Journal of Teikyo Heisei University Vol.20 No.1

帝京平成大学紀要 第20巻 第1号

Records of Three Species of Freshwater Crabs from China

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中国産サワガニ類３種の記録

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摘　要

中国産のサワガニ類３種，すなわち浙江省産の*Sinopotamon chekiangense* Tai & Song, 1975，湖北省産の *S. teritisum* Dai, Chen, Zhang & Lin, 1986 および广西自治区産の *Potamon flexum* Dai, Song, Li & Liang, 1980について，写真を付して再記載を行った。

Abstract

Three species of freshwater crabs (Family Potamidae) from China, *Sinopotamon chekiangense* Tai & Song, 1975 from Zhejiang Province, *S. teritisum* Dai, Chen, Zhang & Lin, 1986 from Hubei Province, and *Potamon flexum* Dai, Song, Li & Liang,1980 from Guangxi Zhuang Autonomous Region, are recorded. Each species is described, with some photographs of the distinguishing characters for further identification.

Keywords : Freshwater crab, *Sinopotamon chekiangense*, *Sinopotamon teritisum*, *Potamon flexum*, Hubei Province, Zhejiang Province, Guangxi Zhuang Autonomous Region, China

Introduction

In 2006 and 2007, under the financial supports from the Ministry of Health, Labor and Welfare of Japan on emerging and reemerging diseases and from the Overseas Research Entrustment Program of the Japan Health Sciences Foundation on emerging and reemerging diseases, the junior authors visited several places in China to collect the freshwater crabs as the second intermediate host of the lung flukes. Most of the crabs collected were exposed to the detection of larval cysts of the parasites, and a pair of each species was transmitted to the senior author to know the scientific names of the crabs as a part of a serial study on the Chinese lung flukes.

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In China, many new species of the freshwater crabs were described from 1970s by Dr. A. Dai of the Academia Sinica and her collaborators under the projects to eradicate the lung flukes, and the results were finally grouped together as one of the serial publication, *Fauna Sinica*, by Dai (1999)[1]. In this book the descriptions of the new taxa and the keys to the species were made in English, but the known species were described in Chinese. All the species were associated with the diagrammatic figures of some characters, but the photographs were insuffient for the subsequent identification. The present records are restricted only to three species, but may be useful for the definite and easy identification of the species from the localities concerned.

The specimens examined are preserved in the collections of the National Museum of Nature and Science, Tokyo. The size of the specimens is shown in millimeters, with the abbreviations cb and cl for the breadth and length of the carapace, respectively.

Taxonomic Accounts

Family Potamidae

Genus ***Sinopotamon*** Bott, 1967

Sinopotamon chekiangense Tai & Song, 1975

(Figs. 1A, 2A, 3)

Material examined. Da Xi Village, Yuyao City, Zhejiang Province, China（浙江省余姚市大溪村）— 1♂ (cb 35.3 mm, cl 27.9 mm), 1♀ (cb 29.5 mm, cl 22.9 mm), Sept. 11, 2006.

Description. Male. Carapace rather narrow, surface flattened and uneven, typically separated to regions with depressed furrows symmetrically arranged; whole surface covered with microscopic pits, anterior and anterolateral regions covered with worn-out granules. Frontorbital margin fringed with confluent granules of good size; median one third of frontal margin weakly concave in dorsal view; supraorbital margin raised without interruption; external orbital angle distinct with angulated granule. Anterolateral margin only weakly arched, with a weak epibranchial notch; anterior margin before epibranchial notch short, fringed with five granules, posterior margin behind epibranchial notch long, armed with ca. 15 sharp granules decreasing size posteriorly. Lateral surface of posterolateral part of carapace covered with short setae.

Both chelipeds of male heavy, not long, similar to, but slightly unequal to each other, with inflated palms; surfaces smooth, but uneven on all surfaces. Ambulatory legs stout, depressed, with many bundles of marginal short setae; anterior margins of meri thickened and fringed with depressed granules; carpi of anterior three pairs with thick anterior margins; each propodus with a longitudinal furrow on upper surface; posterior upper and lower margins armed each with five or six horny spines; dactyli slightly longer than propodi, armed with several and some spines each on anterior upper and lower, and posterior upper and lower margins, respectively.

Male abdomen narrow, tapering from basal segment to sixth segment; terminal segment longer than sixth segment, its lateral margins subparallel to each other for most of length, with rounded distal margin. Male first pleopod stout, with distal segment being one third as long as shaft; shaft with a longitudinal thick ridge at distal inner part on ventral surface; distal segment weakly tapering, incised small at tip, as long as distal ridgeof shaft, only weakly directed outward and dorsad.

Female. A female specimen in hand is slightly smaller than the male examined. The dorsal surface of the carapace is gently separated to the regions; the frontorbital margin is raised, but not so strongly as in the male

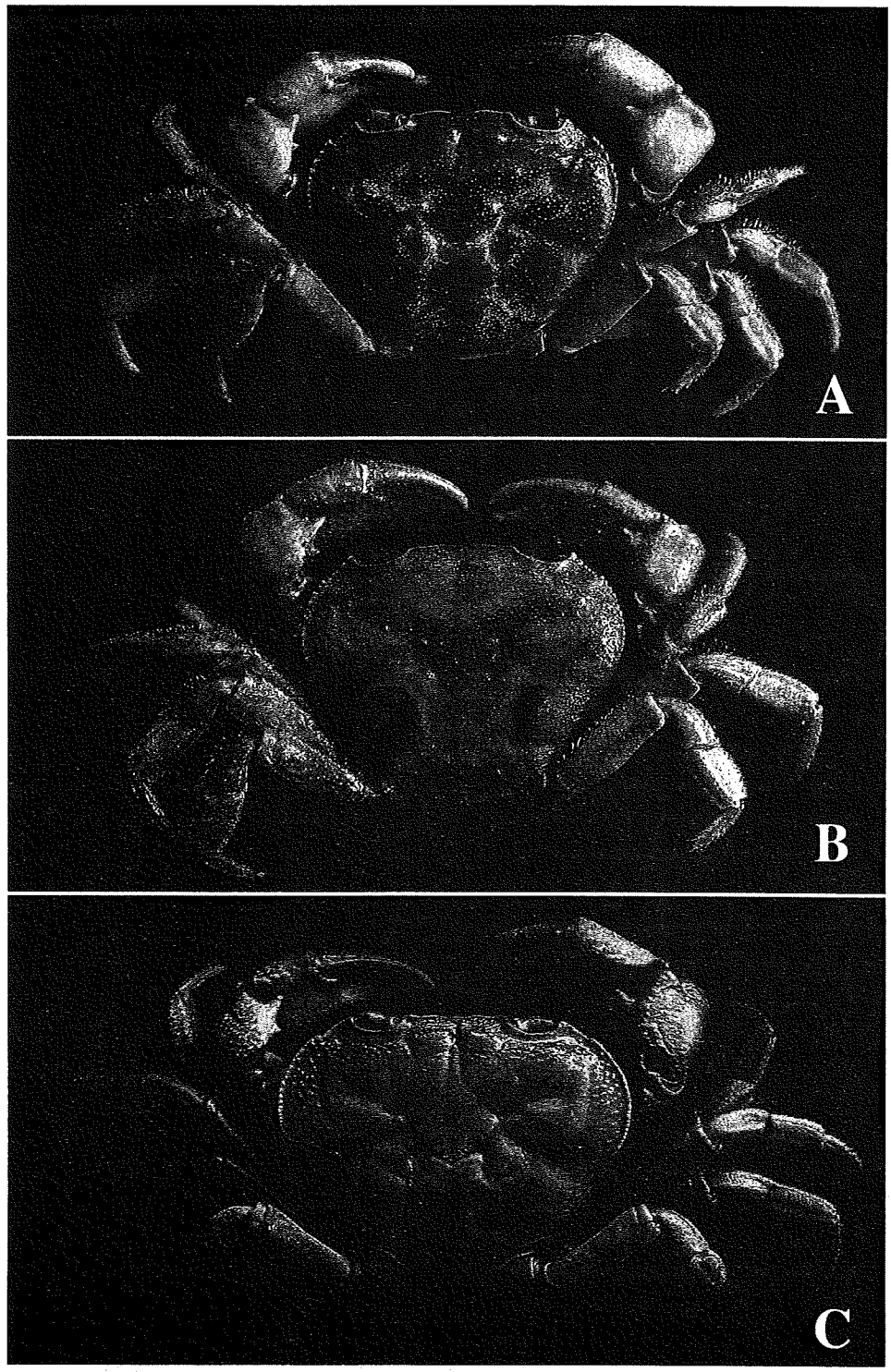

Fig. 1. A: *Sinopotamon chekiangense* Tai & Song, male (cb 35.3 mm) from Yuyao, Zhejiang Province. B: *Sinopotamon teritisum* Dai, Chen, Zhang & Lin, male (cb 31.6 mm) from Danjiangkou, Hubei Province. C: *Potamon flexum* Dai, Song, Li & Liang, male (cb 56.3 mm) from Baiswe, Guangxi Zhuang Autonomous Region. (Preserved specimens)

The chelipeds are very slightly different in size and not much inflated. The armature and hairiness of the chelipeds and ambulatory legs are also weaker than in the male. The abdomen is subovate and covers the whole sternum; the sixth and terminal segments are subequal in length, with the terminal segment is subtruncated at the distal margin. The genital orifice is elliptical, one third as wide as the sternum, occupying the anterior half or more at inner part of the sternum.

Remarks. This species has been known by the original paper (Tai & Sung, 1975[2]) and Dai (1991[3], 1999[1]). The male first pleopod is somewhat different from the schematic figures given by the original authors in having the distal segment not so strongly tapering. The general shape of the carapace is typical for *Sinopotamon*, but the carapace is more or less quadrate, with the weakly convergent posterolateral margins of the carapace. The distal segment of the male abdomen is prominent, not tapering, with rounded terminal margin.

