昭和61年2月17日（月）
（第3種郵便物認可）
北海道

ザンビアの獣医師育成に道産子奮闘

北大獣医学部の支援で、昨年秋に開設されたアフリカ・ザンビアのザンビア大学獣医学部は、本道から派遣された教官が中心となって順調に滑り出した。今年から獣医師の資格をもつ青年海外協力隊員が、大学スタッフに加わることも決まり、また近隣諸国の学生受け入れが検討されるなど、日本の援助がアフリカの大地に大きく根づこうとしている。

昨年秋、学部を開設

現地の期待を担う
北大、帯畜大の教官ら

同大学の獣医学部建設は、六年前に来日したカウンダ大統領の要請に基づき、わが国の無償資金協力で、五十八年に着手された。首都ルサカの大学キャンパスの一角に講義棟、家畜病院、学生寮などを年次計画で建設、総工費約四十億円をかけ、昨年までにすべてが完成し今月末にザンビア側に引き渡されることになっている。

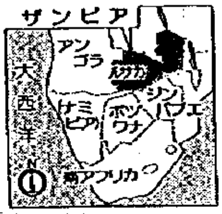
一方、日本国内には、スタッフや運営面など教育態勢を支えていくため、北大を主体とした国内委員会（委員長・尾形学日本獣医学会長）が設置され、初代の教官として昨年八月に四人を派遣した。

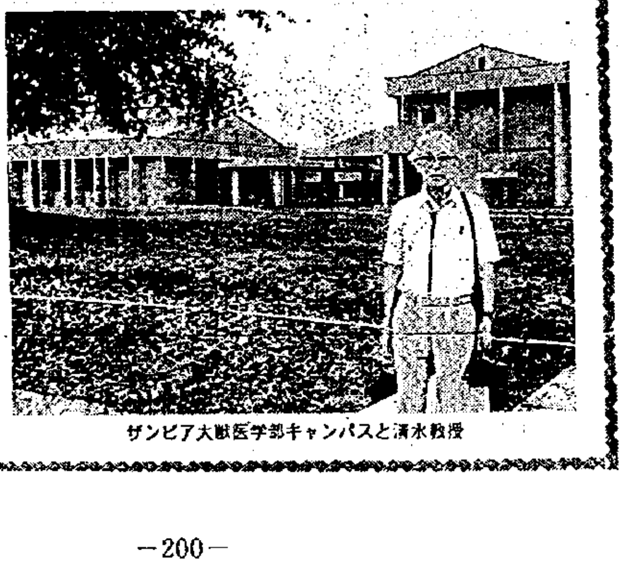
清水亀平次教授は昨年三月に帯広畜産大を退官してアフリカに飛び、石谷類造教授と多田融右講師はともに北大出身、助教授は農水省から派遣されている。また、四月一日付で北大を退官する藤本胖教授（六三）も七月に教授として赴任する。同教授は国内委員会のメンバーで、すでに現地を二度訪問、現役教授は除くという文部省の方針で赴任を一年延期していた。

今後の打ち合わせのため、このほど現地を訪れた北大獣医学部の金川弘司教授によると、現在学生は二年から四年まで合わせて五十一人。教官は日本からの四人のほか、アイルランド共和国、イギリス、現地のスタッフ合わせて十五人ほどで「初の獣医学部で、国全体として畜産に力を入れている時期だけに、期待も大きい」という。

青年海外協力隊員の派遣は、学生に対するきめ細かな指導のためで、今年三人、来年は一人が計画されている。教育面での協力協定は五年間となっているが、金川教授は「学生が指導者として立派に育つまでは、さらに五年以上必要と思われ、協力隊員の参加は心強い」と期待を寄せる。

学生の間では、授業中に時々日本語でしゃべる清水教授の人気が高く、片言の日本語を覚えた学生もいるという。ザンビア側では、獣医学部が軌道に乗りしだい、定員の一〇―一五％を周辺諸国に門戸を開く予定で、本道スタッフは態勢固めに奮闘中だ。





ザンビア大獣医学部キャンパスと清水教授

1987年11月6日　北海道新聞夕刊

アフリカに根付く 本道畜産学

ザンビア大獣医学部支える北大、帯畜大出身スタッフ

来秋、待望の卒業生

北大出身や道産子教授スタッフらの支援でアフリカのザンビア（人口六百四十五万人、カウンダ大統領）に開校されたザンビア大獣医学部が、国の経済悪化に苦しみながらも四年目でついに現地に根を下ろし、初の卒業生を送り出すまであと一年にこぎつけたことが、最近、国際協力事業団（本部・東京）に寄せられた報告で明らかになった。

ザンビア大獣医学部で学生たちに講義する藤本教授（中央）
＝今年8月、大井記者撮影

ザンビア大獣医学部は風土病とされる眠り病を媒介するツエツエバエの繁殖を食い止め、獣医師の養成を通じて立ち遅れている畜産振興を目指す目的で設立。カウンダ大統領自らが日本を訪れて要請、五十八年からわが国の無償による資金協力が始まった。

獣医学部は総工費約四十億円をかけて首都ルサカ市のキャンパス内に講義棟、家畜病院などを新設。スタッフは帯広畜産大を退官した清水悠紀平次教授（六七）＝疾病予防学＝、北大退官の藤本胖（ゆたか）教授（六七）＝臨床基礎講座＝のほか、北大出身の北岡茂男（六二）＝寄生虫学＝、佐藤輝夫（六三）＝臨床病理＝の両教授、多田融右講師（六七）＝寄生虫学＝らいずれも畜産分野では世界の第一線に立つ本道学界の主要メンバーたち。全四講座のうち二部門を日本が担当するが、青年協力隊員としても北大出身の中沢正年（六七）、浦野浩司（六七）、折野宏一（六七）さんや酪農学園大出身で紅一点の岡みさをさん（六七）らが指導の手伝いをしている。

ザンビアは銅の国際価格が低迷、いま国内経済は不振にあえいでいる。同大も学園紛争、学生の中途退学などで学生数が減少、獣医学部は現在、十三人の五年生をふくむ七十一人いるが、国内各地から集まった彼英生たちの卒業時期は予定より三カ月遅れているが、開校から満五年を迎える来年秋には待望の卒業生が誕生する見通しになった。

