FFU) doses of RABV, inoculated by three (SC, IM and IC) routes, were compared. Only one bat (IC inoculated) out of 38 died at day 14 post inoculation and was positive to rabies by FDA, but no clinical signs were observed. The bats in this experiment do not showed clinical signs of the disease, but high titers of antibodies were determined post-inoculation. These data suggest pre-exposure to RABV and decrease of antibodies titers below detection levels previous this study and therefore we consider that inoculation could act as a booster. A high neutralizing activity indicates that the infection is being controlled and animals survive. RABV nested PCR was positive in organs such as brain, stomach, liver and heart in animals that did not show clinical signs of disease and were negative by immunofluorescence. Recent studies have suggested that frequent contact to sub-lethal doses of RABV in wild colonies of bats may result in the establishment of immunity, protecting individuals from subsequent infections. These results suggest that previous non-lethal exposure to RABV somehow protected Artibeus spp. from rabies infection in this study, but the epidemiological role of frugivorous species in the maintenance of rabies virus in the wild remains to be studied.

## ***P29 Ending rabies now – One year of Mission Rabies and the future.***

*Lohr F, Gamble L, Gibson A & Shervell K.*

Rabies is the world's deadliest zoonotic disease. Statistically, at least one child dies of rabies in the world every twenty minutes – and that is almost certainly an underestimation. Once clinical signs manifest, there is no treatment. Over 99% of cases are transmitted through dog bites and the global incidence of the disease is on the increase. To eliminate the disease requires a focused approach of mass vaccination covering 70% of a resident canine population in the targeted area.

Since Mission Rabies was launched (September 2013), one street dog every 4 minutes was vaccinated. The Goa programme, targeted to eliminate the virus from the entire State in three years, just finished its first phase. During the 6 months of the initial programme phase 3-4 confirmed rabid dogs have been picked up every week. Using smart phones to GPS tag vaccinated dogs, as well as post vaccination surveys of the dog population of an area, it can be ensured that the 70% coverage required of each area targeted is hit.

Together with Mission Rabies' sister charity, Worldwide Veterinary Service (WVS), and the help of local and international volunteers, humane canine population control was also conducted, leading to a total of 20,000 street dogs in Goa neutered and vaccinated during the six months of the initial phase of the project.

Additionally, Mission Rabies also launched a concurrent community awareness programme explaining the dangers of rabies and how to avoid dog bites that has reached more than 70,000 children in Goa. The outcome of this programme will be evaluated through various studies.

Mission Rabies' plans for 2015 include launching projects in Malawi, Nepal, Sri Lanka and Ethiopia in addition to the ongoing activities in Goa. Again these projects will be accompanied by scientific studies to ensure the efficacy of the methods used.

# Estado actual de la investigación en rabia

## Current status of rabies research

### Estado atual da pesquisa em raiva

#### *P30 Effects of carcass decomposition on rabies virus infectivity and detection.*

*McElhinney LM, Marston DA, Brookes SM, Fooks AR.*

Carcasses received for rabies diagnosis are occasionally decomposed due to delays in finding, submitting or storing them. Positive diagnostic results from such samples are reliable but negative results may be invalid. Previous studies assessed the effect of decomposition on rabies detection using excised brains. To better reflect decomposition in the field, intact infected mouse carcasses were stored at three temperatures for up to 70 days. The brains were then removed and tested using routine rabies diagnostic assays. Rabies virus was isolated using the Rabies Tissue Culture Inoculation Test (RTCIT) on days 18, 3 and 3 at 4°C, 25°C and 35°C, respectively. The Fluorescent Antibody Test (FAT) detected viral antigen on days 36, 12 and 3, whilst a rabies specific Hemi-nested RT-PCR detected viral RNA on days 70, 48 and 48 at 4°C, 25°C and 35°C, respectively. These findings suggest the persistence of infectious rabies virus in carcasses left for 18 days at cold temperatures (4°C) and up to 3 days in temperatures reaching 35°C. The detection of viral RNA from a carcass decomposing at 35°C for 48 days supports the use of molecular assays to accompany OIE-prescribed rabies diagnostic tests particularly when decomposed samples are likely to be submitted. Such studies also provide essential validation data to support Quality Assurance accreditation to international standards (e.g. ISO17025).

