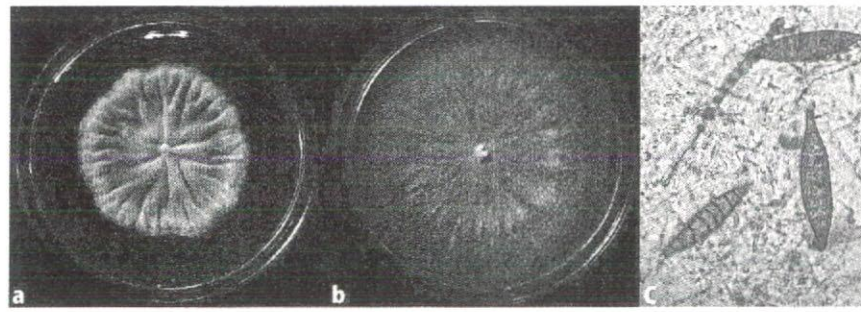

図2　a：原因菌 *Microsporum canis* の集落（左：サブロー寒天培地，25℃，24 日間，右：ポテト・デキストロース寒天培地，25℃，24 日間），b：集落の一部をかきとって観察した大分生子（×200）。

後，培養により *Microsporum canis* 感染が証明されたため，治療可能と説明し，安楽死を回避した。猫は Itraconazole による投薬を 3 か月続け，*Microsporum canis* 陰性を確認した。また，女児も抗真菌剤の内服 3 か月により，毛髪は回復した。

検査：初日	*Microsporum canis*（＋）（図 2）
治療 3 か月後	*Microsporum canis*（－）
休薬 3 か月後	*Microsporum canis*（－）

治　療：Itraconazole 5mg/kg　1 日 2 回，3 か月間

考　察

今回の症例は，ペットショップなどが不適切な助言を与えたことに端緒を発した動物由来性皮膚糸状菌症の事例である。

皮膚糸状菌症は皮膚科・獣医科領域で多く遭遇する共通感染症である[1,2,4]。診断は鏡検や培養で比較的簡単に行える[3]。女性や学童は小動物と直に接することが多く，皮膚糸状菌症に感染する機会が多い。一般的には適切な指導と治療により，悪化することなく治癒する[1,2,4]。しかし，飼い主や医師に共通感染症の意識が向いていないと適切な治療が遅れることがある。

最近の家庭内でのペット飼育に関する意識は，大きく変化し，月数回の入浴，外出後の清拭，獣医師による健康管理の徹底など，飼育動物が清潔に管理され，家族と寝食を共にすることに差し支えないような環境で飼育されている個体も多い。特に，都市部でのペット飼育に関する意識は上記のようなものと推測され，動物由来の皮膚糸状菌症の存在は，特殊な場合に限られていると認識されていると思われた。このような背景も飼育者ならびに数件の皮膚科医が動物由来で皮膚糸状菌症に感染することに思考が至らなかった理由の 1 つと推測している。

今回の事例は動物愛護，生命倫理の問題も含まれていた。動物由来感染症の原因となった飼育動物に対し，治療可能であるにも関わらず，安楽死を勧めた医師もあることが今回の事例から浮き彫りにされた。動物由来感染症への対応は，飼育者，医師，獣医師の連携が重要である。その 1 例が下記の web に掲載されているので，参考にされたい。

ナショナルバイオリソースプロジェクト病原微生物
http://wdcm.nig.ac.jp/byogen/index.html
内人獣共通菌編
http://wdcm.nig.ac.jp/byogen/anime/jinjyukyotu/index.html

平成 18 年 6 月に施行された動物愛護管理法でも動物販売業者や飼い主等は動物による感染症について正しい知識を持ち感染症予防のために必要な注意を払うことと義務付けられている。今後は関連機関への指導や飼い主教育を徹底して，「共に治療する」意識改革を行いたい。

女児への適切な診断治療をご指導頂いた東京都立駒込病院小児科 高山直秀先生，皮膚科 赤城久美子先生に深謝する。

引用文献

1) 長谷川篤彦（2004）：共通感染症ハンドブック，200-201，日本獣医師会．
2) 植村浩一（2003）：動物由来感染症（神山恒夫，山田章雄），237-342，真興交易医書出版部
3) 佐野文子（2006）：*J. Jpn. Small Anim. Vet. Assoc.* 46, 45-60.
4) 高島浩介（1997）：日獣会誌 50, 691-699.

短　報

黒毛和種牛における *Absidia corymbifera* と *Candida tropicalis* の重感染症

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要　約

慢性下痢を呈した生後5カ月齢，雄，黒毛和種牛は対症療法を施されたが，病状に好転がみられず予後不良として安楽殺後剖検に付された．病理組織学的検索では，接合菌感染を伴う慢性壊死性肉芽腫性胃腸炎に加え，全身性に酵母様真菌の感染が認められた．免疫組織化学的検査では，真菌は第四胃では抗*Rhizomucor*抗体陽性，腎臓では抗*Candida*抗体陽性を示した．真菌培養では，小腸から*Candida tropicalis*と*Absidia corymbifera*，腎臓から*C. tropicalis*が分離された．以上の所見から，本症例を子牛の*A. corymbifera*と*C. tropicalis*の重感染症と診断した．
――キーワード：慢性下痢，黒毛和種，真菌感染症．

日獣会誌　60，497～500（2007）

接合菌症は接合菌綱に属する真菌感染による人および動物の疾患である．病原性接合菌綱には*Mucorales*目と*Entomophthorales*目が知られている．*Mucorales*目には，*Absidia*，*Rhizopus*，*Mucor*，*Rhizomucor*，*Mortierella*属が含まれ，主に潰瘍性胃腸炎，肉芽腫性肺炎，リンパ節炎を起こす［1］．全身性感染例では時折流産がみられる．*Entomophthorales*目には*Basidiobolus*，*Conidiobolus*属が含まれ，主に皮下組織，鼻粘膜下組織に限局した慢性好酸球性肉芽腫を形成する［1］．いっぽう，カンジダ属の真菌は人および動物の消化管，呼吸器および生殖器粘膜の常在菌として知られているが，宿主免疫の減弱などに伴って日和見感染を起こす［2，3］．カンジダ症を起こす真菌は200種を超えるが，動物のカンジダ症の原因菌として最も重要なのは*Candida albicans*（*C. albicans*）と*C. tropicalis*である［2］．全身性カンジダ症の報告は犬で多く［4-6］，反芻動物ではまれである［7-9］．今回，*Absidia corymbifera*（*A. corymbifera*）に加え，*C. tropicalis*の重感染による難治性下痢を呈した子牛（黒毛和種）の症例に遭遇したので，その詳細を報告する．

材料および方法

症例および臨床経過：症例は平成17年5月26日生まれ，黒毛和種の雄である．生後1カ月齢から下痢を呈し，抗生剤を投与するが改善はみられず，9月12日に北里大学大動物診療センターに入院した．入院後，輸液，抗プラスミン剤，フルルチアミン製剤の静注，生菌製剤，止瀉剤などの内服を行ったが病状に好転が見られず，血液，粘液を混じた水様性下痢と食欲不振が持続したため，10月28日に予後不良と判断し安楽殺後剖検に付された．なお，糞便検査では線虫，原虫ともに検出されず，BVD-MDの抗体は陰性であった．

検索方法：剖検時に採材した各臓器（胃，小腸，大腸，肝臓，脾臓，腎臓，心臓，肺，骨格筋）を速やかに10％緩衝ホルマリン固定後，常法に従ってパラフィン包埋後薄切切片を作製し，ヘマトキシリン・エオジン染色（HE染色）を施した．第四胃および腎臓の病変部位については抗*Aspergillus*抗体（DAKO，Denmark），抗*Rhizomucor*抗体（DAKO，Denmark）［10］，抗*C. albicans*抗体（Biogenes，Germany）を用いた免疫組

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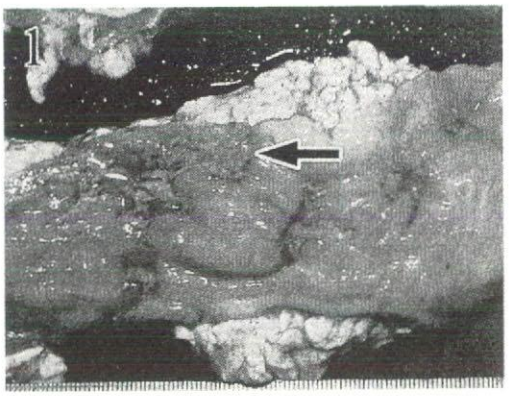