Distribution. Known from many localities in Zhejiang Province and some localities in Fujian Province, living under stone in mountain stream.

Notes. Metacercariae of *Paragonimus westermani* (Kerbert, 1878) were isolated.

Sinopotamon teritisum Dai, Chen, Zhang & Lin, 1986
(Figs. 1B, 2B, 4)

Material examined. Guanshan Town, Danjiangkou City, Hubei Province, China （湖北省牡丹口市官山鎮） – 1♂ (cb 31.6 mm, cl 24.6 mm), 1♀ (cb 25.0 mm, cl 19.7 mm), Sept. 7, 2006.

Description. Male. Carapace rather narrow, and ratio of carapace length to breadth 1.2 (Fig. 1B), not convex dorsally (Fig. 2B); dorsal surface divided into regions with shallow depressions, uniformly and rather densely covered with microscopic pits and short hairs; frontorbital margin thin, with supraorbital margin raised dorsally; a transverse depression behind frontorbital margin followed by obtuse anterior ridges of epigastric, protogastric and branchial regions; anterior part of a longitudinal bifurcation separating gastric regions of both sides distinct and linear, but its posterior half obsolete; lateral margins of mesogastric region linear, deep and longitudinal; cardiac region not convex, as large as mesogastric region; metagastric region outside of cardiac region rounded, surrounded by a shallow depression. Anterolateral margin of carapace with a small interruption at hepatic part, being fringed with a series of sharp granules; external orbital angle sharp, directed forward; posterolateral margin of carapace more or less concave.

Chelipeds of both sides slightly unequal, roughened with sharp granules and short hairs. Ambulatory legs strongly depressed, provided with longish hairs mainly on anterior margins; each carpus with a longitudinal, narrow furrow on middle part of upper surface, each propodus with a longitudinal, wide depression occupying two thirds of upper surface.

Abdomen moderately wide, long; sixth segment 1.5 times as long as fifth segment, 2.3 times as wide as long; terminal segment as long as sixth segment, tongue-shaped, tapering distally, with subacute distal margin, being abruptly widened close to articulation with sixth segment. Male first pleopod stout, with distal segment about one third as long as shaft, directed sternally along sternal trench; ventral surface of distal part of shaft more or less callous and thickened; terminal segment weakly directed outward, tapering distally; semitransparent tip one fourth as long as distal segment, narrowing abruptly toward genital orifice at its top.

Female. The general shape and areolation of the carapace are very close to those of the male examined; the dorsal surface is divided into the regions by depressions, and covered with microscopic pits and short hairs; the epigastric areolae behind the frontal margin is more prominent and advanced than in the male. Both

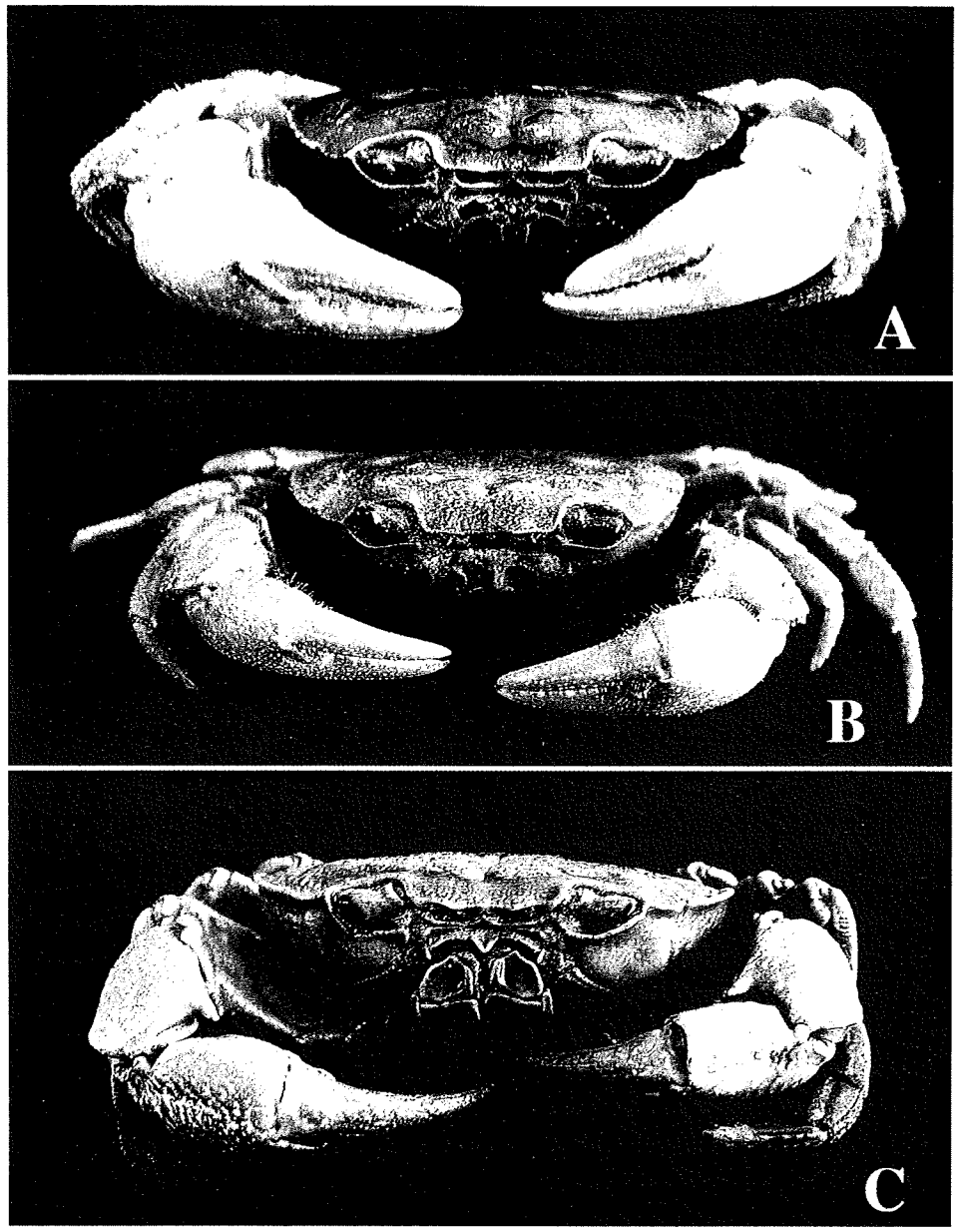

Fig. 2. A: *Sinopotamon chekiangense* Tai & Song, male (cb 35.3 mm) from Yuyao, Zhejiang Province. B: *Sinopotamon teritisum* Dai, Chen, Zhang & Lin, male (cb 31.6 mm) from Danjiangkou, Hubei Province. C: *Potamon flexum* Dai, Song, Li & Liang, male (cb 56.3 mm) from Baiswe, Guangxi Zhuang Autonomous Region.

chelipeds are different in size, but smaller than those of the male. The abdomen is typically ovate and weakly convex along the lateral margins, with the prominent sixth and terminal segments; the sixth segment is one third as long as wide, and the terminal segment is as long as the sixth segment, rather strongly convex forward along the distal margin. The genital orifices are large, occupying almost half, or more, of the sixth sternal segment.

Remarks. This species is generally close to the preceding species, *Sinopotamon chekiangense* Tai & Song,but the carapace is covered with microscopic pits and short hairs, and narrower posteriorly with more strongly

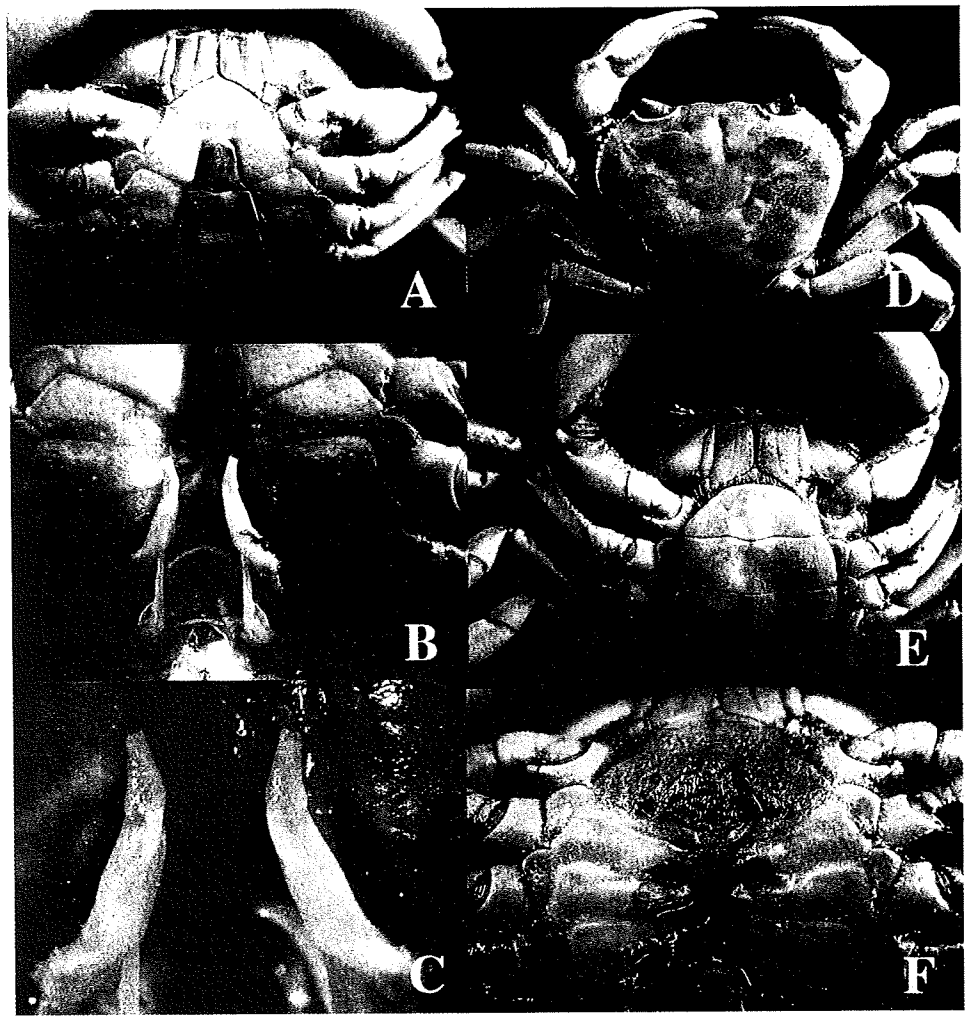

Fig. 3. *Sinopotamon chekiangense* Tai & Song from Yuyao, Zhejiang Province. Male (cb 35.3 mm), abdomen (A) and first pleopod (B, C). Female (cb 29.5 mm), carapace (D), abdomen (E) and genital orifice (F).