## ***P31 “Como células produzem (lyssa)vírus” ou “a aplicação de virologia básica no entendimento da virose que leva à raiva”.***

*Phyllis Catharina Romijn; Leda Maria Silva Kimura.*

A produção de vírus por células animais até a apresentação de um quadro clínico depende de uma série de fatores, sendo em primeira instância determinada pela presença de determinados receptores na célula interagindo com determinado epítipo na partícula viral. Da endocitose da partícula viral nas células de um mamífero até ocorrer uma infecção letal o caminho é longo, havendo inúmeras oportunidades do processo ser abortado. Quando há produção de vírus completo, a exocitose de vírus da célula pode se dar pelo lado apical e/ou basal dependendo do tipo de célula, se epitelial, muscular, nervosa. Assim, no caso da virose por Lyssavirus, dependendo da célula infectada e das células vizinhas a ela, a probabilidade de partículas virais chegar e serem produzidas por células do sistema Nervoso Central e haver o desenvolvimento da doença clinicamente fica bastante reduzida. Pode-se considerar que a maioria das infecções por Lyssavirus são subclínicas, apesar de poder levar a produção de anticorpos detectáveis. Sob este aspecto, a (seleção) genética das espécies suscetíveis exerce um papel relevante, sobrevivendo a subpopulação com menor capacidade em produzir vírus completo e cujo sistema imune mais cedo identificar e eliminar células infectadas.

## ***P32 Standardization of an enzyme immunoassay using rabies virus G recombinant proteins from insect larvae.***

*Rojas-Anaya E, Loza-Rubio E, Escribano-Romero E, Blazquez AB, Saiz-Calahorra JC & Martinez-Escribano JA.*

The objective of this research was to establish the conditions for detection of rabies antibodies by an ELISA assay using recombinant proteins derived from insect larvae. The G gene of the rabies virus was expressed in Bac to Bac system in order to obtain a recombinant baculovirus, and then *Trichoplusia ni* insect larvae were infected. The recombinant protein G (pGr) was semi-purified by nickel columns and identified by western blot using anti-histidine antibodies. 96-well plates were coated with different concentrations of pGr and different dilutions of a positive control antibody were used to establish the suitable concentration. In order to evaluate the ELISA, 60 serum in total (39 positive; 21 negative) corresponding to 23 of human being, 10 to bovine, 11 to dogs and 16 to bats, with vaccination history for human, dogs and bovine. The same sera were tested by the rapid reduction fluorescent focus inhibition test (RFFIT). Western blotting demonstrated the expression of a protein of 65 kDa corresponding to the pGr, obtaining up to 4.5 mg of total protein per 20 larvae. When sera were tested by both serological tests, the result of concordance by the Kappa test was 0.63, demonstrating good concordance between both techniques. Specificity and sensitivity obtained by Chi-square test were 93% and 80%, respectively, with a positive predictive value of 97% and a negative predictive value of 62%, however, the ELISA is unable to detect vaccinated and non-vaccinated animals. We conclude that the use of this immunoassay allows the detection of antibodies of different species that are affected by rabies virus. Moreover, the test of concordance obtained between RFFIT and ELISA using recombinant proteins, it is considered good for literature (Fleiss, 1986), however to enhance this value can increase the sample size, and thus also improve the sensitivity of the test.

### ***P33 Stability of nucleoprotein of rabies virus after serial passages.***

*Helena Beatriz de Carvalho Ruthner Batista, Patricia Mariano Cruz Pereira, Marcelia Emanuele Sad Fernandes, Rafael de Novaes Oliveira, Juliana Galera Castilho, Pedro Carnieli Junior, Paulo Michel Roehle.*