現地入りして三年、ヒゲをたくわえた藤本教授は「基礎教育が不十分なので教えるのに日本の二倍は時間がかかるが、ヨーロッパの政府援助国からも注目されているのでやりがいがある」と意欲的だ。慣れない英語に苦労したという清水教授も「卒業生が出れば、これまでの苦労も報われる」と語っている。

ヨーロッパから派遣された教授陣の中には安い給料に見切りをつけて帰国してしまったケースもあり、教授スタッフの定員三十九人に対して埋まっているのは日本人ら現在は三十人にも満たない。今後の運営は依然として楽観出来ないが、現地で調整に当たる国際協力事業団（JAICA）は「援助は十年、二十年の長期にわたって取り組まなければならないもの。北大はじめ日本側の支援委員会も努力を続けてくれているので力強い」と成功に大きな期待をかけている。

道 新 聞(夕刊)　昭和62年(1987年)11月5日(木曜日)

アフリカに実る「北方」の英知

ザンビア大獣医学部支える道産子スタッフ

北大出身や道産子教授スタッフらの支援でアフリカのザンビア(人口六百四十五万人、カウンダ大統領)に開設されたザンビア大獣医学部が、国の経済悪化に苦しみながらも四年目でついに現地に根を下ろし、初の卒業生を送り出すまであと一年にこぎつけたことが、最近、国際協力事業団(本部・東京)に寄せられた報告で明らかになった。

ザンビア大獣医学部で学生たちに講義する藤本教授(中央立っている)=今年8月、撮影

来年、待望の卒業生

西欧諸国も成果に注目

ザンビア大獣医学部は風土病とされる眠り病を媒介するツエツエバエの繁殖を食い止め、獣医師の養成を通じて立ち遅れている畜産振興を目指す目的で設立、カウンダ大統領自らが日本を訪れて要請したことから具体化し、五十八年からわが国の無償による資金協力が始まった。

獣医学部はザンビア大にとって十一番目の学部で、総工費約四十億円をかけて首都ルサカ市のキャンパス内に研究棟、家畜病院などを新設。スタッフは帯広畜産大を退官した清水亀平次教授(六〇)=疾病予防学=、北大退官の藤本胖(ゆたか)教授(六七)=臨床基礎講座=のほか、北大出身の北岡茂男(六三)=寄生虫学=、佐藤博夫(五七)=臨床病理=の両教授、多田融右講師(五七)=寄生虫学=らいずれも畜産分野では世界の第一線に立つ本道学界の主要メンバーたち。全四講座のうち二部門を日本が担当するが、青年協力隊員としても北大出身の中沢正年(二七)、浦野浩司(二七)、折野宏二(二七)さんや酪農学園大出身で紅一点の岡みささん(二七)らが教授陣の手足となって指導の手伝いをしている。

ザンビアは主要産品の銅の国際価格が低迷、いま国内経済は不振にあえいでいる。同大も学園紛争、学生の中途退学などで学生数が減少。獣医学部は現在、十三人の五年生を含む七十一人いるが、いずれも国内各地から集まった俊英ばかり。学園ストの影響で第一回の卒業時期は予定より三カ月遅れているが、開設から満五年を迎える来年秋には待望の卒業生が誕生する見通しになった。

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今後の運営は依然として楽観出来ないが、現地で援助に当たる国際協力事業団(ＪＡＩＫＡ)は「援助は十年、二十年の長期にわたって取り組まなければならないもの。北大はじめ日本側の支援委員会も努力を続けてくれているので力強い」と成功に大きな期待をかけている。

ゆうかんスペシャル

ザンビアの明日担う獣医さんら

北大に留学中

寒いけど人情温か

アフリカの南部、ザンビア共和国から四人の獣医と獣医の卵が来道、北大大学院獣医学研究科で学んでいる。いずれもわが国の国際協力事業団（JICA）、文部省の奨学生とあって、経済的には不自由はないものの、一番の悩みは本道の寒さ。下着の重ね着などで防寒しているが「寒い、寒い」を連発しながら、本格的な春の訪れを待っている。

フィリップ・ビリさん（[illegible]）、ポール・マセベさん（[illegible]）の二人はJICAの派遣研修生、モハメド・バヤットさん（[illegible]）とマイケル・ンゴマさん（[illegible]）の二人は文部省受け入れの奨学生だ。研修生の二人はザンビア大学の獣医学部講師。昨年八月に来日、一時の東京生活の後、昨秋に来道。今年五月まで北大で寄生虫学、生化学を学ぶ。

一方の奨学生の二人はザンビア大学獣医学部の第一期卒業生。バヤットさんは二年前に来道、北大の日本語コースで六カ月、大学院の研究生として同じく六カ月送ったのち、昨春、念願の大学院生となった。専攻は病理。ンゴマさんはバヤットさんより一年遅れて来道、今春、大学院に入学し公衆衛生を専攻する。二人はザンビア大でビリさんらの講義を受けており、いわば師弟の間柄だ。

ザンビアは英保護領から独立、建国してから三十年に満たない若い国で、日本のちょうど倍の国土（約七十五万平方キロ）に十五分の一（八百七万人）の人口。ザンビア大は、一九六五年に同国最初の大学として開校、八六年、JICAの資金協力で獣医学部が開設された。

広大な国土にウシ二百万頭、ヤギ三十万頭が飼育されているが、伝染病をはじめとする熱帯性疾病の発生率が高いのが悩みの種。しかし、獣医は七十五人しかおらず、ザンビア人医師は十二人にすぎない。

妻も暮らしに慣れた

「異国の生活にも慣れた」と、明るく研究に励む４人

ビリさんら四人の日本留学は、ザンビア大の研究技術を高め、獣医の養成に役立たせるねらい。これに先立ちJICAは、獣医学部の校舎、学生寮、機材などに三十九億円の無償資金協力を行っている。

彼らの教育で生活指導もしているのが獣医学部付属家畜病院の安田準助手（[illegible]）。安田さんは昨年三カ月、ザンビア大で教壇に立っており、彼らとも顔なじみ。「気候、習慣の違う日本で長期の生活をするのは大変」と同情しながら親身の世話をしている。

本道の印象について四人は「広々として自然と触れ合える」（マセベさん）「雪も人も多い」（ビリさん）「北海道の人は親切」（バヤットさん）「地下鉄が素晴らしい」（ンゴマさん）。しかし、全員が「ザンビアも高地なので涼しいが、これほど寒くない」と、本道の寒さが相当こたえている様子だった。