convergent posterolateral margins. The male abdomen is becoming narrower distally in this species, but not tapering in *S. chekiangense*, and the terminal segment of the male first pleopod is tapering and weakly directed outward, but not tapering, incised small at its tip, directed outward and dorsad in *S. chekiangense*.

Distribution. Known by Dai *et al.* (1986)[4]and Dai (1999)[1] who mentioned many localities in Hubei Province and some localities in Sichuan Province. According to them, this species lives in the mountain river at the altitude 700-1400 m.

Notes. Metacercariae of *Paragonimus skrjabini* Chen, 1959 were isolated.

Genus ***Potamon*** Savigny, 1816

Potamon flexum Dai, Song, Li & Liang, 1980

(Figs. 1C, 2C, 5)

Material examined. Guoli, Shanjie Village, Beinan Township, Napo County, Baiswe City, Guangxi Zhuang

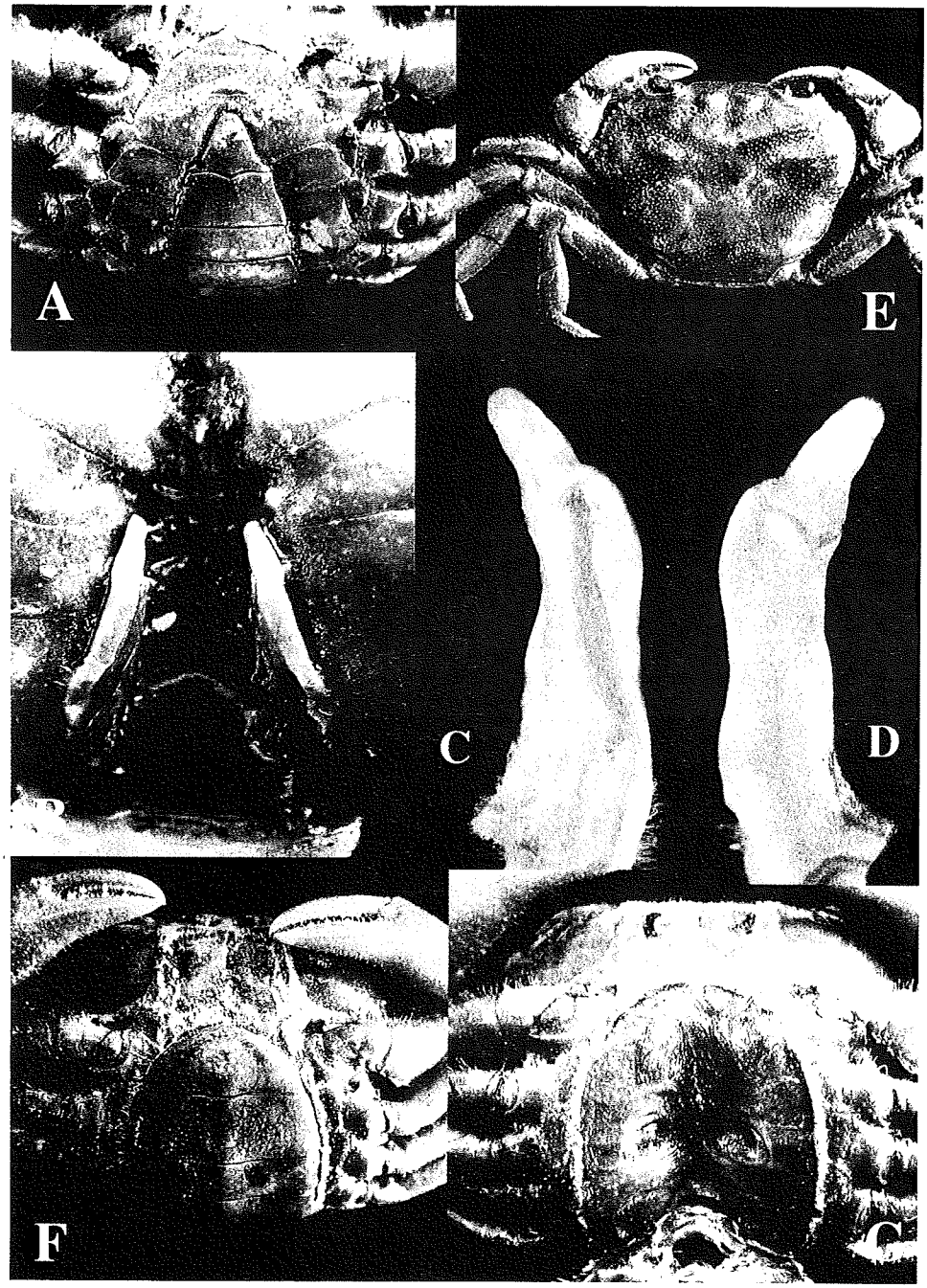

Fig. 4. *Sinopotamon teritisum* Dai, Chen, Zhang & Lin from Danjiangkou, Hubei Province. Male (cb 31.6 mm), abdomen (A), first pleopod (B-D). Female (cb 25.0 mm), carapace (E), abdomen (F) and genital orifice (G).

Autonomous Region, China（广西壮族自治区百色市那坡百南鄉上盖村） — 1♂(cb 56.3 mm, cl 41.3 mm), 1♀(cb 39.5 mm, cl 29.4 mm), May 15, 2007.

Description. Male. Carapace transversely ovate in outline, with convex anterolateral margin rimmed with a narrow edge; dorsal surface rather flattened, roughened with scattered granules of good size mainly on anterior and anterolateral surfaces, uneven, with grooves and depressions symmetrically arranged; frontorbital

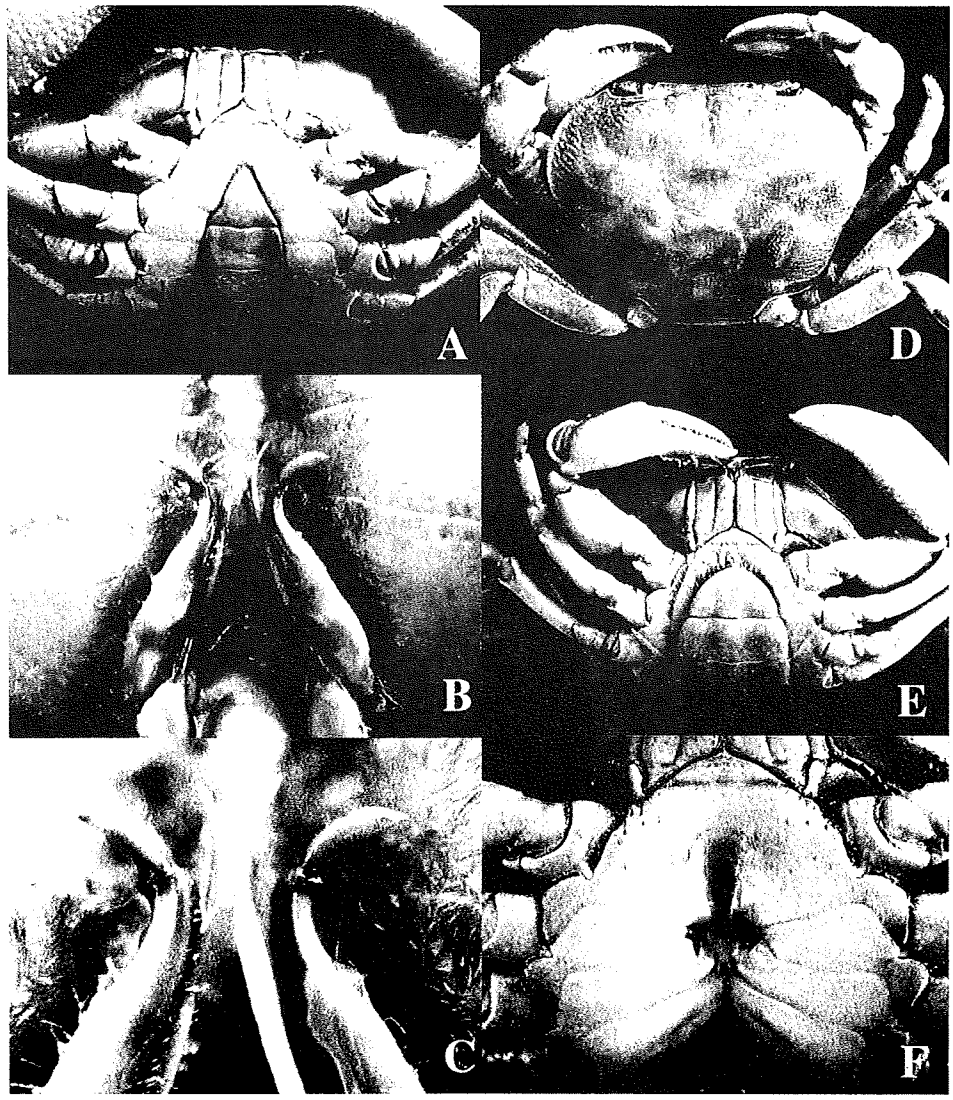

Fig. 5. *Potamon flexum* Dai, Song, Li & Liang from Baiswe, Guangxi Zhuang Autonomous Region. Male (cb 56.3 mm), abdomen (A), first and second pleopods (B, C). Female (cb 39.5 mm), carapace (D), abdomen (E) and genital orifice (F).

margin raised as a narrow ridge, separated from gastric regions by a continuous furrow along margin; some depressions symmetrically arranged around mesogastric region, making an appearance of coarse surface, especially with two prominent furrows running anterolaterally and posterolaterally from lateral angle of mesogastric region. Epibranchial notch small but distinct; anterolateral margin unarmed in front of this notch, anterolateral margin behind this notch strongly arched, fringed with granules which are close together and become smaller posteriorly. Posterolateral margin of carapace strongly convergent, rather concave laterally and dorsally behind rim of anterolateral margin.