Rabies virus (RABV) is the prototype of Lyssavirus genus and is the causative agent of rabies. Five proteins are coding by RABV (nucleoprotein-N, phosphoprotein-P, matrix protein-M, glycoprotein-G and large RNA-polymerase protein- L). The N is a phosphorylated protein with 450 amino acids and is considered the most conserved protein of RABV. Differences in genome of RABV could be identified according to the reservoir and/or geographical distribution, suggesting the adaptation of the viruses to a specific specie or region. These adaptations are very specific however the alterations that define the adaptation of the RABV in each reservoir need more knowledge. This study had the aim of to examine aspects of the antigenic and genetic stability of RABV. For this, 5 serial passages of RABV strain isolated from haematophagous bat *Desmodus rotundus* was held in an experimental host (mice). The stability of RABV was defined by antigenic and genetic characterization of the first and the fifth passages. Antigenic characterization was realized by indirect immunofluorescence with a panel of 8 monoclonal antibodies (Mabs) raised to RABV antigens. Genetic characterization was realized by RT-PCR and sequencing of the whole N gene. Neither significant modification was detected in the antigenic profile of RABV studied after five serial passages, nor than in sequence of the N gene. Our results confirm the high stability of nucleoprotein of RABV and indicate that five serial passages in mice are not enough to change the antigenic and genetic characteristics of RABV isolated from haematophagous bat. These are preliminary results and to confirm the high stability of RABV will be sequenced the complete genome of the virus after serial passages.

## ***P34 Correlation of fluorescence intensity with amount of virus inoculated.***

*Iana Suly, Santos Katz, Karen Miyuki Asano, Keila Yamamoto Nogi, Luciana Botelho Chaves, Adriana Candido Rodrigues Nasraui, Orlando Garcia Ribeiro & Karin Corrêa Scheffer Ferreira.*

Central Nervous System (CNS) isolated from different species can be difficult to adapt in murine neuroblastoma cells (N2A). The objective of this study was to evaluate in different strains of RABV an increase of fluorescence intensity after infection with consecutive passage in N2A. CNS of twelve samples were diagnosed with rabies positive and typed by means of a monoclonal antibody panel against the viral nucleoprotein. Among these samples, two were of *Eptesicus Furinalis* (not compatible with the panel), two were of *Canis familiaris* (Antigenic variant 2 - AgV2), one was of *Cerdocyon Thous* (AgV2), three were of *Bos taurus* (AgV3), two were of *Callithrix jacchus* (not compatible with the panel) and two were of *Homo sapiens* (AgV2). The strains were inoculated in suspension cells, incubated for 96 hours and were submitted to five consecutive passages in N2A cell. Evaluation by direct immunofluorescence (DIF) the increase of fluorescence intensity, express by crosses (+ to ++++). We observed even after successive passages, only three samples presented four crosses, four samples presented two crosses, while four samples were negative. Then, evaluated whether suspensions containing different concentrations of CNS (40%, 20%, 10% and 5%) increased the fluorescence intensity. Our results demonstrated that a suspension containing 40% CNS had higher fluorescence intensity than in other concentrations. Thus, we conclude that the fluorescence intensity of RABV is not correlated with different variants, but associated with amount of virus was inoculated initially. In this context, these results could help in the better diagnosis of rabies. Financial support: Pasteur Institute.

## ***P35 Difference in virulence of wild rabies virus isolates from different reservoirs.***

*Iana Suly Santos Katz, Luciana Botelho Chaves, Adriana Candido Rodrigues Nasraui, Karin Corrêa Scheffer Ferreira, & Orlando Garcia Ribeiro.*

Virulence of Rabies Virus (RABV) has been mainly studied with fixed virus having different degrees of pathogenicity. However, virulence is much different between fixed viruses and wild viruses. Thus, different pathogenicity among RABV strains might be associated with aspects of the complex evolution of rabies viruses in different host-reservoir species. To elucidate this, we compared the virulence of RABV strains isolated from Central Nervous System (CNS) of different reservoirs and CVS/11. RABV isolated from dog (Antigenic Variant 2 (AgV2)), bovine (AgV 3), marmoset (AgVM) and CVS/11 were inoculated intracerebrally in mice. These samples were submitted to six consecutive passages in neuroblastoma cells murine (N2A). We evaluated growth kinetics, cell-to-cell spread and kinetics of virus internalization in N2A cells of these RABV. Our data indicate that after 96h of infection the bovine RABV isolate had titers greater ( $3.88 \times 10^8$  PFU/mL) than with marmoset RABV isolate ( $6.9 \times 10^7$  PFU/mL), dog RABV isolate ( $6.9 \times 10^7$  PFU/mL) and CVS/11 ( $1.2 \times 10^8$  PFU/mL). Additionally, evaluation by IFD revealed differences in the number of cell infected with RABV isolated from dog, bovine, marmoset and CVS/11 at 72 h post infection with an average of 30, 22, 28 and 27 infected cell per focus fluorescent, respectively. Furthermore, we verified that 28.5, 36.1, 31.98 and 33.4 min were the time necessary to RABV isolated from dog, bovine, marmoset and CVS infect 50% of cells. Therefore, the kinetics of virus replication was inversely proportional to uptake and spread of RABV into N2A cells. So, we can speculate if this fact could be due to greater pathogenicity of RABV adapted to the dog and marmoset than variants adapted to hematophagous bat. This work may contribute to elucidation of the pathogenesis in these different hosts and assisting in the rabies control strategy. Financial support: Pasteur Institute.