図1　結腸粘膜の肉眼所見．潰瘍（矢印）と粘膜の肥厚が認められる．

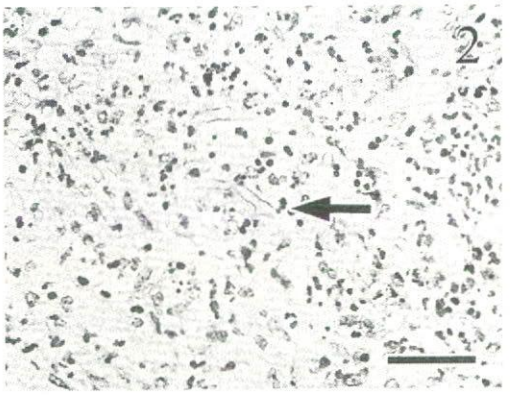

図2　第四胃．好中球およびマクロファージの浸潤を伴う接合菌様真菌（矢印）が散見される．HE染色，bar = 30μm．

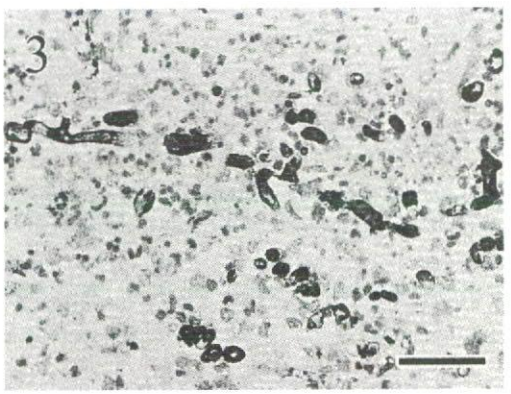

図3　第四胃．接合菌様真菌は抗*Rhizomucor*抗体で陽性を示している．免疫組織化学，bar = 30μm

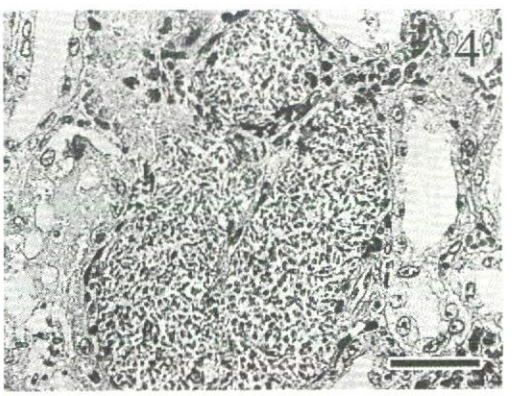

図4　腎臓．尿細管腔にカンジダ様真菌が充満し，尿細管上皮細胞は変性している．HE染色，bar = 30μm．

織化学的検査を実施した．腎臓，小腸の凍結生材料の一部は真菌培養に供した．クロラムフェニコール100mg/*l*を添加したポテトデキストロース寒天平板培地に5ミリ角に細切した凍結組織片を平板1枚あたり数個置き，37℃で7日間培養した．また，消化管，肝臓，脾臓，腎臓，心臓，肺についてはPAS反応とグロコット染色を実施した．

結　　果

剖検所見：剖検時，重度の栄養失調と脱水症状が認められ，舌，第四胃，小腸，大腸に最大2cm大に及ぶ潰瘍が多発性に認められた（図1）．胃腸粘膜の表面には出血壊死を伴う偽膜形成が認められ，粘膜は肥厚し腸間膜脂肪組織はおおむね消失し水腫を呈していた．両側腎臓，心筋，房室弁，大腿部骨格筋には針尖大～粟粒大の境界明瞭な乳白色結節が多数認められた．その他，肝臓の腫大，胆汁貯留および肺水腫が認められた．

病理組織学および免疫組織化学：第四胃と大腸に重度の慢性壊死性胃腸炎がび漫性に認められた．潰瘍周辺の粘膜や粘膜下組織には好中球，リンパ球，マクロファージの浸潤を伴う化膿性壊死性肉芽腫性病変が多発し，一部では肉芽組織に置換していた．線維素血栓や血管周囲炎も時折観察された．第四胃の粘膜下組織では厚い好酸性細胞壁と内部が淡明な球状の接合胞子や時折分岐状構造を示す菌糸様構造物（内径約15μm，長さ約150μm）が多数みられた（図2）．菌糸様構造物は抗*Rhizomucor*抗体で陽性（図3）を示したが，抗*Aspergillus*および*Candida*抗体では陰性であった．

腎臓，肝臓，心筋，肺，骨格筋では，卵円形～瓢箪状の出芽型分生子と細長でまれに分岐を形成する比較的小型な偽菌糸（内径3μm，長さ約35μm）の集簇巣が多発性に認められた．腎臓では，主に尿細管と集合管に集簇巣を形成（図4）する傾向を示したが，肝臓，肺，骨格筋では特異的な増殖傾向は認められなかった．いずれ

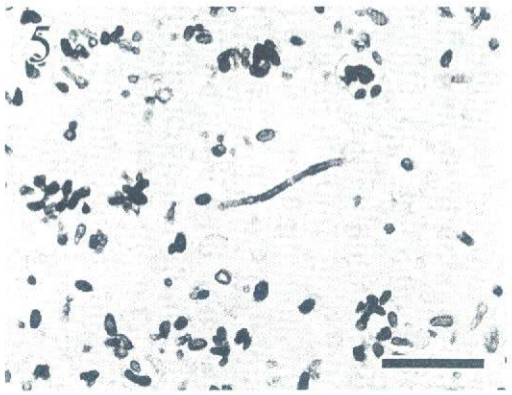

図5　腎臓．カンジダ様真菌はグロコット染色で陽性を示している．bar = 20μm.

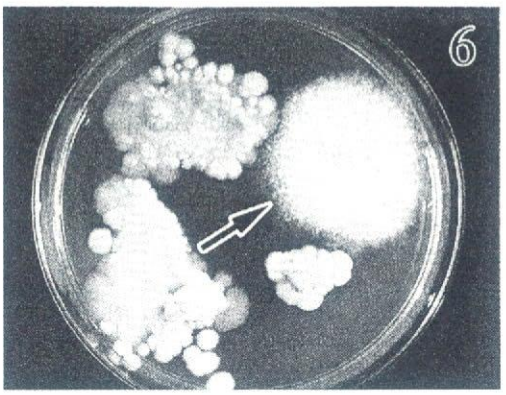

図6　小腸の真菌培養．乳白色～茶褐色の集落（*C. tropicalis*，左）と羊毛状の集落（*A. corymbifera*，矢印）が観察される．

の病変も真菌を取り囲んだ好中球，マクロファージの浸潤から構成され，病変内の実質細胞（尿細管と集合管上皮，肝細胞，肺胞上皮，心筋細胞，骨格筋）は概して凝固壊死を示していた．真菌様構造物は抗*Candida*抗体で陽性を示したが，抗*Aspergillus*，*Rhizomucor*抗体では陰性であった．第四胃と腎臓にみられた病原体はいずれもPAS反応およびグロコット染色（図5）で陽性を示した．

真菌培養：凍結生材料を用いた真菌培養では，小腸から*C. tropicalis*と*A. corymbifera*，腎臓から*C. tropicalis*が分離された．*C. tropicalis*は乳白色～茶褐色で隆起状の大小の集落を形成し，*A. corymbifera*は接合菌に特徴的な白色で羊毛状の集落を形成していた（図6）．

考　　察

牛の消化管における真菌感染の報告は多数あるが[11-17]，重感染を伴った全身感染例は比較的少ない[18]．本症例では小腸から*C. tropicalis*と*A. corymbifera*が分離され，腎臓から*C. tropicalis*が検出された．この事から，本症例を*A. corymbifera*および*C. tropicalis*の重感染症と診断した．病理組織学的に第四胃にみられた真菌は*A. corymbifera*に酷似しており[1]，本菌が重度の壊死性胃腸炎に深く関与していた可能性が示唆された．いっぽう，腎臓，肝臓，心筋，肺および骨格筋の病変は，程度の差はあるものの，すべて真菌を取り囲んだ炎症細胞の浸潤と実質細胞の凝固壊死からなり，真菌の形態は*C. tropicalis*に類似していた[7]．*Candida*は健康動物の口腔，咽頭，腸管，膣，精液，皮膚等に広く分布する酵母様真菌であり，牛では口腔カンジダ症（鵞口瘡，口内炎），胃炎，乳房炎を起こす[3]．通常は病原性が弱いが，宿主免疫の低下に伴って発病する日和見感染症である[3, 12, 13]．代謝性疾患[12, 19]，抗生剤の連用[5-7, 11, 13, 14, 20]，ステロイドの投与[5, 18]が真菌感染症の誘発要因として知られている．本症例では生後1カ月頃から持続的な下痢の治療として，抗生剤投与を行っており，抗生剤の投与が腸内細菌叢を崩壊させ，易感染の要因を与えた可能性が考えられた．しかしながら，*A. corymbifera*と*C. tropicalis*両者の間では体内局在および病変の質において相違点がみられた．すなわち，*A. corymbifera*は消化管において血管炎を伴った陳旧性病変を形成していたが，*C. tropicalis*は消化管以外の全身諸臓器に播種性に伝播し比較的急性の壊死性病変を形成していた．この事から，本症例にみられた慢性下痢の直接的な原因は*A. corymbifera*感染による消化管病変と推測されるが，その後の*C. tropicalis*の日和見感染がさらに病気を増悪させた可能性が想起された．