Chelipeds heavy, distinctly different in size, roughened with granulated wrinkles; inner angle of carpus armed with a strong upper tubercle and a small lower tubercle. Ambulatory legs stout, smooth, sparsely fringed with short setae; each carpus with a longitudinal depression along anterior margin; each propodus

with a longitudinal median furrow along overall length of upper surface.

Abdomen comparatively narrow; length of sixth segment half of its width; terminal segment as long as wide, triangular, with subacute tip. First pleopod stout; shaft tapering, directed obliquely inward, and then strongly and regularly curved outward together with distal segment; ventral surface of shaft swollen longitudinally; distal segment about one fourth as long as shaft, weakly thickened distally to subterminal part, cut out obliquely at distal part, with sharp tip directed almost transversely. Second pleopod elongated, stalk comparatively thick as well as basal half of terminal segment; distal half of terminal segment semitransparent, tapering, weakly curved outward.

Female. The carapace, chelipeds and ambulatory legs are close to those of the male specimen in hand. Although the granules and depressions on the dorsal surface of the carapace are smaller and shallower than those the male, it is not definite that the differences are due to the smaller size of the specimen or the difference of the sex. The chelipeds are comparatively smaller than those of the male, but there may be no definite sexual differences including the asymmetry of both chelipeds between both specimens of different sexes examined. The abdomen is comparatively narrow, with the prominent sixth and terminal segments; the terminal segment is slightly longer than sixth segment, with subacute distal margin. The genital orifice area occupies the inner one fourth of the sixth sternal segment, narrowing outward; its most part convex dorsally as a dome, with the genital orifice at its innermost part directed inward; the front yard of the genital orifice is sunken and expanded anteriorly.

Remarks. This species was described originally by Dai *et al.* (1980: 369, fig. 1, pl. 1 fig. 1)[5] and additionally by Dai (1999: 183, fig. 97, pl. 11 fig. 8)[1] based on the specimens from the Guangxi Zhuang Autonomous Region, living under stone at about 800 m above sea level. According to these records, this species is among the biggest in the genus *Potamon*, with the maximum size of the carapace breadth being 50 and 46.5 mm in the male and the female, respectively. The male specimen in hand is the biggest known to date.

This species is peculiar in having the uneven dorsal surface of the carapace provided especially with two prominent furrows running anterolaterally and posterolaterally from the lateral angle of the mesogastric region, and also the male first pleopod is much more strongly and regularly curved outward at the distal part of the shaft together with the distal segment than those of the related species.

Distribution. Hitherto known from two localities in the Guangxi Zhuang Autonomous Region. Of 13 species and 4 subspecies of the genus *Potamon* enumerated by Dai (1999)[1] from China, *P. flexum* is only the inhabitant outside the Yunnan Province.

Notes : Metacercariae of *Paragonimus heterotremus* Chen & Hsia, 1964 were isolated.

Literature

1) Dai, A., 1999. *Fauna Sinica. Arthropoda, Crustacea, Malacostraca, Decapoda, Parathelphusidae, Potamidae*. Science Press, xiii + 501 pp., 30 pls. (In Chinese with English abstract)

2) Tai, A.-y. & Y.-c. Sung, 1975. A preliminary study of the freshwater crabs as intermediate hosts of lung flukes from China. *Acta Zool. Sinica*, **21**: 169-177, pls. 1-4. (In Chinese with English summary)

3) Dai, A., 1991. Brachyura (freshwater crabs), pp. 387-402. In: *Fauna of Zhejiang, Crustacea*, 481 pp., 4 pls. Zhejiang Science and Technology Publishing House. (In Chinese)

4) Dai, A.-y., G.-x. Chen, S.-q. Zhang & H. Lin, 1986. A study of the freshwater crabs from Hubei Province. *Sinozoologia*, (4): 55-71, pl. 1. (In Chinese with English summary)

5) Dai, A.-y., Y..-z. Song, L.-l. Li & P.-x. Liang, 1980. New species and new record of freshwater crabs from Guangxi. *Acta Zootax. Sinica*, **5**: 369-376. (In Chinese with English summary)

アニサキスの分類学的解析：人への感染源の特定に向けたサバ由来虫体の検索

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Further analysis of Japanese *Anisakis* worms isolated from scombroid fish (mackerel) at the sibling species level

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我が国のアニサキス症は、*Anisakis simplex*を主要病原虫として発生すると考えられてきた。本虫の分類に関しては、これを３種類の同胞種、すなわち*A. pegreffii*、*A. simplex* sensu stricto (s. str.), *A. simplex* Cに分けるという考えが提出されている（Mattiucci and Nascetti, 2006)。

我々は、日本近海に生息する魚類から得た*A. simplex*について、この新しい分類法による同胞種の解析を試みた。その結果、南日本（九州・日本海産のサバ）では*A. pegreffii*が、北日本（北海道・太平洋産のサバ等）では*A. simplex* s. str.が優占することを明らかにした（Umehara *et al.*, 2006)。一方、人体症例由来の虫体（九州と北海道の85名から得た計100虫体）は、*A. pegreffii*を１虫体認めた以外、総て*A. simplex* s. str.と同定された（Umehara *et al.*, 2007)。すなわち九州では、魚に由来する優占種と患者に由来する優占種とが異なるとの結果を得たが、その理由は説明できていない（Umehara *et al.*, 2008)。

人への主たるアニサキスの感染源は、九州ではやはりサバとの成績がある（飯野ら、1992)。そこで今回は更に、九州の市場で日本海産と同様によく流通する東シナ海産のサバを検索対象に選び、アニサキス虫体の検出と同胞種レベルでの同定を試みた。また、千葉県産および新潟県産のサバも対象に、同様の検討を行った。

【材料と方法】

福岡県（東シナ海）で水揚げされたサバ８尾、千葉県（太平洋）のサバ６尾、新潟県（日本海）のサバ16尾から、1,718匹、76匹、32匹のアニサキス虫体を検出した。このうち福岡では152匹を、残りの二県では検出した全虫体を対象とし、１匹ずつDNAを抽出して、同胞種レベルでの分類学的解析を行った。

【結　果】

福岡県（東シナ海）の虫体152匹のうち、150匹は*A. pegreffii*と同定され、*A. simplex* s. str.はわずか１匹であった。残り１匹は本邦で魚類から初めて検出された*A. typica*と同定された。千葉県の虫体76匹は、67匹が*A. simplex* s. str.、９匹が*A. pegreffii*と同定された。新潟県の虫体32匹は、16匹が*A. simplex* s. str.、16匹が*A. pegreffii*と同定された。

【考　察】

九州のアニサキス症例は、地元産ではない魚介類（サバ）を主な原因として発生することが示唆された。今後はサバ以外の魚種について、特に九州の市場で流通するものを対象に、同様の検討を進める予定でいる。

Key words：*A. simplex*, *A. pegreffii*, sibling species

引用文献

Umehara, A. *et al.* H. 2008. Molecular analysis of Japanese *Anisakis simplex* worms. *Southeast Asian J. Trop. Med. Public Health* 39 (Suppl.)：26-31.