一番若いバヤットさんは奥さんと一緒に来道、一年八カ月前に札幌で生まれた長女ファティマちゃんと三人暮らし。「異国生活の寂[illegible]すっかり慣れましたよ」と、札幌暮らしに満足げ。

これと対照的なのがンゴマさんで、今月半ば、父親が病死したとの訃報（ふほう）が届き、四月初めに予定された学会での研究発表が終わり次第「一時帰国して父の墓をお参りしたい」と寂しそうだった。

WORLD ほっかいどう

情報コーナー

★中東問題セミナー

札幌アメリカン・センター（札幌市中央区大通西二八）は二十六日午後二時から同センターで「中東問題セミナー」を開く。

講師は元国務次官補で、イスラエル、エジプト間のキャンプ・デービッド合意の影の立役者といわれるマイケル・スターナー氏。

★独の戦後補償を紹介

札幌国際プラザ（札幌市中央区北一西三）はドイツ紹介シリーズ（1）として四月二十四日午後二時から同プラザで「ドイツの戦後補償」と題する講演会を行う。講師は同プラザ特別スタッフのミリアムさんで、現在、わが国が南北朝鮮、中国などから戦後補償を突き付けられている状況を踏まえながら、ドイツの対ユダヤ人補償などの実態を解説する。聴講は無料。

★日本語教師目指そう

財団法人・北海道国際交流センター（函館市元町一四ノ一）は、来年度の日本語教師養成講座受講生を募集している。応募資格は短大卒業以上（来年度卒業見込みも可）で、定員は二十人。受講期間は七月二十六日から八月七日まで、同センターで。応募締め切りは六月十二日。受講料は十五万円。問い合わせは同センター日本語教師養成講座係（☎0138・22・0770）。

獣医育成を支援

北大とザンビア大 ユニーク交流協定

札幌

【札幌】アフリカ・ザンビア共和国のザンビア大獣医学部の設立時のモデルとなり、その後も教授陣を派遣している北海道大学獣医学部（金川弘司学部長）は、このユニークな関係をさらに強めるため一日付で「学術交流に関する協定」を結んだ。

文部省学術国際局によると、研究者同士の交流でなく、日本側の教授らが開発途上国の大学に長期滞在して現地の学生を直接指導するのは珍しいケースという。

ザンビアはアフリカ南部の内陸に位置し、国内には牛が二百万頭以上いると推定される。ところが獣医は百人以下しかいないため、熱帯性の家畜伝染病に打つ手がなく、獣医の育成が急がれていた。

「獣医学部設立に協力を」との要請を受けた日本は八四年、国際協力事業団を通じて無償資金協力を決定。国立大で唯一獣医学部を持つ北大をモデルとして翌年秋、首都ルサカのザンビア大構内に総工費四十億円で獣医学部が新設された。

同時に北大が中心となって常時十人前後の日本人指導陣を派遣、教官全体の約三分の一を占める。

ザンビア大との交流では北大側にも大きなメリットがある。ツェツェバエが媒介する眠り病、狂犬病など「アフリカは日本では見られなくなった家畜伝染病や寄生虫の〝宝庫〟」（金川学部長）という。病気を教科書で覚えるのではなく、実地に見ることができるのは貴重な経験と、研究者の評判も上々だ。

北大大学院には現在、ザンビア大獣医学部の第一期卒業生二人が留学中。金川学部長は「ザンビア人の獣医が十分に育つまで学術交流を続けたい」と話している。

日本人研究員から牛を使った実習を受けるザンビア大の学生＝87年10月7日、ザンビア大獣医学部

毎日新聞　第3種郵便物認可　1992年（平成4年4月3日）

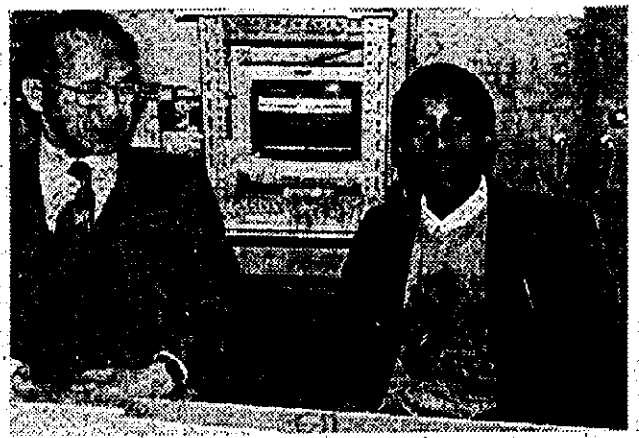
牛の伝染病原菌 見分け方を開発

ザンビアの獣医師チタンボさん

アフリカ中部にあるザンビアから堺市の大阪府立大に留学していた獣医師ハリソン・チタンボさん(32)がアフリカの牛にはやっている伝染病トリパノゾーマの病原菌を見分ける方法の開発に成功した。三年間の府立大留学を終えて一日、母国へ戻った。広大な土地を生かし、肉牛による外貨獲得を目指しているアフリカ諸国にとって伝染病の克服は大きな課題。「周辺国と撲滅チームを作り、経済発展に役立てたい。日本の青年もぜひ手伝ってほしい」と呼びかけている。

帰国し撲滅チーム

府立大農学部で3年間研究



牛の伝染病対策の研究に励んだハリソン・チタンボさん（右）。指導した荒川皓教授も母国での活躍に期待を込める　＝堺市の大阪府立大で

トリパノゾーマは吸血バエとして知られるツエツエバエが媒介する伝染病。牛に感染すると貧血状態になり、死亡することもある。コッパー（銅）ベルト地帯で知られるザンビアはここ数年、国際価格が低下した銅にかわる外貨獲得の目玉として、日本などの援助で、野生牛を家畜化して国内畜産業を育て、牛肉を輸出するプロジェクトに取り組んでいる。

ところが畜産業者が牛の病気について十分な知識をもっておらず、感染した牛に薬を大量投与した結果、薬に強い耐性種の菌がまん延。マラウイ、ジンバブエなど近隣のアフリカ諸国でも被害が深刻化している。