本稿を終えるに当たり，病理学的検査に御協力をいただいた畜農業・食品産業技術総合研究機構動物衛生研究所，播谷　亮先生ならびに真菌同定に御指導いただいた千葉大学真菌医学研究センター，村田佳輝先生に深謝する．

引　用　文　献

[1] Chandler FW, Kaplan W, Ajello L : Color Atlas and Text of the Histopathology of Mycotic Diseases, Chandler FW et al eds, 122-127, Year Book Medical Publishers, Chicago (1980)

[2] Barker IK, Van Dreumel AA, Palmer N : Pathology of Domestic Animals, Jubb KVF et al eds, 4th ed,Vol. 2, 256-257 (1993)

[3] 田淵　清：獣医真菌学，第1版，97-111，148-159，学窓出版，東京（2000）

[4] Brown MR, Thompson CA, Mohamed FM : Systemic candidiasis in an apparently immunocompetent dog, J Vet Diagn Invest, 17, 272-276 (2005)

[5] Heseltine JC, Panciera DL, Saunders GK : Systemic candidiasis in a dog, J Am Vet Med Assoc, 223, 821-824 (2003)

[6] Rodriguez F, Fernández A, Espinosa de los Monteros A, Wohlsein P, Jensen HE : Acute disseminated candidiasis in a puppy associated with parvoviral infection, Vet Rec, 142, 434-436 (1998)
[7] Mills JH, Hirth RS : Systemic candidiasis in calves on prolonged antibiotic therapy, J Am Vet Med Assoc, 150, 862-870 (1967)
[8] McCarty RT : Moniliasis as a systemic infection in cattle : field case reports, Vet Med, 51, 562-564 (1956)
[9] Jubb TF, Baird JD, Percy DH : Systemic candidiasis in a Suffolk ram, Aust Vet J, 65, 333-335 (1988)
[10] Jensen HE, Aalbaek B, Lind P, Krogh HV : Immunohistochemical diagnosis of systemic bovine zygomycosis by murine monoclonal antibodies, Vet Pathol, 33, 176-183 (1996)
[11] Jensen HE, Olsen SN, Aalbaek B : Gastrointestinal aspergillosis and zygomycosis of cattle, Vet Pathol, 31, 28-36 (1994)
[12] Chihaya Y, Matsukawa K, Ohshima K, Matsui Y, Ogasa K, Furusawa Y, Okada H : A pathological study of bovine alimentary mycosis, J Comp Pathol, 107, 195-206 (1992)
[13] Chihaya Y, Furusawa Y, Okada H, Matsukawa K, Matsui Y : Pathological studies on systemic mycoses in calves, J Vet Med Sci, 53, 1051-1058 (1991)
[14] Neitzke JP, Schiefer B : Incidence of mycotic gastritis in calves up to 30 days of age, Can Vet J, 15, 139-143 (1974)
[15] 奈良 博, 田村 哲, 土田 晃 : 犢の真菌症の1例について, 日獣会誌, 25, 166-170 (1972)
[16] 清水祥夫, 黒田俊男, 児玉宜広 : 犢における真菌症の病理組織学的観察, 日獣会誌, 25, 37-40 (1972)
[17] Gitter M, Austwick PKC : The presence of fungi in abomasal ulcers of young calves : a repot of seven cases, Vet Rec, 28, 924-929 (1957)
[18] Waurzyniak BJ, Hoover JP, Clinkenbeard KD, Welsh RD : Dual systemic mycosis caused by *Bipolaris spicifera* and *Torulopsis glabrata* in a dog, Vet Pathol, 29, 566-569 (1992)
[19] Chihaya Y, Matsukawa K, Mizushima S, Matsui Y : Ruminant forestomach and abomasal mucormycosis under rumen acidosis, Vet Pathol, 25, 119-123 (1988)
[20] Chihaya Y, Matsukawa K, Furusawa Y, Hatta Y, Okada H, Arisawa K : Disseminated Aspergillosis with lesions in the central nervous system in a calf, Jpn J Vet Sci, 50, 131-137 (1988)

Dual mycosis caused by *Absidia corymbifera* and *Candida tropicalis* in a Japanese Black calf

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SUMMARY

A male Japanese Black calf, five month old, was presented with history of chronic diarrhea. The calf was necropsied because the diarrhea was continued in spite of symptomatic treatments. Microscopically, chronic necrotizing granulomatous gastroenteritis with zygomycetes and systemic candidiasis were observed. Immunohistochemically, the fungi were positive for anti-*Rhizomucor* antibody in the abomasum and for anti-*Candida* antibody in the kidney. *Absidia corymbifera* and *Candida tropicalis* were isolated from small intestine and kidney by fungal cultures, respectively. This report describes the dual mycosis caused by *Absidia corymbifera* and *Candida tropicalis* in a Japanese Black calf.

Key words : Chronic diarrhea, Japanese Black calf, mycosis.

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短　報

漢方生薬配合薬の抗真菌活性と牛白癬の治療効果

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要　約

漢方生薬配合薬の抗真菌活性を検討したところ，本剤は多くの真菌に対して発育を抑制した．管内肥育牧場で発生した牛白癬3例に応用した結果，本剤の1週間経口投与と漢方生薬10％煎じ液の4日間体表噴霧の併用治療は著効を示した．――キーワード：抗真菌活性，漢方生薬，牛白癬．

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牛皮膚糸状菌症（牛白癬）は，牛だけでなく人への感染も起こす人と動物の共通感染症の一つであり，そのおもな原因菌は*Trichophyton verrucosum*（以下*T. verrucosum*）である［1］．本症は全国的に発生が報告されており［2, 3］，感染性が非常に強く，集団飼育された牛群で発生が多くみられ，発症すると増体に影響を及ぼし経済的損失を招くことが報告されている［3］．本症の治療には，抗真菌剤や消毒薬が有効とされており，経済性と治療効果の確実性および使用の簡便性などが要求されるが，それらの条件に適した薬剤は乏しいのが現状である［4］．

漢方生薬配合薬は，本来動物の消化器疾患治療薬として認可されているが，本剤にはオウバク末，オウゴン末など抗真菌活性を示す成分［5］が含まれている．そこで今回，本剤の抗真菌活性と*T. verrucosum*に感染した牛白癬への治療効果を調査した．

材料および方法

漢方生薬配合薬の抗真菌活性の調査は，2005年3月から2005年5月の間に千葉大学真菌医学研究センターにて保存している人と動物の共通感染症原因真菌9株を用いて，漢方生薬配合薬（新中森獣医散®，中森製薬㈱，宮崎）を0.1％，0.3％，1.0％，3.0％，10％添加して121℃20分オートクレーブ滅菌して作成した1/10サブロー寒天（0.2％グルコース，0.1％ペプトン，1.5％寒天）に各濃度3枚ずつ1白金耳接種した．培養温度は35℃で，培養時間は2～4週間（菌種により異なる）とし，培養後のコロニーの直径を計測した．

牛白癬への治療効果の調査は，管内A肥育牧場（哺育牛導入，肥育素牛販売，300頭飼養）で，2005年6月から2005年11月に*T. verrucosum*が検出された牛白癬症例3頭について実施した．治療方法は，本剤0.05～0.2g/kgの1週間飼料添加と漢方生薬10％煎じ液の連続4日間体表噴霧を行った．なお，漢方生薬10％煎じ液は水分が半分になるまで煮つめて濾過し，皮膚浸透性を高めるためにDimethyl sulfoxide（DMSO）を1％添加して作成した．効果は，治療前，治療最終日，治療終了翌日とその後1週間ごとの患部の観察と患部から採取した鱗屑1白金耳あたりの真菌の培養コロニー数で判定した．また，培養コロニー数カウントの上限は50までとした．

成　績

表1に本剤の各共通感染症原因真菌に対する抗真菌活性の結果を示した．おもに牛・馬に発症する*T. verrucosum*は，漢方生薬濃度0.3％から発育抑制が始まり10％で発育阻止がみられた．また，その他の真菌においても10％で発育阻止がみられた．

表2に牛白癬症例3頭の体表に発現した患部における真菌コロニー数の経時的推移を示した．症例1では真菌コロニー数が治療後1週目から減少し，7週目で急激に発毛が始まり，治療後9週目で完治した（図1）．症例2では，真菌コロニー数が治療後2週目からほぼ消失した．また，治療後5週目で急激に発毛し，治療後7週目で完治した．