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皮膚病診療：31(8)；977～980, 2009

◇虫による皮膚病—2009<臨床例>——⑮

皮膚二核顎口虫症

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Key words
遊走性皮疹，顎口虫症，二核顎口虫，ペルー

・顎口虫症は，野生哺乳動物を終宿主とする顎口虫の第3期後期幼虫が非好適宿主であるヒトに摂取され，人体内を移動することによりさまざまな症状を呈する幼虫移行症である．
・ペルーで生魚料理セビッチェ(ceviche)を食べた後に，遊走性皮疹が出現し，皮膚生検組織内虫体の遺伝子解析により二核顎口虫症と確定診断した1例を経験したので報告する．

症例　44歳，男．海外出張が多い．

初診　2007年10月．

主訴　右下肢の遊走性皮疹．

家族歴，既往歴　特記すべきことはない．

現病歴　2007年9月下旬から10月初旬にペルーへ渡航し，現地の郷土料理であるセビッチェという生魚のマリネを食べた．帰国して数日後に咽頭痛，頭痛が出現した．10月下旬に右膝上方に腫脹，発赤，熱感，軽度の圧痛が出現したため，その2日後に当院整形外科を受診した．白血球とCRPの軽度の上昇（WBC 9,690/μl，Eos 5.6%，CRP 0.75mg/dl）より，蜂窩織炎と診断され，塩酸セフカペンピボキシル(フロモックス)，ロキソプロフェンナトリウム(ロキソニン)にて加療されるも，皮疹が右大腿へ移動してきたため，整形外科受診後8日目に当科を紹介受診した．

当科初診時現症　右大腿前面上方に，熱感・圧痛を伴う限局性の腫脹・発赤を認めた．

臨床検査成績(下線部は異常値)

WBC 9,020/μl(Neut 55.0%，Eos 11.0%)，RBC 550×10⁴/μl，Hb 15.9g/dl，Plt 30.0×10⁴/μl，AST 35 IU/l，ALT 48 IU/l，T-Bil 0.49mg/dl，BUN 14.0mg/dl，Cr 0.94mg/dl，CRP 0.18mg/dl．

鑑別診断

臨床的に遊走性皮疹を呈し，末梢血好酸球の上昇が認められたこと，さらに海外渡航先での魚の生食歴があったことから，皮膚遊走性限局性腫脹をきたす寄生虫疾患を考えた．移動性皮膚病変を呈するものとして，線状疹では顎口虫症，旋尾線虫症や動物由来の鉤虫症，限局性腫脹ではマンソン孤虫症，イヌ糸状虫症，肺吸虫症などが知られている[1]．そこで，国立感染症研究所寄生動物部でドロレス顎口虫，イヌ糸状虫，マンソン孤虫，ウェステルマン肺吸虫，宮崎肺吸虫の5種類の寄生虫抗原を用いてELISA法を行ったところ，ドロレス顎口虫とイヌ糸状虫抗原に対して陽性反応であった．

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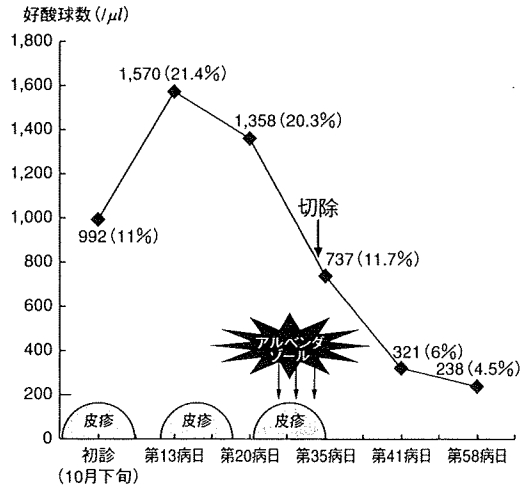

図1　臨床経過．皮疹は約1カ月にわたり消褪と再発を繰り返し移動した．好酸球は21.4%にまで上昇を認めたが，アルベンダゾール投与後に，皮疹を切除した後は順調に低下し，正常化した．

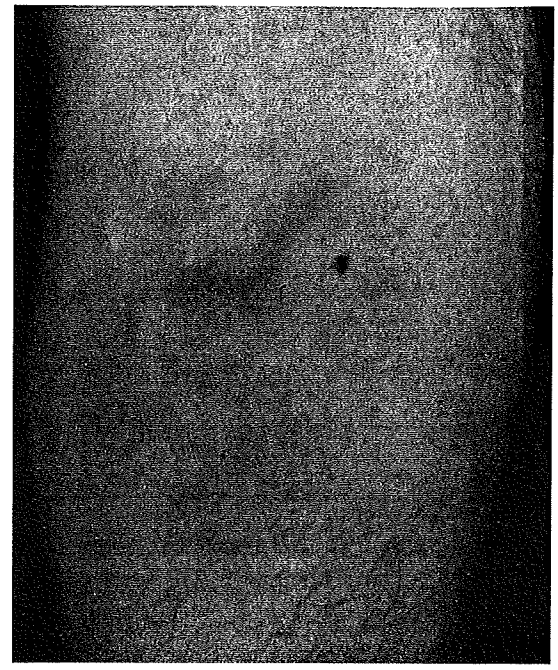

図2　アルベンダゾール開始8日目の臨床像．右下肢腓腹部に線状爬行疹がみられたため，両端を切除した．

旋尾線虫：ホタルイカの生食が感染源であることから否定された．

イヌ糸状虫症：イヌ糸状虫の幼虫に感染したカがヒトを吸血し感染する．臨床症状として咳，痰，胸痛，発熱などが出現する．イヌ糸状虫抗原に対する陽性反応は，イヌ糸状虫と同じ線虫類の粗抗原を用いたことによる交叉反応であると考えられた．

マンソン孤虫症：幼虫であるプロセルコイドが寄生するケンミジンコの摂取，あるいはプレロセルコイドが寄生するヘビ，カエル，地鶏の生食により感染する[2]．臨床症状としては，不規則な発熱や移動性の皮下腫瘤が出現するが，移動速度は遅く，自験例では摂食歴もなく，血清診断結果も陰性であった．

肺吸虫症：サワガニなど淡水産カニに寄生した肺吸虫メタセルカリアを摂取した場合，あるいはサワガニを食したイノシシ筋肉内に寄生した肺吸虫を摂取した場合に感染し，咳・胸痛，血痰，胸水貯留などの症状が出現するほか，皮下腫瘤を形成することもあるが，自験例では摂食歴，臨床症状，血清検査から否定された．

診断，治療と経過

皮膚の発赤，熱感を伴う限局性腫脹は，10月下旬には右膝上方に，その10日後には右大腿上方の前面に，さらにその約2週後にはその後面に，11月下旬には右膝上方の内側に，と約1カ月にわたり消褪と再発を繰り返し移動した．その間，好酸球は21.4%にまで上昇を認めた(図1)．血清診断ELISA法にてドロレス顎口虫抗原に陽性反応を示したことより顎口虫症と考え，アルベンダゾール(エスカゾール)400mg/日を，11月下旬の右膝上方内側の腫脹出現時より3日間投与したところ，内服を開始してから7日目に，皮疹は右下肢腓腹部に移動し再発を認めた．それまで限局性の腫脹を呈していた皮疹は，右下肢腓腹部では線状爬行疹を呈していた(図2)．

皮疹の進行方向が不明であったが，その両端部のいずれかに顎口虫がいると考え，診断確定と治療を兼ね，12月初旬に皮疹の両端部を切除した．線状爬行疹の上方の先端部より生検した標本の真皮中層に虫体を確認でき，虫体周囲には好酸球，リンパ球を主体とする炎症細胞の浸潤を認め，虫体表面には多数の皮棘が密生し，体腔内に大きく突き出した双葉状の側索が観察され，顎口虫幼虫

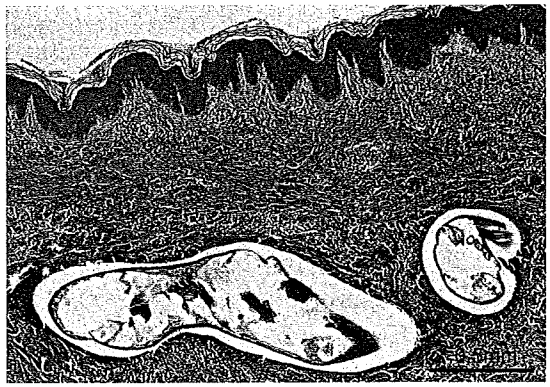

図3　真皮中層に虫体の断面を認め，その周囲には好酸球，リンパ球を中心とする炎症細胞浸潤を認める(H-E染色，×40)．

と同定された(図3)．

そこで，顎口虫の種の鑑別を行うために，国立感染症研究所寄生動物部にて，パラフィン包埋無染色切片内の虫体断端標本を用いて，ミトコンドリアDNAのcytochrome *c* oxidase subunit 1遺伝子(*cox1*)とリボソームRNA遺伝子内のITS-2領域の塩基配列を解析した．その結果，既知の二核顎口虫(*Gnathostoma binucleatum*)の塩基配列と100%一致したことから，本症例を二核顎口虫症と診断した．自験例のDNAデータは以下のアクセッション番号で登録されている(*cox1*はAB374229とAB428417，ITS-2領域はAB428418)．

虫体を摘出した後は，皮膚症状の再燃はなく，好酸球数も順調に低下し12月上旬には正常化した．

考　按

現在，世界には12種の顎口虫が知られており[3,4](表)，人体寄生の報告があるのは，有棘顎口虫，ドロレス顎口虫，日本顎口虫，剛棘顎口虫，二核顎口虫の5種である[3]．本邦では，第二次世界大戦直後の食糧事情が悪かった時期に，雷魚の生食による有棘顎口虫の感染が西日本で流行した．1980年ごろからは，中国，韓国や台湾産の輸入ドジョウの生食による剛棘顎口虫の感染が西日本の大都市を中心に流行した．東北地方では国産ドジョウや，ブラックバスの生食による日本顎口虫の感染が，九州全域，四国，中国地方では渓流魚の生食によるドロレス顎口虫の感染が報告されている[1,3]．

顎口虫成虫は終宿主である哺乳類の胃壁や食道などの消化管粘膜に寄生しており，終宿主の糞便とともに排出された虫卵は水中で発育，孵化し第2期幼虫となり，これが第1中間宿主であるケンミジンコに取り込まれ，その体内で第3期前期幼虫になる．ケンミジンコがさらに第2中間宿主(魚類，両生類)に摂取されると，その体内で第3期後期幼虫となる．ヒトは第3期後期幼虫が寄生した第2中間宿主(魚類，爬虫類)を摂取することで，感染する．