チタンボさんは、日本の国際協力事業団（JICA）の援助で設立されたザンビア大獣医学部を卒業後、英・ウエールズ大に留学、母校の講師をしていたが、八七年にザンビアに派遣されていた府立大農学部の荒川皓教授らと知り合い、研究が進んでいる日本で学ぼうと来日した。

府立大では、感染した牛の血液を空輸して実験を重ね、病原虫のDNA（デオキシリボ核酸）がDAPIと呼ばれる蛍光色素で簡単に染まることを発見。試薬を投与したうえで染色し、分裂するかどうかで、その菌が耐性種かどうか見分けられる方法を開発した。成果は英国の科学雑誌「パラシトロジーリサーチ（寄生虫研究）」など各国の専門誌にも相次いで紹介され、注目を集めた。

「耐性種に感染している個体群が分かれば、政府機関と協力し、移動をストップさせて隔離するなど素早く適切な対策をとることができる」とチタンボさん。

荒川教授は「これまでの研究では、ラジオアイソトープ（同位体）を使うなど、特殊な設備が必要なうえ、見分けるまで数カ月の期間がかかった。この方法は、安い費用で簡単に調べることができ、アフリカ諸国でもすぐ実用化できるだろう」と期待している。

プロジェクト（専門家）関連の学術論文、各種報告、出版物

以下のリストは、関係者の協力により、可能な限りのものを収集して得られたものである。未収載のものがあると思われるため、お気づきの方はお知らせ願いたい。

英文報告

著者（発表者）	標題	年	誌名（学会）	分野
Falade, S., Sato, G., Ulatya, W. and Mwanza, L.	Serovars and antibiotic sensitivity patterns of *Salmonella* strains isolated from domestic animals in Zambia	1989	Zimbabwe Vet. J., 20(1), 19-22	Bacteriology
Pandey, G.S., Shimizu, K., Orino, K. and Schneebeli, M.	Preliminary observations on ovine paratuberculosis (Johne's diseases) in Zambia	1989	Revue Elev. Med. vet. Pays trop., 42(4), 515-516	Bacteriology
Ishihara,T., Hagiwara, H., Kitada, R., Korematsu, K. and Sato, T.	Tuberculin test results in cattle in Southern Province of Zambia	1992	UNZA Veterinarian, 3(2), 3-6	Bacteriology
Ngoma, M., Suzuki, A., Takashima, I. and Sato, G.	Antibiotic resistance of *Escherihia coli* and Salmonella from apparently healthy slaughtered cattle and pigs, and deseased animals in Zambia	1993	Jpn. J. Vet. Res., 41(1), 1-10	Bacteriology
Takashima, I., Ngoma, M. and Hashimoto, N.	Antimicrobial effects of a new carboxyquinolone drug, Q-35, on five serogroups of *Leptospira interrogans*	1993	Antimicrobial Agents and Chemotherapy, 37(4), 901-902	Bacteriology
Sato, Y., Schneebeli, M., Matsukawa, K., Chimana, H., Sinsungwe, H. and Sato, G.	Outbreaks of *Salmonella* Dublin infection among calves on a dairy farm applying *Salmonella* Bacterins in Zambia	1993	J. Vet. Med. Sci., 55(3), 511-513	Bacteriology
Orino, K., Hasebe, F., Kitada, R., Miyazaki, T., Matsushita, F., Mubiana, M., Sato, T. and Naiki, M.	Prevalence of bovine brucellosis in Southern Province of Zambia	1994	UNZA Veterinarian, 5(2), 5-7	Bacteriology
Orino, K., Korematsu, K., Ulaya, W., Mwanza, L. and Naiki, M.	Persistence of antibody in cattle after *Brucella abortus* Strain 19 vaccination in Mazabuka District of Zambia	1995	UNZA Veterinarian, 6(1), 3	Bacteriology
Ngoma, M., Pandey, G.S., Suzuki, A., Sato, G. and Chimana, H.	Prevalence of *Salmonella* in apparently healthy slaughtered cattle and pigs in Zambia	1996	Indian J. Anim. Hlth, 35(2), 197-200	Bacteriology
Orino, K., Gabber, K.M.A., Ulaya, W. and Shimizu, K.	Bacteriological studies of pneumonia in Zambia	1988	Unpublished	Bacteriology
Orino, K. and Shimizu, K.	Serological survey on bovine Paratuberculosis in Zambia	1988	Unpublished	Bacteriology
Sitima, A.M.C., pandey, G.S. and Fujikura, T.	Viability of Mycobacterium bovis in traditionally processed sour milk and the prevalence of bovine tuberculosis in Namwala District of Zambia	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Bacteriology, Public Health
M'ule, D., Lungu, J., Arumugam, G., Namangala, B. and Nagabayashi, T.	Aerobic intestinal and faecal flora among wild animals under natural and ranched conditions	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Bacteriology, Wildlife