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表1　漢方生薬配合薬濃度別にみた原因真菌コロニーの直径（cm）

菌名	菌株番号	漢方生薬配合薬濃度（%）					
		0	0.1	0.3	1.0	3.0	10.0
Trichophyton verrucosum	IFM 46012	3.5	2.8	2.7	0.2	0.2	0
		(3.4-3.6)	(2.8)	(2.5-2.8)	(0-0.7)	(0-0.4)	0
		100	80.0	77.1	5.7	5.7	0
T. verrucosum	IFM 46798	1.9	2.7	0.5	0.3	0.1	0
		(1.8-2.0)	(2.6-2.8)	(0-1.6)	(0-0.9)	(0-0.1)	0
		100	142	26.3	15.8	5.3	0
T. mentagrophytes	IFM 53814	3.3	3.5	3.4	2.5	2.4	0
		(3.2-3.4)	(3.0-4.2)	(3.0-4.2)	(2.2-2.8)	(2.0-2.8)	0
		100	106	103	75.8	72.7	0
T. mentagrophytes var. *erinacei*	IFM 50998	6.6	7.2	6.7	6.7	5.9	2.7
		(6.2-6.8)	(7.0-7.4)	(6.4-6.8)	(6.6-6.8)	(5.8-6.0)	(2.6-2.8)
		100	109	102	102	89.4	40.9
T. rubrum	IFM 53813	6.0	5.2	0	0	0	0
		(5.8-6.2)	(4.7-5.7)	0	0	0	0
		100	86.7	0	0	0	0
T. tonsurans	IFM 52825	4.5	4.0	3.4	2.1	2.1	0
		(4.1-4.9)	(3.7-4.2)	(2.8-3.9)	(1.8-2.4)	(2.0-2.2)	0
		100	88.9	75.6	35.0	35.0	0
Microsporum canis	IFM 53931	6.6	6.4	5.6	4.4	0	0
		(6.5-6.8)	(6.4-6.5)	(5.3-6.0)	(4.4)	0	0
		100	97.0	84.8	66.7	0	0
M. canis	IFM 54149	5.7	5.7	5.2	0	0	0
		(5.5-5.8)	(5.5-5.8)	(4.5-5.3)	0	0	0
		100	100	91.2	0	0	0
M. gypseum	IFM 53792	7.3	7.1	6.0	3.0	1.6	0.5
		(7.2-7.4)	(7.0-7.2)	(5.8-6.2)	(2.8-3.2)	(1.4-1.8)	(0-0.8)
		100	97.3	82.2	41.1	21.9	6.8

注：最上段は3枚の培地の平均値を示す．最下段は本剤を添加しないときの発育直径を100%としたときの%を示す．

表2　症例の真菌コロニー数の推移

採材日	供試牛		
	症例1	症例2	症例3
治療前	>50	>50	>50
治療最終日	>50	>50	>50
治療終了翌日	>50	>50	>50
1週目	28	>50	>50
2週目	46	0	7
3週目	21	7	>50
4週目	2	7	>50
5週目	10	0	>50
6週目	3	1	39
7週目	4	治癒	>50（再治療）
8週目	2		>50
9週目	治癒		7
10週目			20
11週目			1
12週目			4
13週目			1
14週目			4
15週目			7
16週目			4
20週目			治癒

治療効果出現が遅延した症例3では，治療後3週目から真菌コロニー数の上昇を示し，治療後5週目を経過しても体毛再生がみられなかった．そこで，治療後7週目に再度，漢方生薬10%煎じ液の4日間連続体表噴霧のみを行ったところ，治療後9週目から真菌コロニー数の減少がみられ，体毛再生も始まり，治療後20週目で完治した．

考　　察

今回の調査により，漢方生薬配合薬は，人と動物の共通感染症の原因菌である多くの真菌に対して発育を抑制することが明らかになった．特に，人の水虫の原因菌とされる*T. rubrum* [6] に対しては，漢方生薬濃度0.3%以上で発育阻止を示し，強い抗真菌活性がみられた．また，菌種によって感受性は異なるものの，多くの真菌において漢方生薬濃度10%で発育阻止を示すことが明らかになった．

牛白癬は，若齢牛や栄養不良の老齢牛に好発し，牛どうしの接触や作業器具などから間接的に感染し，春から夏にかけて多発するといわれている [7]．その主要な原

図1　症例1の治癒経過（左は治療前，右は治療後9週目）

因菌は*T. verrucosum*である［1］．今回，*T. verrucosum*に感染した牛白癬症例に対して，本剤の経口投与と煎じ液の体表噴霧の併用治療は，有効であった．症例1，2においては，治癒に至るまで約2カ月間を要し，塩化ジデシルジメチルアンモニウム溶液を用いて治療した報告［2, 3］と同等の治療期間であった．また，真菌コロニー数の減少に対して体毛再生の開始は数週間遅延して生じることが明らかになった．いっぽう，治療効果出現が遅延した症例3では，治療後3週目から真菌コロニー数の増加がみられたが，その原因として，接触による再感染が考えられた．したがって，4日間連続体表噴霧後5週目を目安に症状の改善がみられない場合は，本剤の再噴霧が必要であると考えられた．

以上のことから，牛白癬に対し漢方生薬配合薬を，10％煎じ液として病変部に直接噴霧し，経口投与することが有効であることが分かった．今回の試験では，煎じ液の体表噴霧のみの効果について調査しなかったが，症例3の再治療で体表噴霧のみで完治したことから，今回の治療効果の主要部分は煎じ液を外用で用いたことによると考えられた．

引用文献

［1］清水悠紀臣，鹿江雅光，田澗　清，平棟孝志，見上彪：獣医伝染病学，第5版，143，近代出版，東京（2000）
［2］亀山光博，中馬猛久，岡本嘉六，高木光博，岡本光司：白癬の多発する育成牛群に対する塩化ジデシルジメチルアンモニウムの消毒効果，日獣会誌，59，343-348（2006）
［3］新保秋雄，後藤純子，永吉　勝，藤原直躬：牛の皮膚糸状菌症に関する検討，家畜診療，47，827-832（2000）
［4］高島浩介：野外における家畜病原性真菌（11），畜産の研究，42，606-608（1988）
［5］中山医学院編：漢薬の臨床応用，神戸中医学研究会訳，97-101，医歯薬出版株式会社（1979）
［6］南山堂医学大辞典，第8版，1667，南山堂，東京（1983）
［7］高島浩介：野外における家畜病原性真菌（10），畜産の研究，42，492-494（1988）

Anti-Fungal Effect of Chinese Herbal Combination Drug and Efficacy with Cow Ringworm

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SUMMARY

The objective was to examine the anti-fungal effects of a Chinese herbal combination drug. The results demonstrated that this drug was effective in controlling many fungus growths. The drug was applied to three calves with cow ringworm on a local fattening ranch. The combined treatment of external applications of a 10% Chinese herbal infusion liquid solution over four days and internal administration of the Chinese herbal drug for one week showed remarkable results. —— Key words : Anti-fungal, Chinese herbal medicine, cow ringworm.

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夕&Eye

病を知る　動物からの感染症⑦

皮膚糸状菌症

イヌ・ネコに触れ発疹や化膿

人由来の場合と区別を

動物にすみつく真菌（カビの仲間）が人にうつり、皮膚の炎症を起こす「皮膚糸状菌症」。シャンプーなどペットの衛生習慣の広がりでイヌ・ネコからの感染は減っているが、ハリネズミなど珍種の動物から新たな感染例が報告されている。千葉大学真菌医学研究センターの佐野文子・助教授（獣医師）は「人からうつる真菌症とまぎらわしい場合があり、受診時にペットを飼っていると告げることが重要」と指摘する。

――どのような病気ですか。

「動物がもつ皮膚糸状菌が接触によって人にうつり、発疹（ほっしん）や化膿（かのう）を起こす病気です。人の水虫・タムシ菌はもっぱら人同士で感染しますが、同じように動物の間でだけ広がる真菌がある。それが本来感染する動物以外にうつると、激しい炎症を起こすのです」

「原因菌としてはイヌやネコに多いイヌ小胞子菌がよく知られています。ほかにもウサギやネズミなどがもつ毛瘡（もうそう）菌による感染例もあります」

――特徴的な症状は？

「皮膚に境界のはっきりした輪状の炎症が生じ、かゆみを伴います。白癬（はくせん）と同じ症状ですが、人の水虫やタムシが足の裏や股（こ）間など目立たない場所にできるのに対し、動物由来の真菌症は顔や首、手足などに生じやすい。動物を抱いたとき、衣服で覆われていない場所にうつるためでしょう」