## ***P36 Rabies seroprevalence in *Desmodus rotundus* feeding on dogs in periurban area from São Paulo city, Brazil.***

*Martorelli LFA, Rosa AR, Almeida MF, Barone GT, Oliveira DC & Uieda W.*

In 2012, the Zoonosis Control Center from Sao Paulo city (CCZ/SP), received a citizen's complain from a periurban area of the city about hematophagous bats attacking their dogs. Two surveys were performed and mist nets were put around the kennel. Five *Desmodus rotundus* were captured and sent to rabies diagnosis by Fluorescent Antibody Technique and Mouse Inoculation Test. As the bat's bites on dogs persisted, educational leaflets were distributed at the condominium to become aware owners of the problem and to ask them to inform another similar occurrence. One resident, after reading the leaflet, reported an abandoned house with bats. In the shelter indicated, 64 bats were captured. Blood samples were collected from all bats and the sera were analyzed to rabies antibodies using the Rapid Fluorescent Focus Inhibition Test modified. None bats were positive for rabies. The prevalence of rabies antibodies was 65.6% using 0.5IU/mL as a cut-off. Considering the presence of antibodies, without cut-off, four sera had titers to 0.16UI/mL, 18 had titer between 0.25 and 0.33UI/mL and 42 sera showed titers between 0.5 and 1.3 UI/mL. The expressive levels of rabies antibodies verified in these bats indicate that rabies virus circulated actively among them. This situation is worrying due to the proximity of these areas inhabited by the hematophagous bats and urban areas. Although the rabies had been under control in the city since 1981, there is always the risk of no-vaccinated stray animals to come into contact with an infected bat.

## ***P37 Ventajas y limitantes de la detección del virus de la rabia por inmunohistoquímica (dRIT).***

*María Susana Moreno Fernández.*

**Introducción:** La Inmunohistoquímica (dRIT) es una técnica que se caracteriza por ser rápida, de bajo costo, accesible y confiable para la identificación del virus rábico. Es un proceso en trámite de aprobación y validación en los EUA y aun no reconocido para su uso generalizado pero su utilización es recomendada por el CDC y el USDA. La dRIT puede ser usada para fortalecer la vigilancia de campo apoyando los programas de vacunación, no debe ser usada para vigilancia en salud pública. Sin embargo, dicha técnica se puede realizar donde se recolecta la muestra sospechosa, con el fin de emitir un resultado preliminar, sin necesidad de esperar que llegue al laboratorio para ser analizada.

**Método:** Se realiza a partir de improntas de encéfalos, donde incluye controles positivos y negativos para su validación. Se analizaron 250 encéfalos de perro, 45 encéfalos de gato y 5 murciélagos, muestras recibidas durante 2013 y comparadas con los resultados obtenidos por IFD.

**Resultados:** Fueron procesadas 300 muestras que previamente se analizaron con IFD en el Laboratorio Estatal, se obtuvo un 100% de concordancia en resultados de las 299 muestras negativas y 1 muestra positiva la cual era de murciélago, así como el 100% de concordancia con los controles positivos y negativos utilizados en cada corrida.

**Conclusiones:** Se observó gran sensibilidad y concordancia, la técnica de Inmunohistoquímica, es una técnica útil para campo, es una alternativa para detección del virus de la rabia, al ser rápida, de bajo costo, segura, accesible, confiable y de gran sensibilidad, lo que facilita en gran medida el trabajo operativo en campo o laboratorio, con disminución del costo de reactivos.

## ***P38 Use of filter paper blood samples for rabies antibody detection in foxes and raccoon dogs.***

*Marine Wasniewski, Jacques Barrat, Benoit Combes, Anne-Laure Guiot & Florence Cliquet.*

The effectiveness of oral rabies vaccination in wildlife is usually evaluated by the detection of rabies antibodies. However, the assessment of rabies antibodies has several technical difficulties in the field, such as the collection, storage, transport and titration of blood samples, often of poor quality.