Arumugam, G., Mubita, C.M., M'ule, D., Lungu, J., Namangala, B. and Nagabayashi, T.	Distribution and composition of aerobic bacterial flora in the small and large intestine of Hippopotami (*Hippopotamus amphibius*)	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Bacteriology, Wildlife
Shimizu, K., Saigawa, K., Orino, K. and Benkele.W.	Outbreak of anthrax among the wildlife in South Luangwa National Park, Zambia	1987	Unpublished	Bacteriology, Wildlife
Samui, K.L., Inoue, S., Mweene, A.S., Nambota, A.M., Mlangwa, J.E.D., Chilonda, P., Onuma, M. and Morita, C.	Distribution of Rift Valley Fever among cattle in Zambia	1997	Jpn. J. Med. Sci. Biol., 50, 73-77	Virology
Samui, K.L., Mweene, A.S., Nambota, A.M., Mlangwa, J.E>D., Chilonda, P., Inoue, S., Morita, C., Okabayashi, T. and Hasebe, F.	The distribution of Rift Valley Fever and other hitherto unrecognised vector-borne infections among cattle in Zambia	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Virology, Epidemiology
Morita, C.	Prevalence of Rift Valley Fever in Lusaka and Mazabuka - Zambia	1988	J. Vet. Med. B 35, 157-160	Virology, Epidemiology
Hasebe, F., Sato, T., Ulaya, W.D., Nyambe, I. and Morita, C.	Seroepidemiological survey on Rift Valley Fever in Zambia	1989	J. Vet. Med. B 36, 317-319	Virology, Epidemiology
Samui, K.L., Inoue, S., Mweene, A.S., Nambota, A.M., Mlangwa, J.E.D., Chilonda, P. Onuma, M. and Morita, C.	Distribution of Rift Valley Fever among cattle in Zambia	1997	Jpn. J. Med. Sci.Biol., 50, 73-77	Virology, Epidemiology
Samui, K.L., Nambota, A.M., Mweene A.S. and Onuma, M.	African swine fever in Zambia: Potential financial and production consequences for the commercial sector	1996	Jpn. J. Vet. Res., 44(2), 119-124	Virology, Epidemiology
Kadohira, M., Samui, K.L. and Mlangwa, J.E.D.	The health and productivity of traditionally managed cattle in Lusaka Province, Zambia: Results of a questionnaire survey	1996	Zambian J. Veterinary Science, Dec. 1996, 11-20	Epidemiology
Kadohira, M., Kitala, P., Samui, K. and Mcdermott, J.	Sampling strategies for disease studies in tropical countries: Examples of multi-stage cluster sampling and its application in Zambia and Kenya	1997	Epidemiol. sante anim., 1997, 31-32, 02.A.15	Epidemiology
Okabayashi, T., Hasebe, F., Samui, K.L., Mweene A.S., Pandey, G.S. and Morita C.	Prevalence of antibodies against Spotted Fever group *Rickettsia*, Murine Typhus, and Q Fever in Zambia	1997	Epidemiol. sante anim., 1997, 31-32, 02.A.12	Rickettsia, Epidemiology
Orino, K. and Shimizu, K.	Serological survey on bovine Anaplasmosis in Zambia	1987	Unpublished	Rickettsia
Orino, K. and Shimizu, K.	Serological survey of Toxoplasmosis	1987	Unpublished	Parasitology
Chitambo, H. and Arakawa, A.	Therapeutic effect of Berenil and Samorin in mice infected with four trypanosome populations isolated from Zambian cattle	1991	Veterinary Parasitology, 39, 43-52	Parasitology
Chitambo, H. and Arakawa, A.	*Trypanosoma congolense*: the in vitro akinetoplastic induction sensitivity assay	1991	Parasitol. Res., 78, 136-141	Parasitology
Tada, Y., Nakazawa,M., Chisembe,S., Phiri,P.G. and Chota, A.	Eficacies of Oxfendazole, Fenbendazole and Ivermectin against gastro-intesyinal nematodes of Sheep and Goat in Zambia	1992	Bull. Anim. Prod. Afr., 40, 287-288	Parasitology

Chitambo, H., Arakawa, A. and Ono, T.	In vivo assesment of drug sensitivity of African trypanosomes using the akinetoplastic induction test.	1992	Research in Veterinary Science, 52, 243-249	Parasitology
Chitambo, H. and Arakawa, A.	*Trypanosoma congolense*: The use of 4,6-Diamidino-2-Phenylindole (DAPI) in the akinetoplastic induction sensitivity test	1992	J. Vet. Med. Sci., 54(4), 773-775	Parasitology
Chitambo, H. and Arakawa, A.	*Trypanosoma congolense*: Manifestation of resistance to Berenil and Samorin in cloned trypanosomes isolated from Zambian cattle	1992	Zbl. Bakt., 277, 371-381	Parasitology
Tada, Y., Nakazawa,M., Urano, K., Chisembe,S., Phiri,P.G. and Chota, A.	Pasture infestation with infective larvae of *Haemonchus contortus* on a sheep farm in Zambia	1994	UNZA Veterinarian, 5(2), 3-4	Parasitology
Nambota, A., Samui, K., Sugimoto, C., Kakuta, T. and Onuma, M.	Theileriosis in Zambia: Etiology, epidemiology and control measures.	1994	Jpn. J. Vet. Res., 42(1), 1-18	Parasitology
Tada, Y., Nakazawa, M., Chisembe, S., Phiri, P.G. and Chota, A.	Helminth infection of some domestic and wild animals in Zambia	1995	UNZA Veterinarian, 6(1), 8	Parasitology
Syakalima, M., Yasuda, J. and Hashimoto, A.	Preliminary efficacy trial of cymelarsan in mice artificiallyinfected with *Trypanosoma brucei brucei* isolated from a dog in Zambia	1995	Jpn. J. Vet. Res. 43(2), 93-97	Parasitology
Ono, T., Ohnishi, Y., Takami, K., Yamamoto, M., Chitambo, H. and Arakawa, A.	Effect of Berenil on the kinetoplast of *Trypanosoma gambiense*, Pararosaniline sensitive and resistant clone in mice	1995	Jpn. J. Parasitol., 44(2), 106-111	Parasitology
Matsukawa, K., Chiti,L., Yoshima,M. and Sayer, P.D.	The First Reported Case of Canine Visceral Leishmaniasis in Zambia	1995	Proceedings of the Scientific Meeting of the Veterinary Association of Zambia, 20-21 April,1995, Lusaka, Zambia	Parasitology
Nambota, A.M., Lovelace, C.E.A., Chitambo, H., Kakuda, T., Sugimoto, C. and Onuma, M.	Characterization of some *Theileria parva* stocks from Zambia using monoclonal antibodies	1996	J. Vet. Med. Sci., 59(1), 1-4	Parasitology
Nambota, A.M., Lovelace, C.E.A., Chitambo, H., Kakuda, T., Sugimoto, C. and Onuma, M.	Characterisation of some *Theileria parva* from Zambia using monoclonal antibodies	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Parasitology
Katakura, K., Lubinga, C., Chitambo, H. and Tada, Y.	Detection of *Trypanosoma congolense* and *T. brucei* subspecies in cattle in Zabia by polymerase chain reaction from blood collected on a filter paper	1997	Parasitol. Res. 83, 241-245	Parasitology
Pandey, G.S., Inoue, N., Ohshima, K., Okada, K., Chihaya, Y. and Fujimoto, Y.	Poxvirus infection in Nile crocodiles (*Crokodylus niloticus*)	1990	Research in Veterinary Science 1990,49, 171-176	Pathology
Musonda, M. M.	Pathological Study on Swine Lymphosarcoma	1993	Bull. Azabu Univ., Vet. Med. 14(1-2), 17-29	Pathology
Schneebeli, M., Inoue,S. and Madarame, H.	Hydranencephaly in newborn calves in Zambia	1993	J. Vet. Med. Sci. 55(3), 515-517	Pathology
Matsukawa, K., Yoshima, M. and Yoshida, M.	Orchiepididymitis due to *Brucella* in a Kafue Lechewe	1995	Proceedings of the symposium, The effects of enlargement of domestic animal pasture on the wildlife in Zambia, 22 March 1995, Lusaka, Zambia	Pathology
Matsukawa, K., Yoshima, M., Mwase,	Acute theileriosis in a 4-month-old eland	1995	Proceedings of the symposium,	Pathology