顔に輪状の炎症が生じた例（きさらづ皮膚科クリニックの高橋容子氏提供）

千葉大学真菌医学研究センター助教授
佐野　文子氏

「イヌ小胞子菌の場合、名前とは違ってネコが感染源になることが多い。ネコは感染しても一般に症状は軽い。人の頭部にできた白癬が重症になると脱毛を伴うケルズス禿瘡（とくそう）になり、治療に数カ月かかることもあります」

――どんな動物に注意が必要ですか。

「イヌ小胞子菌は日本では高度経済成長期に輸入高級ネコが持ち込み、イヌにも広がったと言われます。しかし、その後はペットにシャンプーをする習慣の広がりなどで保菌率はかなり下がり、現在は一％以下と考えられます」

「一方でエキゾチックペット（珍種の外来動物）からの感染が増えています。毛瘡菌はハムスターやチンチラなどから分離され、私たちの調査ではハリネズミの三八％から菌が検出されました。感染すると手がチクチクして水疱（すいほう）ができたり、皮がむけたりします」

――治療法は？

「通常は抗真菌薬を患部に塗ればよくなります。頭部白癬は内服薬との併用が必要になることもある。数週間で治りますが、ただ、最近は症例が減って動物由来の真菌症を知らない医師が増え、ケルズス禿瘡のように重症化する例がある。受診したら『動物を飼っている』と告げてください」

――予防法や注意点は？

「まず、動物に触れたら手をせっけんなどで十分に洗うこと。飼い主がはっきりしない動物には触れないことも重要です。動物は土壌から感染することが多いので、外で遊ばせている場合には注意したほうがよい。タオルや寝具などの共用は避けるべきです」

「柔道やレスリングをする人はいわゆる『マット菌』による感染とまぎらわしいので注意してください。練習場のマットや対戦者に触れた手足や顔などにトリコフィトン・トンズランスという菌が感染して起きます。発症部位や症状が動物由来の場合と似ていて区別が難しい。薬の処方が違うので、医師に状況を伝えることが重要になります」

（聞き手は編集委員　久保田啓介）

人の菌も動物に重症になる例も

動物から人への感染とは逆に、人の水虫菌もペットの動物にうつり皮膚病を起こすことがある。素足で愛犬をかまった人の水虫菌がイヌにうつり、脱毛など激しい症状を示した例が国内でも報告されている。

真菌が本来感染する動物（固有宿主）にうつっても症状は軽いが、まれにそれ以外の動物にうつると劇症型になりやすい。本来の宿主は殺さないようにして自身が生き延びようとする真菌の生存戦略にかかわると考えられる。

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Biological characters of bats in relation to natural reservoir of emerging viruses

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Abstract

Many investigators focused on bats (Chiroptera) for their specific character, i.e. echolocation system, phylogenic tree, food practice and unique reproduction. However, most of basic information about the vital functions related to anti-viral activity has been unclear. For evaluating some animals as a natural reservoir or host of infectious pathogens, it is necessary that not only their immune system but also their biology, the environment of their living, food habits and physiological features should be clarified and they should be analyzed from these multi-view points. The majority of current studies on infectious diseases have been conducted for the elucidation of viral virulence using experimental animals or viral gene function in vitro, but in a few case, researchers focused on wild animal itself. In this paper, we described basic information about bats as follows; genetic background, character of the immunological factors, histological character of immune organs, the physiological function and sensitivity of bat cells to viral infection.

Keywords: Chiroptera; Mitochondrial DNA; Immune factors; Body temperature; Spleen; Retina; ELISA; YOKV

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Résumé

De nombreux chercheurs se sont intéressés aux chauves-souris (Chiroptères) pour leurs particularités, notamment pour leur système d'écholocalisation, leur phylogénèse, leurs habitudes alimentaires ou leur mode de reproduction unique. Néanmoins nous n'avons presque pas d'informations de base quant à leurs fonctions vitales en ce qui concerne leur résistance aux virus. Il est indispensable, lorsqu'on considère une espèce animale réservoir naturel d'agents pathogènes, de ne pas se limiter à la seule étude de son système immunitaire, mais de faire également des analyses portant sur plusieurs points de vue: biologie, habitat, habitudes alimentaires et caractéristiques physiologiques. La plupart des études récentes portant sur des maladies infectieuses consistent en des analyses in vitro des fonctions génétiques virales ou en des études pathologiques sur des animaux de laboratoire. Ainsi les chercheurs ne s'intéressent que très rarement aux animaux sauvages directement. La présente étude se propose donc de donner des informations de base sur les chauves-souris, en particulier leur background génétique, les caractéristiques de leurs facteurs immunitaires, les caractéristiques histologiques de leurs organes immunitaires, leurs fonctions physiologiques, leur sensibilité cellulaire face aux infections virales.

Mots-clés: Chauves-souris (Chiroptères); ADN mitochondrial; Facteurs immunitaires; Température corporelle; Rate; Rétine; ELISA; Virus YOKV

1. Introduction

Bats (Mammalian: Chiroptera) have been studied exclusively on their specific character, i.e. echolocation system, phylogenic tree, food practice and unique reproduction [1–5]. However, it is recently reported that bats are also natural reservoirs or natural hosts of the emerging and re-emerging infectious disease, for examples Nipah virus, Hendra virus and lyssavirus infections including rabies, which lead to serious disorders in humans as well as in some animals [6–8]. Moreover, bat-SARS-CoV and Ebola virus were also isolated from bats, and the infectious disease derived from bats has been increasing in number [9–11]. Among these viruses, few investigators reported the result of experimental infection to bats and especially for Ebola virus, it was reported that bats supported the replication and circulation of high titers of this virus without any illness [12]. Many investigators focused on bats from the above-mentioned standpoints, however, most of basic information about their vital functions, such as the specific immune system and others associated with anti-viral activity, has been unclear until now. Therefore, basic researches for immunology in bats are important to advance investigation of the bat-derived infectious diseases.

The majority of current studies on infectious diseases have been aimed at the elucidation of viral virulence using experimental animals or viral gene function using cell lines in vitro, but in a few case, researchers focused on wild animal itself [13,14]. In this paper, we describe basic information about bats as follows: genetic background, character of the immunological factors and the physiological function.

The study is conducted using the genus *Rousette* (Chiroptera: Megachiroptera) which includes Egyptian and Leschenault's rousettes.

2. Biological characters of the rousette bats

In the basic studies on bats, to elucidate their biological character is the most important subject, although there is a little information about it. Therefore, we investigated the bats from the genetic, histological, molecular biological and physiological points of view.

2.1. Phylogenic analysis using the mitochondria DNA

Bats consist of the second-ranking big order in mammals, having about 1000 species, and live around the world except for the northern and southern polar areas. They is only one mammal with the capacity of powered flight and are divided into two suborders, Microchiroptera and Megachiroptera. The formers have various food habits, which are mainly insectivorous, and have the ability of the laryngeal echolocation but the latter is a fruit eater and have a comprehensive visual perception similar to the non-human primate and human being [15].

The phylogenic position or the relationship of bats among other mammalians has been studied using both morphological and molecular techniques. Some morphological studies have supported the idea that bat is included in the superorder Archonta, which also include Primate, Dermoptera and Scandentia [16,17]. However, from the molecular point of view, bat is thought to be within Laurasiatheria including Cetartiodactyla, Perissodactyla, Carnivora, Pholidota and Eulipotyphla [18]. Moreover, several investigators place bats as a sister group of Fereuungulata and latest studies support that bat is the monophyletic group within Fereuungulata, by mitochondria DNA (mtDNA) sequence data [19,20].

Although Megachiroptera is more closely related to primates than to Microchiroptera based on an anatomical pattern of neural projections from eye balls to prestrinate cortex via geniculate bodies [21,22], molecular studies reject this hypothesis. The phylogenetic position of Megachiroptera within bats is placed neighboring to Rhinolophoidea [15]. However, it is not clear which species of Megachiroptera is the most close relatives to Microchiroptera, especially for Rhinolophoidea. Thus, we determined the complete mtDNA sequence of Egyptian rousette, *Rousettus aegyptiacus*, which inhabits from South Africa to Egypt, Pakistan and Cyprus, by the direct and the shotgun sequencing methods. The phylogenic analysis was conducted to decide the position of Egyptian rousette within Chiroptera and among other animals.