The objective of this study was to assess the feasibility of collecting blood on filter paper (FP) coupled with enzyme-linked immunosorbent assay (ELISA) titration of rabies antibodies in raccoon dogs and red foxes.

The FP blood sampling method was found highly specific and repeatable in both species. Overall, results obtained with the FP sampling method were highly concordant with the conventional (venipuncture) sampling methods. Blood eluates from FP samples from foxes and raccoon dogs tested using ELISA showed concordance values of 92% and 95%, respectively, with serum samples tested using the seroneutralisation test and values of 95% and 91%, respectively, when the ELISA assay was used on both types of sample.

The use of FP blood sampling coupled with the titration of rabies antibodies by ELISA provides a reliable alternative to conventional blood sampling and serum testing by seroneutralisation. This simple procedure is particularly attractive and cost-effective for assessing the effectiveness of oral rabies vaccination in field conditions.

## ***P39 Vacunación antirrábica y estrategias para valorar la efectividad en camélidos sudamericanos.***

*Martín Ledesma, Matías Micucci, Oscar Pérez, Gabriela Calamante, Flavia Zanetti, Juliana Leoni & Alejandro Ferrari.*

En Argentina, la cría de Camélidos Sudamericanos (CS) está en aumento por el aprovechamiento de su fibra. Es un ganado crucial para las economías del norte del país, que es a su vez el territorio con mayor circulación de virus rábico (RV). Las estadísticas de organismos oficiales indican entre 15 y 60 casos anuales en los últimos 4 años. Este aumento en la cría implica que comiencen a ser criada fuera de su hábitat normal, extendiendo su distribución hacia el centro del país. El cambio climático generó cambios en los patrones migratorios de los murciélagos, generándose nuevas zonas vulnerables. No existen reportes oficiales de rabia en CS en Argentina, pero se registraron brotes en Bolivia. Para la Argentina es importante disponer de una estrategia de vacunación efectiva junto con metodologías distintas al ensayo de seroneutralización (ESN) in vivo o in vitro, que permitan valorar el estado inmunitario de animales vacunados. Así se desarrolló un ELISA contra el virus entero y el test de avidéz en el contexto del mismo ELISA. Se evaluaron en dos llamas cada una monodosis (3 UI) dos vacunas antirrábicas. (RV amplificado en BHK e inactivado (PV-BHK Malbrán) y poxvirus recombinante que expresa la glicoproteína de RV (CNPV-RG)). Los resultados del ESN muestran que CNPV-RG brinda protección frente al virus (0,57 y 0,82 UI/mL), mientras que PV-BHK Malbrán no (0,39 y 0,31 UI/mL), correlacionándose con los valores de avidéz y DO combinados (el suero de mayor protección es el de mayor avidéz con una DO media, el de protección cercana a la referencia tiene avidéz media con DO alta, mientras que los que están por debajo, muestran valores medios/bajos de DO y avidéz). La próxima etapa de este proyecto es evaluar un número más grande de animales, ante los promisorios resultados obtenidos hasta el momento.

## ***P40 Uso de yoduro de propidio en la inmunofluorescencia directa como herramienta para el diagnóstico de rabia en muestras antemortem en casos de humanos en México.***

*Mauricio Gómez, David-Martínez, Irma Leticia-Padilla, Susana-Chávez, Martin-Melo, Nidia-Aréchiga, Albert-Sandoval, Israel Animas, Alfredo-Gudiño, Beatriz Escamilla & Miguel Iguale-Vidales.*