M. and Sharma, N.	in Zambia		The effects of enlargement of domestic animal pasture on the wildlife in Zambia, 22 March 1995, Lusaka, Zambia	
Bhaiyat, M.I., Matsukawa, K. and Bar, S.	Globoid cell leukodystrophy in a maltese dog	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Pathology
Matsukawa, K. and Bhaiyat, M.I.	Ovine verminous encephalomyelitis: Setariosis?	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Pathology
Matsukawa, K., Bhaiyat, M.I., Chiti, L. and Sayer, P.D.	The first case of canine visceral leishmaniasis in Zambia: Pathological findings	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Pathology
Matsukawa, K., Chiti, L., Yoshima, M. and Sayer, P.D.	Canine visceral leishmaniasis: first case in Zambia	1997	Onderstepoort Journal of Veterinary Research, 64, 77-79	Pathology
Bhaiyat, M.I., Matsukawa, K., Kaneuchi, C. and Chiti, L.	Significance of occurrence of canine salmonelosis in Lusaka	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Pathology, Bacteriology
Nalubamba, K., Tembo, A., Hashimoto, H., Lovelace, C.E. and Aziz, D.	An outbreak of aflatoxicosis in swine: clinical p[athological	1997	School Seminar for Veterinary Practitioners, June 1997	Poisoning
Inoue, S.	Aspect of Newcastle disease in Zambia	1990	UNZA Veterinarian, 1(1),7	Poultry Disease
Sato, Y. and Phiri, I.	Avian diseases diagnosed at the School of Veterinary Medicine	1991	UNZA Veterinarian, 2(1), 3-4	Poultry Disease
Sato, Y., Schneebeli, M. and Sato,G.	An occurrence of vitamin A deficiency in chickens in Zambia	1992	J. Vet. Med. Sci., 54(3), 601-603	Poultry Disease
Sato, Y., Schneebeli,M. and Sato, G.	An Occurrence of Vitamine A Deficiency in Chicken in Zambia	1992	J. Vet. Med. Sci. 54(3), 601-603	Poultry Disease
Nyeleti, C., Baba, E. and Pandey, G.S.	The effect of starvation and *Escherichia coli* infection on yolk sac retention in chicks	1994	UNZA Veterinarian, 5(2), 9-11	Poultry Disease
Hirai, M., Baba, E. and Pandey, G.S.	A five-year summary of disease of chickens diagnosed in the School of Veterinary Medicine at the University of Zambia, Lusaka	1994	UNZA Veterinarian, 5(2), 8-9	Poultry Disease
Tuchili, L.M., Kodama, H., Izumoto, Y., Mukamoto, M., Fukata, T. and Baba, T.	Detection of *Salmonella Gallinarum* and *Typhimurium* DNA in expetimentally infected chicks by polymerase chain reaction	1994	J. Vet. Med. Sci., 57(1), 59-63	Poultry Disease Bacteriology

Hasegawa, M.	An epidemiological survey of avian infectious diseases in a small village in Zambia	1994	UNZA Veterinarian, 5(2), 7-8	Poultry Disease, Epidemiology
Pandey,G.S., Kobayashi, K., Hitrai, H. and Musonda, M.M.	Bilateral duplication of the ceca in a domestic chicken	1994	Avian Diseases, 38, 201-202	Poultry Disease
Sato, Y., Yasuda, J., Sinsungwe, H., Chimana, H. and Sato, G.	An occurrence of stomach impaction in ostriches (*Struthio camerus*) on a farm in Zambia associated with high mortality	1994	J. vet. Med. Sci. 56(4), 783-784	Poultry Disease
Hasegawa, M., Tuchili, L.M., Pandey, G.S.	Epidemiological survey of poultry diseases in commercial breeding farms in Zambia	1995	UNZA Veterinarian, 6(1), 5-7	Poultry Disease
Tuchili,L.M., Kaneuchi, C. and Ulaya, W.	Recent Sero-characterization of *Salmonella* Straines Isolated from Chickens in Zambia	1995	Proceedings of the Scientific meeting of the Veterinary Association of Zambia, 20-21 April, 1995, Lusaka, Zambia	Poultry Disease
Tuchili, L., Ulaya, W., Kato, Y. and Kaneuchi, C.	Recent characterisation *Salmonella* strains isolated from chickens in Zambia	1996	J. Vet. Med. Sci. 58(1), 77-78	Poultry Disease
Tuchili, L.M., Kodama, H., Sharma, R.N., Takatori,I., Pandey, G.S., Kabilika, S., Mukamoto, M., Tsuji, S. and Baba, T.	Detection of *Salmonella* DNA in chicken embryos and environmental samples by polymerase chain reaction	1996	J. Vet. Med. Sci., 58(9), 881-884	Poultry Disease, Bacteriology
S. Takahashi, A.K.Suzuki, G.S.Pandey and T.Kaji	Serological Diagosis of Newcastle Disease between Guinea Fowl and Chicken in Developing Country	1996	The 8th Animal Science Congress, The Asian - Australian Association of Animal Production Societies, Oct. 13-18,1996, Makuhari, Chiba, Japan	Poultry Disease
Sato, Y., Sato, G., Tuchili, L., Pandey, G.S., Nakajima, A., Chimana, H. and Sinsungwe, H.	Status of *Salmonella gallinarum-pullorum* infections in poultry in Zambia	1997	Avian Diseases, 41, 490-495	Poultry Disease, Bacteriology
Pandey, G.S. and Hasegawa, H.	Serological survey of *mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in chickens in Zambia	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Poultry Disease
Tuchili, L.M., Kodama, H., Sharma, R.N., Takatori, I., Pandey, G.S., Kabilika, S., Mukamoto, M., Tsuji, S. and Baba, T.	Detection of *Salmonella* DNA in chicken embryos and environmental samples by polymerase chain reaction	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Poultry Disease, Bacteriology
Muangandu, H., Nakanta, P., Bwalya, J., Ulaya, W.D., Sakala, R.M. and Fujikura, T.	Dog ecology surveyllance and research for canine and human rabies control in Zambia	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Public Health