The number of the nucleotide base pairs of mtDNA of Egyptian rousette was 16,706. The phylogenic tree was made using maximum-likelihood method based on the amino acid sequences of 13 proteins encoded by mtDNA. The sequence was compared with two other Megachiroteras, five Microchiropteras and other mammals. The results suggested that Chiroptera was monophylic group and more closely related to the large clade including Carnivora, Artiodactyla and Perissodactyla. Moreover,

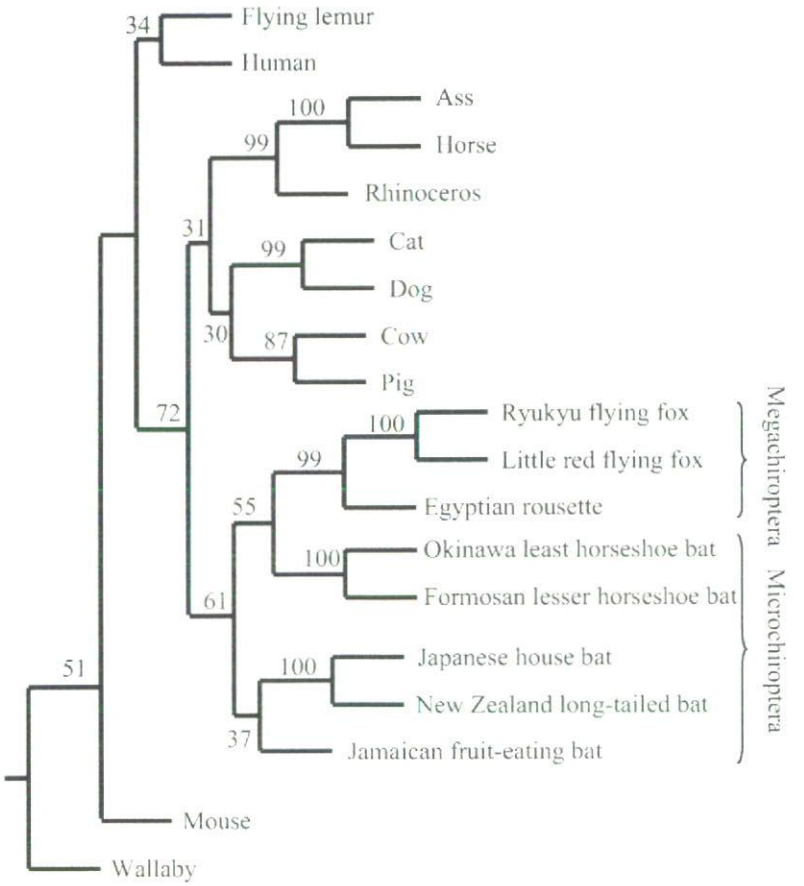

Fig. 1. Maximum likelihood tree constructed by PHYLIP using the sequence of nucleotide 13 protein genes encoded by mtDNA. Numbers at nodes indicate bootstrap value for maximum likelihood method.

within Chiroptera group, Egyptian rousette might be divided earlier from Rhinolophoidae (Chiroptera: Microchiroptera) than two other Megachiropteras, the little red flying fox and the Ryukyu flying fox (Fig. 1).

Our data supported two former hypotheses. One is that Megachiroptera makes a monophyletic group together with Microchiroptera. The second is that Chiroptera share a common ancestor with the large cluster including Carnivora, Perissodactyla and Artiodactyla, and Chiroptera diverged from a common ancestor before the large cluster diverged [23.24]. Moreover, this result created a new possibility that the genus *Rousette* may be the prototype of the suborder of Megachiroptera which was divided from Microchiroptera (one of missing rings of the small bats to mega bats). Thus, it is considered that the genus *Rousette* is the most suitable group for comparative studies on bat species.

2.2. Immunological cross-reactivity of bat IgG epitopes

The difference of antigenic determinants in serum proteins is a convenient tool for examining the molecular evolution of a certain protein among animal species [25–27]. A polyclonal antibody includes different kinds of antibodies recognizing

different epitopes, and it can specifically bind to the definite antigenic determinants of the serum protein. A high degree of cross-reactivity reflects similarity of antigenic determinants on the target protein among different species, which might have been conserved through the course of evolution. Therefore, we quantified the difference of antigen determinants using bat immunoglobulin G (IgG) within Chiroptera as well as between Chiropera and other closely related species based on the cross-reactivity of polyclonal anti-bat IgG antibody.

The rabbit anti-bat IgG was prepared using purified bat IgG obtained from the serum of *Rousettes aegyptiacus* as antigen. The purification of bat IgG was checked by SDS-PAGE. To assess the specificity of the anti-bat IgG antibody, we performed western blot analysis. Only one band with a molecular weight of about 160 kDa was detected in both bat serum and purified bat IgG fraction. These results suggested that the prepared anti-bat IgG antibody had high specificity. To compare the specificity, sera were obtained from four orders, i.e. Primate (one squirrel monkey, two lemurian monkeys and one human), Carnivora (four dogs), Insectivora (five large Japanese moles and four house shrews) and Chiroptera including two Rousettes, one Microchiroptera and three other Megachriopteras. The cross-reactivity of IgG epitopes in each serum sample using anti-bat IgG antibody was determined by the competitive enzyme-linked immunosorbent assay (ELISA). Fetal calf serum (FCS) was used as negative control. As a result, all serum samples obtained from Chiroptera showed high cross-reactivity, over 95% of inhibition, and the cross-reactivity of other animal samples was very low, mostly less than 20% of inhibition. The mean and standard deviation of percent inhibition of sera from Primates, Carnivores, Insectivores and Chiroptera were 15.2 ± 4.6, 12.2 ± 2.8, 8.4 ± 2.2 and 100.9 ± 4.5, respectively.

This result indicated that the anti-Rousette IgG polyclonal antibody had a high specificity to Chiroptera IgG, and will be useful for the screening and detection of pathogens. It supported also that two suborders of Chiroptera were closely related to each other or are monophyletic. The relationship between Chiroptera and other species, which have been considered as close relatives of bats such as Primate, Insectivora and Carnivora, were obviously distinct. However, the present result indicated that Primate and Carnivora might be more closely related to Chiroptera than Insectivora ($p < 0.05$).

It is considered that the large difference between Chiroptera species and Primate, Carnivora or Insectivora was caused by their own evolution as well as living environment; i.e. the former basically lives in the sky and the latter on or in the ground. The result also suggested that Chiroptera might be monophyly.

2.3. Phylogenic analysis using anatomical and histological methods

The echolocation system, which is the main recognition ability of Microchiroptera, has been reported frequently [28–30]. The cognitive methods of two suborders within Chiroptera are completely different, i.e. Microchiroptera uses mainly echolocation, and Megachiroptera uses visual perception. In the former all fibers of the optic nerve entered into the contralateral optic tract, however, in the