La rabia en el hombre se caracteriza por la aparición de un cuadro clínico de encefalitis cuyos síntomas varían según el individuo, por lo que el diagnóstico diferencial con respecto a otras encefalitis virales es difícil o imposible, bajo estas condiciones solo la inmunofluorescencia directa IFD permite un diagnóstico seguro. La IFD es un procedimiento utilizado para diagnosticar en casos sospechosos de rabia, confirma o descarta la presencia del virus en tejidos del SNC (hipocampo, cerebelo, medula espinal y corteza cerebral), en casos de humanos las muestras antemortem son (improntas de córnea, hisopo sublingual, biopsia de cuero cabelludo y líquido cefalorraquídeo) y postmortem el encéfalo completo. Esta técnica se basa en mezclas anticuerpos monoclonales marcados con fluoresceína dirigidos contra la proteína N del virus de la rabia, presenta una sensibilidad y especificidad 99.99%. El diagnóstico de rabia por IFD tiene sus inicios en el InDRE/SSA/México en 1992, las nuevas tecnologías han mejorado la eficiencia diagnóstica, se trabajó con conjugados antirrábicos policlonales en los 90s y conjugados a base de mezclas de monoclonales a partir 1996, ahora incluyen colorante de contraste y en la actualidad se incorpora el Ioduro de Propidio como colorante de núcleos celulares, esto para evidenciar células de descamación de la córnea en muestras antemortem. Para el DX por IFD en encéfalos se realizan impresiones de los diferentes tejidos del SNC (medula espinal, asta de Amón cerebelo y corteza cerebral) en portaobjetos mismos que se fijan en acetona durante 2 horas dejándolas secar al aire, agregamos conjugado antirrábico con un título de 1:64 y diluido con solución de Ioduro de Propidio al 0.0005mg/mL, se incubó a 37 °C, 30 minutos quitando el exceso de conjugado con PBS y agua destilada, los portaobjetos se observan en microscopio de epifluorescencia a 200 y 400 aumentos, para muestras antemortem hablando exclusivamente de improntas de córnea se realiza la IFD, para muestras de biopsia de cuero cabelludo, LCR, e hisopo sublingual se inoculan directamente en células de neuroblastoma murino CCL-131 ATCC (se realiza una suspensión al 20% de tejido en D-MEM 10% y centrifugamos a 2500 rpm por 15 minutos, 1mL del sobrenadante es inoculado en un cultivo con  $3.5 \times 10^6$  células, realizando la primera lectura por IFD a los 3 días de incubación de las células y la segunda lectura a los 5 días. De 1992- 1996 se trabajó con conjugados a base de anticuerpos policlonales marcados con fluoresceína mismos que se adsorbían en suspensiones al 20 % de cerebros, una de ratón infectados y otra de ratones sanos con la finalidad de captar a los anticuerpos inespecíficos este tipo de conjugados dejaba atrapados en las improntas de córnea precipitados de fluoresceína que comprometían el DX al observar fluorescencia en las impresiones, de 1996-2001 se trabajó con conjugados a base de mezclas de monoclonales de origen de hibridomas productores de anticuerpos, lo que eliminó el uso de suspensiones de adsorción y eliminó el problema de las fluorescencias inespecíficas, aun con estas nuevas tecnologías el diagnóstico en improntas de córnea se tenía dificultades de interpretación por no asegurar que en la impronta se encontrarán células de descamación de la córnea, en los últimos años se trabaja con conjugados que incluyen azul de Evans como colorante de contraste y de 2011-2014 el laboratorio incorporó el Ioduro de Propidio a una concentración de 0.0005 mg/mL para evidenciar los núcleos de células. De 1992-2012 se trabajaron un total de 260 muestras ante-mortem en humanos, siendo positivas el 96% de los cerebros procesados, 7% de Improntas de córnea, 4.6% líquido cefalorraquídeo y 2.3% biopsias de cuero cabelludo, muestras de hisopo sublingual no se han diagnosticado como positivas. Concluimos que el uso de Yoduro de propidio ayuda en la identificación de los núcleos celulares y asegura que la calidad de la impronta es la adecuada.

## ***P41 Rapid immunohistochemistry technique to detection of rabies virus antigen - study of two methods for samples fixation.***

*Natalia Helena Frada Centoamore, Samira Maria Achkar, Elaine Raniero Fernandes, Maria Irma Seixas Duarte & Fernanda Guedes.*