Tuchili, L.M., Bwalya, J., Ulaya, W.D., Fujikura, T., Bbalo, G.C., Masumbu, A., Tembo, W., Zambara, m., Dyaunka, V.C. and Mkumba, P.	Isolation of *Bacillus anthrasis* from environmental samples collected from anthrax endemic areas of Western Province, Zambia	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Public Health
Sakala, R.M., Bwalya, J.M., Pandey, G.S., Fujikura, T., Siame, C.M., Mululuma, G., Lubasi, M.M. and Hachitema, D.	Preliminary survey of traditional farming villages in Mazabuka District: A veterinary public health approach	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Public health
Makondo, K., Mwanza, A., Zulu, V.C., Patel, O.V. and Hishinuma, H.	Gross reproductive anomalies and chromosomal chimerism in a freemartin heifer - A case report	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Reproduction
Omamegbe, J.O., Chiti, L., Muleya, J., Hashimoto, H. and Hankanga, C.	Orthopaedic conditions of the pelvic girdle in companion animals	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Surgery
Hashimoto, H., Nalumbamba, K., Chiti, L., Thornicroft, T. and Koie, H.	Surgical Approaches and repair of iliac fracture and sacroiliac separation in dogs	1997	An international scientific symposium in commemoration of the 10th anniversary of the opening of the Samora Machel School of Veterinary Medicine, 9-11th April 1997, Lusaka, Zambia	Surgery
Hashimoto, H., Nalumbamba, K., Chiti, L., Thornicroft, T., Hankangar, C.H., Phiri, B., Kubi, C. and Omamegbe, J.O.	Surgical repair of fractures using bone plates and screws in dogs	1997	School Seminar for Veterinary Practitioners, June 1997	Surgery
Kubi, C., Hashimoto, H. and Nalubamba, K.	Repair of multiple pelvic fractures in a dog	1997	School Seminar for Veterinary Practitioners, June 1997	Surgery
Hankanga C., Hashimoto, H., Kubi, C. and Phiri, B.	A case report; wound management using Chitipack®	1997	School Seminar for Veterinary Practitioners, June 1997	Surgery
A.Suzuki	Contact Between Domestic animals and Wildlife Using Computer Imaging Analysis in Lochinvar National Park of Zambia	1995	Symposium on Effects of Enlargement of Domestic Animal Pasture on the Wildlife in Zambia, 25 March 1995, Lusaka, Zambia	Wildlife
Matsukawa, K.	Brucellosis in Kafue lechwe and East Coast Fever in Eland	1995	Symposium on Effects of Enlargement of Domestic Animal Pasture on the Wildlife in Zambia, 25 March 1995, Lusaka, Zambia	Wildlife
G.S.Pandey, A.Mweene, A.K.Suzuki, A.Nambota, and T.Kaji	Dermatophilosis (Cutaneous Streptothricosis) in Kafue Lechwe (*Kobus*	1994	Journal of Wildlife Diseases, 30(4), 586-588	

	lechwe kafuensis)			
Pandey, G.S., Suzuki,A., Kaji,T. and Takahashi, S.	Emerging Diseases of Livestock and Wildlife through Their Mixing and Game Ranching in Zambia	1995	World Veterinary Congress, 3-9 Sept. 1995, Yokohama, Japan	Wildlife
Suzuki, A., Pandey, G.S., Matsukawa, K., Takahashi, S., Nambota, A. and Kaji, T.	Emerging problems on diseases of livestock and wildlife due to their mixing in the developing country	1996	The 8th AAAP Animal Science Congress, Tokyo, Japan, Vol. 2, 1068-1069	Wildlife

邦文

著者	標題	年	誌名（出版）	分野・分類
佐藤良彦、Sinsungwe, H.、Phiri, J.、Chimana, H.、Ulaya, W.、佐藤儀平	ザンビア国のブロイラーにみられた鶏脳軟化症の一例	1992	畜産の研究、46(3), 368-370	鶏病
高橋慎司、林谷秀樹、G.S.Pandey、E.T.Mwase、梶隆、鈴木明	ザンビア共和国のニワトリにおけるニューカッスル病及びひな白痢菌の血清学的診断	1992	第85回日本畜産学会	鶏病
多田融右 Persson, B., Chisembe, S., Phiri, P.G.右、中沢正年、Chisembe, S., Phiri, P.G. and Chota, A.	ザンビア国おける家畜の寄生蠕虫について	1989	第107回日本獣医学会	寄生虫学
中沢正年、多田融右、Lovelace, C.E., Persson, E., Chisembe, S., Phiri, P.G. and Chota, A.	ザンビアにおける羊、山羊の消化管寄生線虫の季節的変化について	1989	第07回日本獣医学会	寄生虫学
多田融右、中沢正年、浦野浩司、Chisembe, S., Phiri, P.G. and Chota, A.	ザンビアの羊放牧地での放牧、休牧期における感染仔虫数の消長について	1989	第108回日本獣医学会	寄生虫学
多田融右、Phiri, P.G., Chota, A., Munyenyembe, F., 西川一夫	ザンビアの野生動物の寄生蠕虫	1989	第36回日本寄生虫学会北日本支部大会	寄生虫学
菱沼貢、門平睦代、鈴木和男、Zulu, V.、岡田幸助、藤田正一、森田千春、関根純二郎	カバ (*Hyppopotamus amphibius*) における精子形成	1997	第93回日本畜産学会大会、VI27 a 5	繁殖学
菱沼貢、門平睦代、鈴木和男、Zulu, V.、岡田幸助、藤田正一、森田千春、関根純二郎	カバ (*Hyppopotamus amphibius*) における卵巣および胎子の観察	1997	第124回日本獣医学会、P-120	繁殖学
折野宏一、長谷部太、佐藤輝男、宮崎敏次、Mukendua Mubiana	ザンビアの牛および人におけるブルセラ抗体調査	1989	第107回日本獣医学会	微生物学
鈴木明、高橋慎司、林谷秀樹、G.S.Pandey、E.T.Mwase、梶隆	ザンビアで飲用されているサワーミルクにおける人畜共通感染症起因菌の汚染状況	1994	第88回日本畜産学会	公衆衛生
佐藤儀平	ザンビアの衛生事情	1992	日本細菌学会北海道支部会報、1992年第1号、3-5	公衆衛生
鈴木明、G.S.Pandey, A.Nambota, K.Matusukawa, 高橋慎司	家畜と野生動物の混在に関する研究ーカフエ・レーチェのブルセラ病について	1996	第91回日本畜産学会	
鈴木明	ザンビアにおける家畜と野生動物の関係	1991	国立環境研究所ニュース、Vol.10, No.2, 9	野生動物
鈴木明	野生動物と家畜の共存条件を求めて	1996	国立環境研究所ニュース、Vol.15, No.3, 7-8	野生動物
鈴木明	野生動物と獣医学＋αについて一開発途上国・ザンビアでは	1995	AD＆S（アドス）、Associate Doctors and Students, Vol.1, No.2	野生動物
鈴木明、高橋慎司、林谷秀樹、G.S.Pandey、E.T.Mwase、梶隆	ザンビア共和国における家畜と野生動物の接触について	1992	第85回日本畜産学会	野生動物
鈴木明、高橋慎司、林谷秀樹、G.S.Pandey、E.T.Mwase、梶隆	ザンビアにおける家畜と野生動物の接触の程度について	1992	第113回日本獣医学会	野生動物
佐藤輝夫	ザンビアにおける畜産事情	1991		畜産
佐藤良彦	ザンビア共和国の農業畜産事情	1992	畜産の研究、46(5), 69-75	畜産・農業
門平睦代	ザンビアの家畜衛生畜産事情	1996	畜産技術1996年7月号	畜産・家畜衛生
浜名克己	ザンビアの畜産と獣医臨床	1992	鹿児島大学獣医学研究会、1992年11月2日	畜産・臨床
浜名克己	ザンビアの畜産と獣医臨床	1992	日本産業動物獣医学会中国地区大会	畜産・臨床
浜名克己	ザンビアでの臨床経験	1993	鹿児島県家畜疾病診断研究	臨床