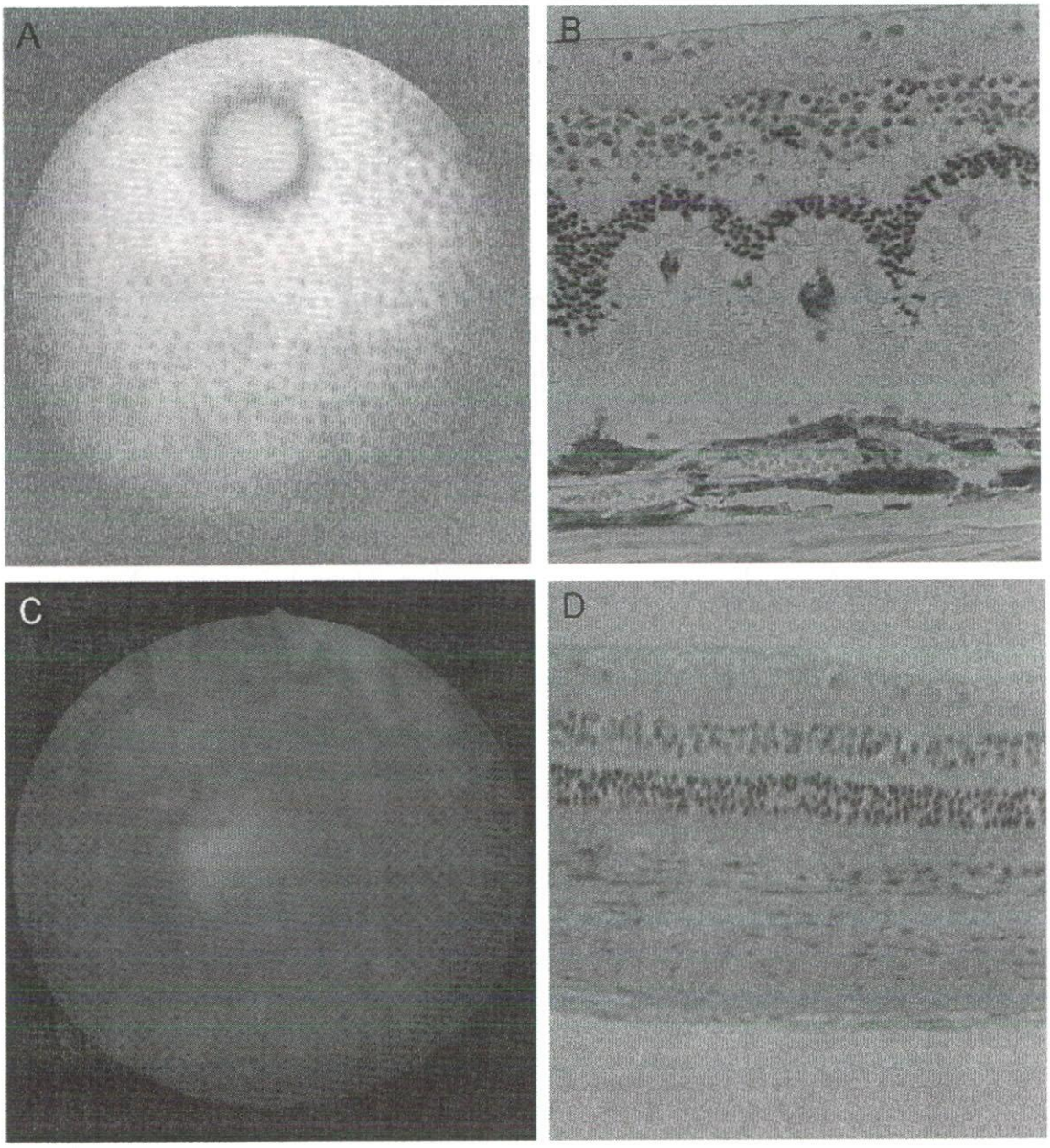

Fig. 2. Anatomical and histopathological analysis of retina of Egyptian rousette, *Rousettus aegyptiacus*: (A, C) funduscopy of eye ball, (B, D) histopathology of retina, (A) Tinny spots were observed in bats retina, (B) papillary structure was observed between outer granular layer and retinal pigment epithelium and (C, D) funduscopy and histopathology of guinea-pig, as a control.

latter the optic nerve connect to both contrarateral and ipsilateral superior colliculus, being similar to the projection pattern of primates. Thus, we investigated on the optical system of *R. aegyptiacus* and structure of their retina in comparison with those of Michrochiroptera.

In the funduscopy finding of the eye balls of Egyptian rousette, there were large numbers of tiny spots, which scatter onto the whole retina except for the optic disc. This peculiar structure could not be observed onto other animals' retina including

small bats. Although blood vessels distributed from the optic nerve papilla are observed in the retina of other mammals, in the case of rousette retina, the blood vessels were not observed at all. By the histological examination, rousette retina had a papillary structure between outer granular layer and retinal pigment epithelium, unlike other animals' smooth-layered retina including small bats. In the immunohistological examinations, rhodopsin could be detected from the outer segment of the internal photoreceptor matrix (IPM) to outer nuclear layer (ONL) but mainly in the outer segment of IPM, and recoverin-positive cells were detected from inner segment of IPM to ONL. Although both recoverin and rhodopsin proteins were expressed in the same area as those in the rat retina, the ratio of rhodopsin-positive area to that of recoverin in the Egyptian rousette retina was wider than that in rat retina (Fig. 2).

These results suggested that the tiny spots observed by the funduscopy on the retina of Egyptian rousette assumed to be due to the papillary protrusion of pigment epithelium, which might result in enlargement of the surface area receiving light wave, because they need to increase the number of photoreceptor or the light-receiving area for gathering information in the dark night regarding the environment, such as enemies or prey without tapetamu lucidum structures or only simple tongue echolocation system.

2.4. *Change of body temperature*

Body temperature regulates the basic metabolic rate and various body activities, but it is settled within narrow range in majority of mammalians by the homeostatic control, except for some emergencies such as viral or bacterial infections. In the virus replication process, it has been reported that the ratio of virus production is dependent on the body temperature [31]. It is noted that bats, especially for Microchirotpera, have specific character, torpor, which is similar to hibernation, when they are exposed in low temperature [32]. It was also reported that some kinds of Megachiroptera showed torpor-like response under low-temperature condition [33]. In torpor and hibernation, the lowering of the internal body temperature is observed and animals get large tolerance to nuclear radiation, cancerogenic substance and infectious diseases. Whereas the investigation about torpor in bats had already reported, there was no report on circadian rhythm of the internal body temperature of bats using a telemeter. We examined the internal body temperature of *Rousettus leschenaulti* using telemetry system. In order to know environmental temperature effects, the rousette bat was kept in an incubator, which was settled light and dark cycle consisted of 12 h of light per day (light on from 08:00 to 20:00) and the thermal conditions were settled following three conditions; 24 °C constant, from 10:00 to 16:00 at 30 °C and another time at 24 °C, from 10:00 to 16:00 at 33 °C and another time at 27 °C.

Under the first condition, 24 °C constant, rousette bat body temperature was around 36 °C in light period when bats took a rest, but in dark period when bats became active, the body temperature was up to and sometime over 39 °C. Under the second and third conditions, in the dark phase the average of their body temperature

were over 38 °C, but in the light phase was depended on the environmental temperature (from 36 to 38 °C). In the case of the second condition, the gap of body temperature between light and dark phase was more tight than others. These results indicated that the thermal gap of body temperature between the rest and active phase was wider than other mammals, and that the infected virus might be difficult to replicate constantly in rousette bats. It was thought that higher body temperature in dark phase over 38 °C was due to the movement.

3. Evaluation of the immune systems of bats

Bats are thought to be a natural reservoir or vector of rabies virus, Nipah virus, Hendra virus, European bat lyssavirus types 1 and 2 and Australian bat lyssavirus [34–38]. In addition, other viruses related to the emerging and re-emerging infectious diseases were also isolated from bats, for examples Ebola virus and bat-SARS-CoV [9–11]. In the case of experimental infection of Ebola virus, bats supported the replication and the circulation of high titers of virus without any clinical signs [39].

It has already been reported that bats have three types of immunocompetent cells, i.e. plastic adherent cells with pesudopodia, nylon wool adherent cells with small microvilli and nylon nonadherent cells with comparatively smooth surfaces. These cell types resemble to macrophage, B cells and T cells of other mammals, respectively [40]. However, there is a little basic information about the immune system of bats and the comprehensive studies in vitro are not conducted. So, we examined the molecular and biological characters of their immune factors and performed histopathological and immunohistochemical analyses of their immune organs.

3.1. Molecular and biological characters of bat immune factors

The nucleotide sequences of almost all immune factors of bats are not identified until now. We determined the nucleotide sequences of several immune factors including full open reading frame (ORF) of CD4 on the helper T cells, which induces the acquire immunity, and IFN-α and β, which relate to the innate immunity. The nucleotide and deduced amino acid sequences of these molecules were analyzed by phylogenic and molecular biological point of view.

In the acquired immunity, the antigen recognition between T cells and antigen-presenting cells (APC) requires interaction of T cell receptor (TCR) and major histocompatibility complex (MHC) molecules. The CD4 (MHC class II) and CD8 (MHC class I) molecules are the surface key proteins engaged in immune reactions. Thus, the surfaces of mature T cells expressed either CD4 or CD8 [41,42]. CD4-expressing T cells react to the antigen presented by the MHC classII molecule of APC [43]. CD4 molecule is a glycoprotein belonging to an adherent molecule group classified to the immunoglobulin superfamily. It has four Ig-like domains in the extracellular region and intracellular region connects to tyrosine kinase, Lck, belonging to the Src family specific for lymphoid cells [44]. We sequenced the full