ヒトが感染すると，前駆症状として微熱，感冒様症状，倦怠感，食欲不振などを呈する．またヒトに経口摂取された幼虫は，腸管から一度腹腔に出て肝臓を通過するので肝障害が出現することもある[5]．皮膚症状は感染の約3週間後より出現し，線状爬行疹や限局性腫脹を呈する[3]．皮疹は痒みや軽度の疼痛を伴いながら移動し，数カ月～数年間持続する．そのほか，歩行・運動障害，痙攣，意識障害，気胸，イレウス，腹膜炎，心筋梗塞，眼科・耳鼻科・泌尿器科的障害など，さまざまな症状を呈する[6]．

遊走性皮疹などの臨床所見，魚類，両生類などの生食歴，海外居住歴・海外渡航歴などの詳細な問診，末梢血好酸球数や血清IgE値の上昇等から本疾患を疑い，皮内反応やELISA法による免疫学的血清反応を行う．末梢血好酸球数や血清IgE値は，感染期間や個体により差異がみられる．本疾患患者におけるドロレス顎口虫抗原を用いた皮内反応では，陽性率82.4%，偽陽性16.6%，またELISA法では陽性率93.7%，偽陽性27.7%で，感度は高いが，特異性はやや低いとする調査結果もある[7]．免疫学的血清反応は，各種線虫類抗原間で交叉反応がみられ，また検査方法や評価が各検査機関で一定していないなどの欠点があり，あくまで補助診断であり，診断確定には治療を兼ねて虫体を摘出する方法が考えられるが，虫体検出はむずかしい．虫体が摘出された場合には，頭球鉤の形態や数，腸管上皮細胞の形態や核数の観察により顎口虫の種の同定が可能であるが[8]，虫体断端によっては，これらの形態が観察されるとは限らないので，自験例のように遺伝子解析が種の同定に有用である．

表　顎口虫属の独立種(赤羽，岩田〈2005〉を改変)

学名，和名	終宿主	寄生部	分布地
G. spinigerum Owen, 1836　有棘顎口虫	ネコ科，イヌ科	胃壁	アジア，オセアニア
G. hispidum Fedtschenko, 1872　剛棘顎口虫	ブタ，野生ブタ	胃壁	アジア，ヨーロッパ
G. turgidum Stossich, 1902　オポッサム顎口虫	オポッサム	胃壁	アメリカ大陸
G. americanum Travassos, 1925　アメリカ顎口虫	ネコ科	胃壁	南米
G. doloresi Tubangui, 1925　ドロレス顎口虫	ブタ，イノシシ	胃壁	アジア，オセアニア
G. nipponicum Yamaguti, 1941　日本顎口虫	イタチ	食道壁	日本
G. procyonis Chandler, 1942　アライグマ顎口虫	アライグマ	胃壁	アメリカ大陸
G. miyazakii Anderson, 1964　宮崎顎口虫	カワウソ	腎臓	アメリカ大陸
G. malaysiae Miyazaki et Dunn, 1965　マレーシア顎口虫	ネズミ	胃壁	マレーシア，タイ国
G. vietnamicum Le-Van-Hoa, 1965　ベトナム顎口虫	カワウソ	腎臓	ベトナム，タイ国
G. binucleatum Almeyda-Artigas, 1991　二核顎口虫	ネコ科，イヌ科	胃壁	メキシコ以南のアメリカ大陸
G. lamothei Bertoni-Ruiz et al, 2005　和名なし	アライグマ	胃壁	メキシコ

治療は，虫体の摘出がもっとも確実だが，虫体の移動速度が速く[9]，深部皮下組織に迷入することもあること，などから，皮疹部のみを生検しても虫体が摘出できないことも多い．皮疹の進行部先端のいまだ正常にみえる皮膚をできるかぎり大きく切除することが推奨されている[10]．駆虫剤としてアルベンダゾールが使用されることが多いが[1,6,8~12]，単独では奏効率が低い．駆虫剤の内服により虫体の移動速度が下がり，また虫体が皮膚浅層に遊走してくる傾向があるので，摘出に成功する可能性が高くなる[12]．

経過・予後は種によって異なるが，皮膚症状は数カ月～数年間持続する例が多く，中枢神経系に迷入すれば致命的となることもある．

自験例の原因種となった二核顎口虫は，1991年にメキシコで山猫から成虫が発見され[13]，ヒトへの感染源としてテラピアなどの淡水魚の生食が指摘されている．二核顎口虫の形態は，有棘顎口虫と類似しているが，腸管上皮細胞内の核数が，二核顎口虫では平均2個であるのに対し，有棘顎口虫では平均3～4個と報告されているが[8]，自験例では確認できなかった．ペルーでの顎口虫症感染例はこれまでに2例報告されているが[8,14]，いずれも種の同定には至っておらず，自験例は病理組織標本を用いた遺伝子解析により二核顎口虫症と確定診断された本邦初の報告例，世界でも2番目の報告例である．

近年，海外渡航者数の増加に伴い，自験例のように邦人が海外で感染し，帰国後国内で発症する，いわゆる輸入寄生虫症例の報告が相次いでいることから，われわれ皮膚科医も寄生虫疾患の可能性を念頭に置いて診断する必要があると考えられた．

本研究の一部は厚生労働科学研究費補助金　新興・再興感染症研究事業「顧みられない病気に関する研究」(H20-新興-一般-016)によって行われた．

本論文の要旨は，第406回日本皮膚科学会大阪地方会において発表した．

＜文　献＞

1) 緒方克己：最新皮膚科学大系　16，中山書店，東京，p.116，2003
2) 藤田紘一郎：成人病と生活習慣病　33：882，2003
3) 赤羽啓栄，岩田久寿郎：現代寄生虫病事情，医学のあゆみ別冊，医歯薬出版，東京，p.783，2004
4) Bertoni-Ruiz, F. et al.：J Parasitol　91：1143，2005
5) 寺西好治ほか：皮膚臨床　36：473，1994
6) 村上京子ほか：皮膚病診療　9：509，1987
7) 三森龍之ほか：Jpn J Trop Med Hyg　15：191，1987
8) 平島昌生ほか：皮膚臨床　45：1541，2003
9) Jelinek, T. et al.：DMW日本語翻訳版　16：1746，1994
10) 河村七美ほか：日皮会誌　113：389，2003
11) 鎌倉知子，池田政身：皮膚臨床　45：121，2003
12) 坂本光男ほか：感染症学雑誌　78：442，2004
13) Almeyda-Artigas, R.J.：An Inst Cienc del Mar y Limnol Univ Nat Auton Mexico　18：137，1991
14) Chappuis, F. et al.：Clin Infect Dis　33：E17，2001

Journal of Medical Microbiology (2009), 58, 1227–1230 DOI 10.1099/jmm.0.011528-0

Human leptospirosis cases and the prevalence of rats harbouring *Leptospira interrogans* in urban areas of Tokyo, Japan

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Thirteen patients with leptospirosis were identified, as confirmed by laboratory analysis during the last 5 years in our laboratory, who came from urban areas of Tokyo, Japan. All of the patients came into contact with rats before the onset of illness. Seventeen per cent of Norway rats captured in the inner cities of Tokyo carried leptospires in their kidneys. Most of these rat isolates were *Leptospira interrogans* serovar Copenhageni/Icterohaemorrhagiae. Antibodies against these serovars and their DNA were detected in the patients. This suggests that rats are important reservoirs of leptospirosis, and that rat-borne leptospires occur in urban areas of Tokyo.

Received 1 April 2009
Accepted 28 May 2009

INTRODUCTION

Leptospirosis is caused by infection with pathogenic *Leptospira*. It is a globally important zoonotic disease that affects humans in rural and urban settings, in both industrialized and developing countries (Bharti *et al.*, 2003; Levett, 2001; McBride *et al.*, 2005). Transmission of *Leptospira* pathogens to humans occurs mainly through indirect contact with water or soil contaminated by the urine of infected animals (Faine *et al.*, 1999). Leptospirosis has become an important public health problem in Asia and Latin America. In these tropical areas, large outbreaks of leptospirosis are most likely to occur after floods, hurricanes or other disasters. Leptospirosis has also become an urban problem in developing countries. Outbreaks occur in poor urban slum communities during seasonal periods of heavy rainfall (Johnson *et al.*, 2004; Ko *et al.*, 1999; LaRocque *et al.*, 2005). The risk of infection in urban inhabitants is not limited to developing countries because the importance of urban leptospirosis has already been recognized in inner-city populations of the USA (Vinetz *et al.*, 1996). In the present study, we report the presence of leptospirosis and rat reservoirs of leptospires in urban areas of Tokyo, Japan.

METHODS

Serodiagnosis of patients with clinically suspected leptospirosis. The microscopic agglutination test (MAT) for detection of anti-*Leptospira* antibodies in patient serum samples was performed (Faine *et al.*, 1999) using a battery of reference strains described previously (Koizumi *et al.*, 2008). These reference strains were cultivated in liquid modified Korthof's medium with 10% rabbit serum at 30 °C (Faine *et al.*, 1999). Detection of IgM was also carried out by IgM dot enzyme-linked immunoassay (*Dip-S-Ticks*; PanBio) for cases 6 and 8 (Supplementary Table S1 available with the online journal).

Isolation of leptospires from rats. Norway rats (*Rattus norvegicus*) were captured using live traps at 14 locations in urban areas of Tokyo from 2002 to 2007. For the isolation of leptospires, rat kidneys were inoculated into medium and cultivated as described above.

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Abbreviation: MAT, microscopic agglutination test.

The GenBank/EMBL/DDBJ accession numbers for the *flaB* sequences of rat isolates and patient samples are AB454100–AB454125.

A table of detection test data and a figure of PFGE results are available as supplementary data with the online version of this paper.