Direct Immunofluorescence Antibody Test (dFAT) is the gold standard technique used for rabies diagnosis; however, there are some drawbacks that can limit its usefulness as its high cost and potential exposure of laboratory staff. Therefore, in developing countries rabies surveillance and diagnosis in suspected animals are constrained. The immunohistochemistry technique (IHQT) has lower cost when compared with dFAT. The objectives of the present study were to standardize the rapid immunohistochemistry technique (rIHQT) in brain samples of bovines for rabies diagnostic and to compare two methods for samples fixation. Were selected five brain samples of bovines with positive diagnostic by dFAT. Touch impressions of two fragments of central nervous system (CNS) were made in labeled glass microscope slides: cerebellum and hippocampus. As fixatives solutions were used the 10% buffered formalin for 10 minutes, 20 minutes and 16 hours, and 100% acetone for 25 and 45 minutes. After fixation, endogenous peroxidase was blocked with 3% hydrogen peroxidase for 10-minutes. Polyclonal mouse anti-rabies virus proteins primary antibody at a 1:150 dilution was incubated for 30 minutes room temperature followed by two 15-minutes incubations: biotinylated goat anti-mouse immunoglobulins and streptavidin conjugated to horseradish peroxidase. The reactions were visualized using 3,3'-diaminobenzidine-tetrahydrochloride chromogen for 2 minutes and counterstained with Harris haematoxylin. Negative controls included parallel sections from fragments of CNS processed as described above, omitting the primary antibody. All samples evaluated presented positive immunolabeling for rabies virus antigen with 100% agreement with dFAT, independent of the fixative used. These partial results suggest that this protocol for rIHQT could be applied in a larger number of samples and in other species for rabies diagnosis, confirming its high specificity and sensitivity. In addition, it is shown that this diagnostic methodology could to contribute in epidemiologic surveillance in regions where the diagnostic is limited or is not carried out.

## ***P42 Rabies surveillance during 2013 and 2014 performed at InDRE.***

*Nidia Aréchiga-Ceballos, Susana Cháves-López, Albert Sandoval-Borja, Irma Padilla-Medina, Martín Melo-Munguía, David Martínez-Solís, Rita Terán-Toledo, Israel Animas Vargas, Mauricio Gómez-Sierra, Alfredo Gudiño-Vázquez, Beatriz Escamilla-Ríos & Miguel Iguala-Vidales.*

During 2013, 4140 samples were submitted for testing to the Rabies Laboratory at the Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE). 147 samples were not suitable for rabies testing and therefore were rejected. The majority of the samples received 98% (n= 4074) were brains samples: 3254 dogs (79%); 391 bovines (9.5%); 219 domestic cats (6%); 150 bats (4%); 20 horses (0.5%); 17 skunks (0.4%); 6 pigs, 4 lambs, 4 goats; other wild-living animals also tested were: rabbits (n=2), coyotes (n=2), llamas (n=2), foxes (n=2), coati (n=1), squirrel (n=1), bobcat (n=1), field rat (n=1) and donkey (n=1). 245 out of 4074 brains samples tested positive in the fluorescent antibody test (FAT).

All positive samples were characterized by monoclonal antibodies. Relative contributions by the major animal groups were as follows: 169 were cattle (69%); 32 bats (13%); 10 dogs (4%) the rest of the reported rabid animals were wildlife. In addition to the animal samples 10 human suspect cases were tested by FAT and/or RT-PCR or RTCIT when suitable: sublingual swab (n=3), cerebrospinal fluid (n=2), corneal imprint (n=2), nuchal skin biopsy (n=2) were submitted for rabies testing corresponding to 4 suspected cases from Tamaulipas (1), Jalisco (2) and Estado de México (1) all were negative for rabies. In 2014 until July 31, 1644 samples have been submitted: (94%) brain samples (n=1561) (4.3%); human sera (n=71); sublingual swab (n=4), corneal imprint (n=3), nuchal skin biopsy (n=3) cerebrospinal fluid (n=2) of four suspected cases from Nayarit (1), Hidalgo (1), Yucatan (1) and Tamaulipas (1). So far, no human cases has been reported in Mexico since 2012.

## ***P43 The role of economic analysis in measuring rabies impacts, interventions and elimination efforts.***

*Stephanie A. Shwiff, Aaron Anderson, Brody Hatch, MA & Steven S. Shwiff.*

Economic analyses of rabies impacts and interventions have become increasingly prevalent in the literature. Demand for economic analysis is driven by many factors, including the need to document the impact of rabies on affected groups and justify rabies intervention efforts to myriad stakeholder groups. As alternative funding streams such as development and social impact bonds become available to pay for rabies intervention and elimination efforts, additional emphasis is being placed on economic analyses that can measure the broader economic impacts of rabies intervention and quantify both private and public return on investment. This article examines the potential outputs from currently available economic models and discusses the potential synergism between regional economic models and intervention-based models. This examination provides a comprehensive view of the benefits and costs of economic modeling to further rabies elimination efforts.