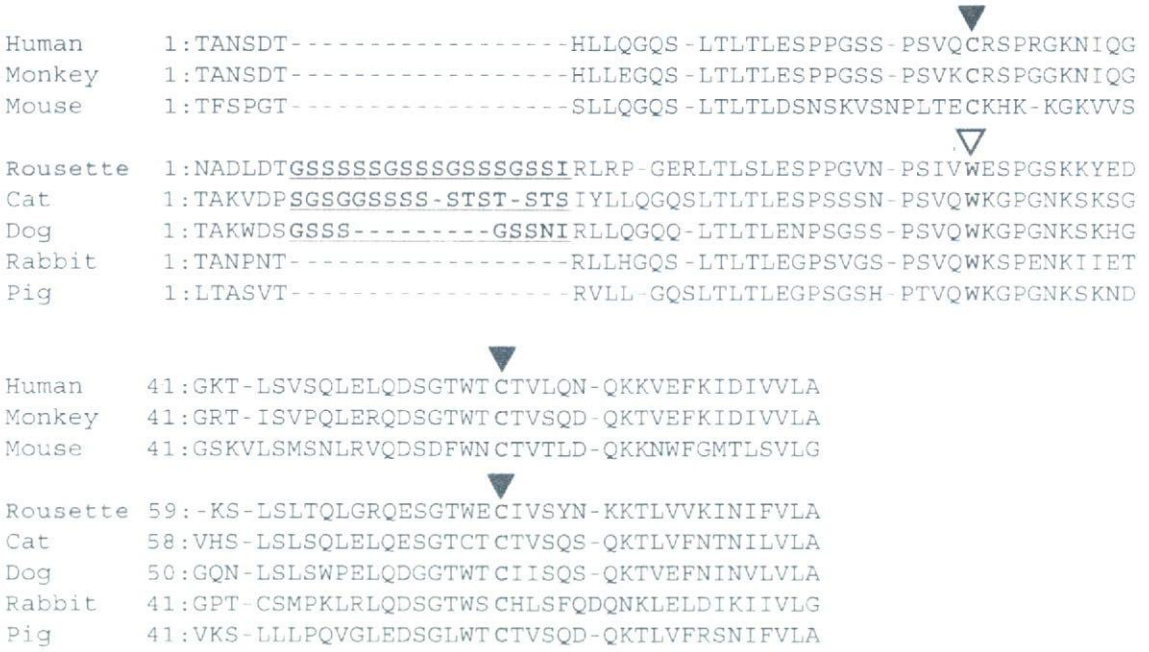

Fig. 3. Comparison of the CD4 Ig-like C region between the bat and human, monkey mouse, cat, pig, dog and whale. Identical amino acid residues are indicated by dots (•) and gaps are indicated by bars (–). The cystein residues consisting of the disulfide bond are indicated by closed triangles. The residues in which tryptophan is substituted for cystein are indicated by open triangles. Underlines show amino acids insertions provided CD4 of human and mouse being as a standard.

ORF of CD4, and analyzed the bat CD4 from phylogenic and comparative zoological viewpoints.

The nucleotide sequence of bat CD4 consisted of a total of 1905 bases, in which 1419 nucleotides encoded 472 amino acids. When the homology of the nucleotide and the deduced amino acid sequences were compared between the bat CD4 and those of other animals, such as human, mouse, cat, dog and chicken, bat CD4 had a higher homology to both cat and dog CD4 than those of other animals. The phylogenic tree using neighbor-joining method with the amino acid sequence of CD4 showed that bat CD4 was closely related to cat and dog CD4 and was distant from duck and chicken, which agreed with the homology of the nucleotide and the amino acid sequence. This result supported that Chiroptera was included in the same clade including cat, dog, pig and whale [45,46]. Moreover, the amino acid sequence of the CD4 Ig-like C-type 1 domain was compared between bat and other animals. In bat CD4, there was an insertion of 18 extra amino acids in the beginning of this domain, where dog and cat CD4 have 9 and 16 amino acids insertions, respectively, provided CD4 of human and mouse being as a standard. The insertion might influence the relationship between V-type domain and C-type 1 domain. In the N-terminus side, cystein pair might be lost because one cystein was replaced by tryptophan in the bat CD4, whereas in the case of human, monkey and mouse CD4 it was still cystein. The latter animals might have a disulfide bond formed by the two cysteins in this region. However, bat CD4 lacked the disulfide bond, as in cat, dog and whale. As a result

the conformation of bat CD4 Ig-like C-type 1 region might be different from that of the human and mouse (Fig. 3). The conformational change of the bat CD4 might influence the antigen presentation process between APC and helper T cells by means of CD4–MHC classII connection [47,48].

In the innate immunity, virus-infected cells provoke many responses to viral infection. One of these responses is to secrete type-I (α/β) interferons (IFNs) [49]. Type-I INFs are antiviral cytokines, and composed of the multiple subtypes of α and the single type of β. When some microbes infect to cells or are detected by anti-microbe intercellular or extracellular sensors, type-I IFNs are secreted from most of the cells, especially from dendritic cells. Secreted type-I IFNs bind to the interferon receptor (IFNAR) and activate the expression of numerous interferon-stimulated genes (ISGs), such as the protein kinase R (PKR), the 2′–5′ oligoadenylate synthetases (OAS), the myxovirus resistance gene (Mx), which have anti-viral activities [50]. However, the nucleotide sequences of type-I IFNs of bats were not determined until now.

The ORFs of IFN-α and β had 562 base pairs that encoded 187 amino acids, 558 base pairs that encoded 186 amino acids, respectively. Since IFN-α genes have many subtypes, it is difficult to compare its nucleotide sequence among several animal species. Therefore, we checked homology of the bat IFN-β to those of human, pig, cat, horse and mouse. The homologies were 77.5%, 82.0%, 78.3%, 77.5% and 66.5% at nucleotide level, and 64.2%, 72.0%, 61.8%, 61.3% and 49.5% at amino acid level, respectively. The phylogenic tree was constructed based on the amino acid sequences obtained from the bat INF ORFs with several representative eutherian type-I IFNs as well as chicken type-I IFN using maximum likelihood method. The results showed that both bat IFN-α and bat IFN-β were included in each type of mammalian IFN group, but far from those in avian. Moreover, both bat IFN-α and β were more closely related to pig IFN-α and β than the other mammal ones.

There have been few studies on the immune systems of the bat that has been thought to be an important vector or a natural host of various pathogenic microbes. We believe that these basic information will help to elucidate the ecology of infectious agents derived from bats as wells as our understanding of bat immunological factors.

3.2. Histopathological and immunohistochemical analyses of the immune organs

It has been reported that many microbes were isolated from bats in field sampling and the antibody to several pathogens was detected in the epidemiological studies using viral neutralizing test. In these epidemiological studies and experimental infections with microbes in vivo, the histopathological finding is one of the most important information for investigating whether and how they react to the invading microbes. However, there are limited numbers of reports about their normal histology, especially for the immune organs. We examined histopathologically on thymus, spleen and mesenteric lymph nodes, which are the main immune organs, of six normal *R. aegyptiacus*, which were kept at conventional containment grade in our laboratory. Their thymus, spleen and lymph nudes were sampled, fixed with 4%

paraformaldehyde, embedded in paraffin and sliced. We examined their normal histology using hematoxylin–eosin staining, Masson–trichrome staining and immunostaining with anti-bat IgG rabbit polyclonal antibody.

In the hematoxylin–eosin staining, thymus structure was not different from other animals, but most of all germinal centers in splenic white pulp and lymph node of all samples were hypertrophic. By Masson–trichrome staining, these hypertrophic germinal centers were not for the deposition of fibrin but for cells with abundant cytoplasm. In the immunostaing using the anti-bat IgG antibody, these cells correspond to B cells which were positive to anti-bat IgG antibody. This result indicated that the immunological cells, especially for B cells, in Rousette bats kept in our laboratory under the conventional condition were naturally activated, or they might be coexisted with some microbes, which were apparently no pathogenic to Rousette bat. It is needed further study on natural state of T and B cells in different species of bats at various ages for basic information.

4. The susceptibility of bat to virus infection

There are also a few studies on the experimental infection of virus, which was isolated from bats or antibody positive to the virus in the field survey and/or virus was thought to be concerned in the infectious cycles [51–53]. Thus, we established primary cell cultures from rousette bat kidneys and evaluated their susceptibility to the virus infections compared with the commercially available bat lung cell line, Tb-1 Lu.

4.1. Sensitivity of bats cells to viruses in vitro

Only a few cases of experimental infection of virus to bats in vivo or cell lines derived from bats have been documented, and the established and available cell line from bat is only Tb-1 Lu, which is the alveolar epithelial cell lines [54]. Therefore, first we tried to prepare bat primary kidney cells (BPKC) and examined the sensitivity of them to some viruses.

Viruses used in the experiment were following; Yokose virus (YOKV; family Flaviviridae), which is only one virus isolated from Japanese Microhiroptera, Akabane virus (AKAV; family Bunyaviridae), Fukuoka virus (FUKV; family Rhabdovirus), Kawanabe virus (KAWV; family Reoviridae), canine parainfluenza virus (CPIV; family Paramyxoviridae), pseudorabies virus (PRV; family Herpesvidirae) and Japanese encephalitis virus (JEV; family Flaviviridae). All of these viruses had the ability to infect and replicate in both BPKC and Tb-1 Lu, and in BPKC these viruses, except for JEV, developed a cytopathic effect (CPE) and the infected cells were dead until 3 days after infection (d.a.i.). Meanwhile, Tb-1 Lu cell lines released the infectious virus, although it showed lower levels of all viruses replication than BPKC without CPE. JEV could infect and produce the infectious virus in BPKC showing a peak at 2 d.a.i., but the virus did not induce any CPE until 7 d.a.i.

To examine whether the presence or absence of CPE depend on the cell type with IFN, we treated polyinosinic–polycytidylic acid [poly(I:C)] to two types of cell and examined the difference of type-I IFN expression. Poly(I:C) is synthetic mimetic viral