PCR. DNA was extracted from *Leptospira* isolates, and the blood and urine samples of patients, using a DNeasy tissue kit (Qiagen). Extracted DNAs were subjected to PCR to detect the *Leptospira flaB* gene (*flaB*-PCR; Kawabata *et al.*, 2001; Koizumi *et al.*, 2003). Sequencing of amplicons was performed by the dideoxynucleotide chain-termination method using a BigDye terminator v1.1 cycle sequencing kit (Applied Biosystems).

Identification of serogroups of rat isolates. The serogroups of the isolates were identified by MAT using a panel of anti-*Leptospira* rabbit sera for serovars Australis, Autumnalis, Canicola, Copenhageni, Hebdomadis and Icterohaemorrhagiae, which are present in the main island of Japan.

PFGE. PFGE of rat isolates was carried out as described previously (Koizumi *et al.*, 2009).

RESULTS AND DISCUSSION

Human leptospirosis cases in urban areas of Tokyo

In the last 5 years (from the first case on 4 September 2003 to the last on 18 September 2008), we carried out laboratory examinations for leptospirosis for 55 cases. According to their physicians in Tokyo, the symptoms in these patients matched those of leptospirosis. A total of 16 cases were revealed to be positive for leptospirosis during the period of the study; 13 were from Tokyo (Table 1, Supplementary Table S1 available with the online journal) and the other 3 cases were from Bali (Indonesia), Borneo (Malaysia) and Fiji (data not shown). Among the 13 cases in Tokyo, 12 patients were definitively diagnosed by MAT (fourfold increase in antibody titre between acute and convalescent serum samples or reciprocal MAT titre of at least 400 in a single serum sample), including 4 patients who were also positive for the leptospiral *flaB* gene by PCR using urine or blood specimens. One probable case was demonstrated by anti-leptospiral IgM and a MAT titre of 1 : 160. All patients were hospitalized with severe manifestations, such as acute renal failure and jaundice, indicating that they had contracted Weil's disease (a severe type of leptospirosis). All patients declared that they had come into contact with rats (Table 1). Two patients (nos 1 and 2) worked at a place where they came in contact with rats, and rats were frequently found in the houses or stores of other patients (nos 3–13). The patients neither performed agricultural work nor undertook recreational activity in an endemic area, nor were they exposed to possible reservoir animals other than rats, which are generally considered as high risk behaviours. Among the 39 leptospirosis-negative cases, only 4 patients came in contact with rats (1 patient was a construction worker, and rats were seen at the houses of the other 3 patients). This indicated that the ratio of contact with rats among leptospirosis-positive cases was significantly higher than that among leptospirosis-negative cases in urban areas of Tokyo (Fisher's exact test, $P<0.01$). Since leptospirosis became a reportable disease in Japan (from November 2003), another laboratory-confirmed case other than the 13 cases mentioned above was reported from a regional medical centre in Tokyo in September 2006. The patient saw rats at his restaurant (T. Iida, personal communication).

Isolation and characterization of *Leptospira interrogans* from Norway rats captured in urban areas of Tokyo

We captured 127 Norway rats (*R. norvegicus*) at 14 locations in urban areas of Tokyo from 2002 to 2007. Leptospires were isolated from 22 of the 127 rats from 6 of

Table 1. Human leptospirosis in urban areas of Tokyo diagnosed in our laboratory from 2003 to 2008

Patient no.	Year	Sex	Age (years)	Occupation	Association with rats
1	2003	M	66	Construction worker	Probable environmental contamination with rat urine
2	2004	M	35	Sewer worker	Probable environmental contamination with rat urine
3	2005	F	53	Housewife	Rats appeared frequently in patient's house
4	2005	M	65	Butcher	Patient cleaned the urine and faeces of rats in his store without wearing gloves
5	2006	M	51	Fish dealer	Rats appeared frequently in patient's store
6	2006	M	62	Unknown	Rats appeared frequently in patient's house
7	2006	M	54	Fish dealer	Patient cleaned the urine and faeces of rats in his store without wearing gloves
8	2007	M	51	Day worker	Rats appeared frequently in patient's house (he had been bitten by a rat at his house)
9	2007	F	57	Restaurant worker	Rats appeared frequently in patient's restaurant
10	2008	M	57	Supermarket salesman	Patient was involved in killing rats captured at his store
11	2008	M	56	Fish market worker	Rats appeared frequently in the fish market
12	2008	M	58	Unknown	Patient had contact with rat urine in his house
13	2008	M	68	Restaurant chef	Patient had been bitten by a rat in his restaurant

F, Female; M, male.

the 14 places (Table 2). Nucleotide sequences of the partial *flaB* gene from 18 rats captured at locations F, G, H, K and M were identical (GenBank accession numbers AB454100–AB454117) and those from 4 rats at location A (the *flaB* sequences were identical among the four; GenBank accession numbers AB454118–AB454121) were different in six bases from those described above. The sequences from the 18 rats were identical to those of the reference strains of *L. interrogans* serovar Copenhageni and Icterohaemorrhagiae, suggesting that all the isolates were *L. interrogans*. These isolates reacted equally with anti-Copenhageni and anti-Icterohaemorrhagiae sera, but not with the other sera (data not shown). The *Not*I restriction patterns of the genomes of the isolates from the 18 rats were identical on PFGE not only to each other, but also to the reference strains of serovars Copenhageni and Icterohaemorrhagiae (Supplementary Fig. S1 available with the online journal). It has been determined by PFGE that the genomes of leptospiral serovars have been remarkably conserved over time and across a wide geographical distribution (Herrmann *et al.*, 1991, 1992). Most (but not all) serovars give unique patterns on PFGE carried out using the restriction enzyme *Not*I, although the *L. interrogans* serovars Copenhageni and Icterohaemorrhagiae are indistinguishable. These two serovars are also very difficult to distinguish by serological methods (Kobayashi *et al.*, 1984). These results indicate that isolates from the 18 rats belonged to *L. interrogans* serovar Copenhageni or Icterohaemorrhagiae, which are known to frequently cause Weil's disease in Japan and other countries. We could not carry out MAT for serogroup identification and PFGE on the four isolates at location A due to poor growth.

Table 2. Isolation of leptospires from rats captured in urban areas of Tokyo

Location*	No. of rats captured	No. of rats from which *Leptospira* was isolated (%)
A – park	15	4 (27)
B – park	2	0
C – street	4	0
D – street	8	0
E – building	12	0
F – street	29	12 (41)
G – street	1	1 (100)
H – garden (house)	4	3 (75)
I – park	1	0
J – street	12	0
K – street	13	1 (8)
L – street	5	0
M – store	2	1 (50)
N – street	19	0
Total	127	22 (17)

*Location M was a store in which patient 7 used to work; other locations are not related to the putative exposure sites of the patients.

Conclusion

In 5 of the 13 human leptospirosis cases (cases 1–3, 6 and 7), the patients were infected with serovar Copenhageni or Icterohaemorrhagiae as shown by serological and PCR-based evidence (Supplementary Table S1 available with the online journal). Cross-agglutination and even paradoxical reactions are observed in MAT, but the existence of antibodies against serovars Copenhageni and Icterohaemorrhagiae in all other serum samples suggests the possibility of infection with these serovars (Supplementary Table S1 available with the online journal). Nucleotide sequences of the partial *flaB* gene from urine and blood (patients 1, 2, 3 and 7; GenBank accession numbers AB454122–AB454125) were identical to those from the rat isolates. In particular, *Leptospira* was isolated from a rat captured at the store where patient 7 previously worked. Although there is a possibility of recall bias, all the patients said they had rat contact (Table 1). Dogs and cats may also serve as reservoir hosts in urban settings. We attempted to isolate leptospires from the kidney tissues of stray or abandoned dogs and cats in Tokyo (304 dogs and 77 cats), but *Leptospira* was not obtained. These results strongly suggest that the patients contracted leptospirosis (Weil's disease) from rats in urban areas of Tokyo, though the possibility of involvement of other reservoir animals cannot be excluded.

Outbreaks of leptospirosis are recognized through occupational exposure, such as rice farming and other agricultural activities in rural areas of the tropics (Tangkanakul *et al.*, 2000). Leptospirosis has also become a health problem in urban slums in developing countries (Johnson *et al.*, 2004; Ko *et al.*, 1999; LaRocque *et al.*, 2005). In 'developed countries', recreational activities have recently been identified as a significant risk factor for leptospirosis (Bharti *et al.*, 2003; Levett, 2001; McBride *et al.*, 2005). The present study suggests that humans could contract leptospirosis through occupational exposure or exposure during activities of daily life in environments contaminated with rat urine containing leptospires in urban areas in Tokyo. Leptospirosis constitutes one of the neglected diseases in Japan except for Okinawa Prefecture (Nakamura *et al.*, 2006; Narita *et al.*, 2005). This is one of the reasons why few cases have been identified over the 5 year period despite the high carriage of leptospires in rats in Tokyo. A high prevalence of *Leptospira* spp. in Norway rats from urban settings has also been reported from temperate and tropical endemic countries, but human leptospirosis in urban areas is underreported even in an endemic country (Ariyapruchya *et al.*, 2003; Demers *et al.*, 1985; Doungchawee *et al.*, 2005; Easterbrook *et al.*, 2007; Krøjgaard *et al.*, 2009). Physicians and public health authorities should, therefore, be aware of the severe risk of contracting leptospirosis associated with rats in urban areas.

ACKNOWLEDGEMENTS

We are grateful to H. Obe and M. Uchida for their technical assistance. This work was supported in part by a Health Sciences

Research Grant-in-Aid for Emerging and Re-emerging Infectious Diseases (H13-Shinkou-4, H16-Shinkou-Ippan-033 and H18-Shinkou-8) from the Ministry of Health, Labour and Welfare of Japan.

REFERENCES

Ariyapruchya, B., Sungkanuparph, S. & Dumrongkitchaiporn, S. (2003). Clinical presentation and medical complication in 59 cases of laboratory-confirmed leptospirosis in Bangkok. *Southeast Asian J Trop Med Public Health* **34**, 159–164.