## ***P44 Resultados y perspectivas de la titulación de anticuerpos antirrábicos en México.***

*Israel Nicolás-Reyes & Juan Antonio Montaña-Hirose.*

Algunos países de la Unión Europea están libres de rabia urbana y en muchos otros realizan un tremendo esfuerzo para controlar y erradicar la enfermedad, por ello, los viajes con mascotas han sido sometidos a leyes y regulaciones estrictas, las cuales aplican a la circulación de perros, gatos y hurones y dependen de la situación de la rabia en el país de origen. México ha conseguido controlar esta enfermedad por medio de campañas de vacunación canina y felina, en particular en lo que se refiere a casos de rabia canina y consecuentemente la rabia humana. Gracias a esto, México figura entre los países considerados por el Parlamento Europeo con una situación favorable (Regulation (EC) N° 998/2003). En 2011, el CENASA participó en la prueba Internacional de desempeño para la serología rabia, organizada por el Laboratorio de ANSES, Nancy, Francia, que es el Laboratorio de referencia para Rabia de la OIE. El 11 de junio de 2012 CENASA recibió la autorización para realizar la prueba serológica para el monitoreo de la efectividad de la vacuna contra la rabia. Con esto se evita que las mascotas se sometan a cuarentena en la Unión Europea. Esta técnica también ha sido utilizada en colaboración con la Secretaria de Salud para evaluar si un fármaco que es aplicado simultáneamente con la vacuna antirrábica afecta la respuesta inmunitaria. A través de la difusión y promoción de la prueba en el CENASA, se ha incrementado a la fecha considerablemente el número de usuarios a nivel nacional e internacional, ya que también se han recibido muestras de diferentes países como Guatemala, Cuba, El Salvador y Costa Rica, además de que recientemente, los gobiernos de Corea del Sur, Australia y Taiwán reconocen los resultados del CENASA relacionados con la serología de rabia. Entre las perspectivas a corto plazo que se tienen son: monitoreo de la campaña de vacunación nacional antirrábica canina y la apertura y difusión a todo Centro América.

## ***P45 Global gene expression of innate and adaptative immune response in mice experimentally infected with RABV variants isolated from different animal species.***

*Susan Dora Allendorf, Camila M. Appolinário, Glenda Nicioli da Silva, Acácia Ferreira Vicente, João Marcelo A. P. Antunes, Marina G. Peres, Mateus S. R. Mioni, Bruna Letícia Devidé, Clóvis Reynaldo S. Fonseca & Jane Megid.*

Rabies virus (RABV) isolated from different mammals seem to have unique characteristics that influences the outcome after animals get infected. RABV circulates in nature and is maintained by reservoirs that are responsible for persuading the disease for almost 4,000 years. Considering the different pattern of pathogenicity of RABV strains in naturally and experimentally infected animals the samples were related to a dog (variant 2), bovine (variant 3), crab eating fox, marmoset and *Myotis* spp. The aim of this study was to identify the differentially expressed genes in an experimental mouse model inoculated with five Rabies virus (RABV) variants that are circulating in different reservoirs in Brazil. The present work used RT2 Profiler™ PCR Array (SABiosciences/Qiagen) in a pathway related to Innate and Adaptative Immune response. The obtained data provide a global screening of the individual profile from each sample in two moments, one before development of clinical signs and the other during the final stage of disease. The identification of genes with different expression may help to find the abnormal intracellular pathway that could be responsible for the death and survival of RABV infected patients. The results showed that rate of replication does not correlate with pathogenicity. Moreover, different viruses also showed to have individual strategies to infect each host and for being maintained circulating in a susceptible population since the genes expressed differentially in the five studied samples. Immune response events may be involved and influence the outcome of infection and differences in gene expression found in experimentally infected mice with street RABV virus demonstrate that the variants had independent characteristic that determine the clinical evolution and survival of the infected mice. Wild rabies virus induced up-regulation of several genes related to inflammatory response and downregulation of IFN stimulated genes in mice experimentally infected with different variant of rabies virus suggesting evasion of immune response.