Bharti, A. R., Nally, J. E., Ricaldi, J. N., Matthias, M. A., Diaz, M. M., Lovett, M. A., Levett, P. N., Gilman, R. H., Willig, M. R. & other authors (2003). Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis* **3**, 757–771.

Demers, R. Y., Frank, R., Demers, P. & Clay, M. (1985). Leptospiral exposure in Detroit rodent control workers. *Am J Public Health* **75**, 1090–1091.

Doungchawee, G., Phulsuksombat, D., Naigowit, P., Khoaprasert, Y., Sangjun, N., Kongtim, S. & Smythe, L. (2005). Survey of leptospirosis of small mammals in Thailand. *Southeast Asian J Trop Med Public Health* **36**, 1516–1522.

Easterbrook, J. D., Kaplan, J. B., Vanasco, N. B., Reeves, W. K., Purcell, R. H., Kosoy, M. Y., Glass, G. E., Watson, J. & Klein, S. L. (2007). A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol Infect* **135**, 1192–1199.

Faine, S., Adler, B., Bolin, C. & Perolat, P. (1999). *Leptospira and Leptospirosis*, 2nd edn. Melbourne: MediSci.

Herrmann, J. L., Baril, C., Bellenger, E., Perolat, P., Baranton, G. & Saint Girons, I. (1991). Genome conservation in isolates of *Leptospira interrogans*. *J Bacteriol* **173**, 7582–7588.

Herrmann, J. L., Bellenger, E., Perolat, P., Baranton, G. & Saint Girons, I. (1992). Pulsed-field gel electrophoresis of *Not*I digests of leptospiral DNA: a new rapid method of serovar identification. *J Clin Microbiol* **30**, 1696–1702.

Johnson, M. A. S., Smith, H., Joseph, P., Gilman, R. H., Bautista, C. T., Campos, K. J., Cespedes, M., Klatsky, P., Vidal, C. & other authors (2004). Environmental exposure and leptospirosis, Peru. *Emerg Infect Dis* **10**, 1016–1022.

Kawabata, H., Dancel, L. A., Villanueva, S. Y., Yanagihara, Y., Koizumi, N. & Watanabe, H. (2001). *fla*B-polymerase chain reaction (*fla*B-PCR) and its restriction fragment length polymorphism (RFLP) analysis are an efficient tool for detection and identification of *Leptospira* spp. *Microbiol Immunol* **45**, 491–496.

Ko, A. I., Galvão Reis, M., Ribeiro Dourado, C. M., Johnson, W. D., Jr & Riley, L. W. (1999). Urban epidemic of severe leptospirosis in Brazil. *Lancet* **354**, 820–825.

Kobayashi, Y., Tamai, T., Oyama, T., Hasegawa, H., Sada, E., Kusaba, T. & Hamaji, M. (1984). Characterization of monoclonal antibodies against etiological agents of Weil's disease. *Microbiol Immunol* **28**, 359–370.

Koizumi, N., Watanabe, H., Umezawa, K., Iiduka, T. & Inokuchi, S. (2003). A case of leptospirosis diagnosed early by *flaB*-PCR. *Kansenshogaku Zasshi* **77**, 627–630 (in Japanese).

Koizumi, N., Muto, M., Yamamoto, S., Baba, Y., Kudo, M., Tamae, Y., Shimomura, K., Takatori, I., Iwakiri, A. & other authors (2008). Investigation of reservoir animals of *Leptospira* in the northern part of Miyazaki prefecture. *Jpn J Infect Dis* **61**, 465–468.

Koizumi, N., Uchida, M., Makino, T., Taguri, T., Kuroki, T., Muto, M., Kato, Y. & Watanabe, H. (2009). Isolation and characterization of *Leptospira* spp. from raccoons in Japan. *J Vet Med Sci* **71**, 425–429.

Krøjgaard, L. H., Villumsen, S., Markussen, M. D. K., Jensen, J. S., Leirs, H. & Heiberg, A.-C. (2009). High prevalence of *Leptospira* spp. in sewer rats (*Rattus norvegicus*). *Epidemiol Infect* (in press). doi:10.1017/S0950268809002647

LaRocque, R. C., Breiman, R. F., Ari, M. D., Morey, R. E., Janan, F. A., Hayes, J. M., Hossain, M. A., Brooks, W. A. & Levett, P. N. (2005). Leptospirosis during dengue outbreak, Bangladesh. *Emerg Infect Dis* **11**, 766–769.

Levett, P. N. (2001). Leptospirosis. *Clin Microbiol Rev* **14**, 296–326.

McBride, A. J. L., Athanazio, D. A., Reis, M. G. & Ko, A. I. (2005). Leptospirosis. *Curr Opin Infect Dis* **18**, 376–386.

Nakamura, M., Taira, K., Itokazu, K., Kudaka, J., Asato, R., Kise, T. & Koizumi, N. (2006). Sporadic cases and an outbreak of leptospirosis probably associated with recreational activities in rivers in the northern part of Okinawa Main Island. *J Vet Med Sci* **68**, 83–85.

Narita, M., Fujitani, S., Haake, D. A. & Paterson, D. L. (2005). Leptospirosis after recreational exposure to water in the Yaeyama Islands, Japan. *Am J Trop Med Hyg* **73**, 652–656.

Tangkanakul, W., Tharmaphornpil, P., Plikaytis, B. D., Bragg, S., Poonsuksombat, D., Choomkasien, P., Kingnate, D. & Ashford, D. A. (2000). Risk factors associated with leptospirosis in northeastern Thailand, 1998. *Am J Trop Med Hyg* **63**, 204–208.

Vinetz, J. M., Glass, G. E., Flexner, C. E., Mueller, P. & Kaslow, D. C. (1996). Sporadic urban leptospirosis. *Ann Intern Med* **125**, 794–798.

原著

犬と猫に寄生するイヌノミおよびネコノミに対するフィプロニルを有効成分とする2種の滴下投与用液剤の駆除効果 —賦形剤の違いは効果に影響を及ぼすか？—

Efficacy of 2 kinds of the spot formulation containing fipronil as an active ingredient against the dog flea *Ctenocephalides canis* and the cat flea *C. felis* on dogs and domestic cats: Dose the difference in the vehicle influence the efficacy?

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要　約

犬と猫に寄生するイヌノミ *Ctenocephalides canis* およびネコノミ *C. felis* に対するフィプロニルを有効成分とする2種の滴下投与用液剤の駆除効果を比較した。これらのノミの自然感染を受けている犬について，無投薬対照群30例，フロントラインスポットオンドッグ（メリアル・ジャパン株式会社）投与群30例，マイフリーガード犬用（フジタ製薬株式会社）投与群30例を設定し，投薬群2群の犬に対しては各々の供試薬剤を有効成分として6.7 mg/kgの用量で投与した。また，同様にノミの自然感染を受けている猫についても，無投薬対照群30例，フロントラインスポットオンキャット（メリアル・ジャパン株式会社）投与群30例，マイフリーガード猫用（フジタ製薬株式会社）投与群30例を設定し，投薬群2群の猫に対しては各々の供試薬剤を有効成分として10 mg/kgの用量で投与した。その結果，いずれの薬剤を投与した場合にも，ほとんどの犬と猫でノミは投薬の翌日までに完全に消失し，翌日にノミが残存していた例においても，投薬後2-3日目までにはノミは完全に駆除されていた。この際，フロントラインスポットオン製剤とマイフリーガード製剤のノミ駆除効果に有意な差異は認められず，さらに残効性および犬と猫に対する安全性に関しても，両薬剤に差異は確認できなかった。このことから，マイフリーガード製剤は，フロントライン製剤と治療学的に同等であると結論した。

SUMMARY

Efficacy of 2 kinds of the spot formulation containing fipronil as an active ingredient was evaluated against the dog flea *Ctenocephalides canis* and the cat flea *C. felis* on dogs and domestic cats in a period from June to August of 2009 in Japan. Dogs spontaneously infested by these species of fleas were randomly assigned to 3 groups, consisted of 30 animals each: An unmedicated control and 2 medicated groups treated with FRONTLINE SPOT ON FOR DOGS (Merial Japan Ltd., Japan) and MY FREE GUARD FOR DOGS (Fujita Pharmaceutical Co., Ltd., Japan). The dogs of the 2 medicated groups were administered with a respective remedy at a dose of 6.7 mg active ingredient per kg body weight. Cats spontaneously infested by these species of fleas were also randomly assigned to 3 groups, consisted of 30 animals each: An unmedicated control and 2 medicated groups treated with FRONTLINE SPOT ON FOR CATS (Merial Japan Ltd., Japan) and MY FREE GUARD FOR CATS (Fujita Pharmaceutical Co., Ltd., Japan). The cats of the 2 medicated groups were administered with a respective remedy at a dose of 10 mg/kg. The fleas were completely eliminated from most of the dogs and cats by the day following to the treatment and from the other animals by 2 - 3 days after treatment, demonstrating no significant difference between FRONTLINE SPOT ON products and MY FREE GUARD products concerning to the efficacy against fleas. No differences were also confirmed between FRONTLINE SPOT ON products and MY FREE GUARD products concerning to the residual efficacy against fleas and the safety to dogs and cats. It is concluded that MY FREE GUARD FOR DOGS and MY FREE GUARD FOR CATS are therapeutically equal to FRONTLINE SPOT ON FOR DOGS and FRONTLINE SPOT ON FOR CATS, respectively.

KeyWords：cat, *Ctenocepalides canis*, *Ctenocephalides felis*, dog, fipronil, flea, FRONTLINE SPOT ON, MY FREE GUARD

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