			会会報第４５号、1-3	
安田準、松川清	ザンビアの獣医学教育と獣医事情	1995	動物臨床医学 4(1), 1-8	臨床・獣医学教育
藤本肆	ザンビア大学獣医学部技術協力からみた今後の獣医学教育の在り方	1991	第１１２回日本獣医学会、教育特別講演	獣医学教育
佐藤儀平、松坂尚典	ザンビア大学獣医学部における獣医公衆衛生学教科の授業（1）	1993	北海道獣医師会雑誌 37,376-380	獣医学教育
佐藤儀平、松坂尚典	ザンビア大学獣医学部における獣医公衆衛生学教科の授業（2）	1993	北海道獣医師会雑誌 37,409-415	獣医学教育
佐藤儀平	ザンビア大学獣医学部への懸念	1996	北海道大学獣医学部同窓会報、第３９号	獣医学教育
佐藤良彦	ザンビア共和国の教育事情	1991	日本獣医師会雑誌、44(10),1024-1025	教育
藤本肆	ザンビア大学獣医学部技術協力によせて	1985	北海道大学獣医学部関東支部会報、p 5-8	技術協力
藤本肆	アフリカ、ザンビア大学獣医学部技術協力によせて	1985	北海道大学新聞 323 号	技術協力
藤本肆	ザンビア大学獣医学部の技術協力の現状について	1988	日本獣医師会雑誌、41,207-212	技術協力
藤本肆	アフリカ・ザンビアの国際技術協力から帰って	1991	北海道大学札幌同窓会誌第１０号	技術協力
藤本肆	ザンビア大学獣医学部プロジェクトの技術協力	1991	国際協力研究、7(1),41-52	技術協力
浜名克己	国際技術協力：ザンビア大学獣医学部	1992	鹿児島大学報	技術協力
浜名克己	ザンビア大学獣医学部プロジェクト	1992	鹿児島県獣医師会会報 5(2),67-69	技術協力
藤本肆	国際技術協力ーザンビア大学獣医学部	1992	楡の風　NO. 21，北大工学部同窓会楡工会	技術協力
佐藤良彦	ザンビア大学獣医学部プロジェクトと国際協力	1992	臨床獣医、10(8), 1850-1855	技術協力
藤本肆	ザンビア大学獣医学部の創設から今日までの流れ	1996	北海道大学獣医学部同窓会報第３９号、25-26	技術協力
安田準	日本の獣医学と国際貢献	1997	SAC-Small Animal Clinic, No.109、共立商事（株）	技術協力
大島寛一	ザンビア大学獣医学部とその周辺	1988	岩獣会報 14, 90-94	紹介記事
安田準	コンボカセレモニー	1991	北海道獣医師会雑誌 35, 322-323	紹介記事
鈴木明	ザンビアにおける衛生事情と対策	1992	海外学術調査ニュースレター、No.21、文部省科学研究費・国際学術研究総括班	紹介記事
佐藤儀平	アフリカの思い出ーバオバブ序説	1993	巌鷲寮誌・平成４年度号、18-21	紹介記事
小林好作	ザンビア便り	1993	東京エルム新聞第 387 号	紹介記事
鈴木明	家畜放牧のもたらすもの・アフリカ・ザンビアの野生動物と家畜の関係	1994	グローバルネット 47 号、地球・人間環境フォーラム	紹介記事
佐藤儀平	ザンビアの若き獣医学徒 Michael Ngoma 君の氏を悼む	1994	北海道獣医師会雑誌 38, 153-157	紹介記事
佐藤儀平	既刊及び新刊のザンビア紹介図書について	1994	北海道獣医師会雑誌 38, 20-21	紹介記事
小林好作	ザンビア大学獣医学部での１年	1994	麻布大学学園情報第 100 号、3-4	紹介記事
小林好作	ザンビアでの１年	1994	東京エルム新聞第 400 号	紹介記事
小林好作	「シマ」を食べる	1994	月刊 NOSAI、1994 年８月号、p 1	紹介記事

岡田幸助	ジャカランダの咲く頃　ザンビアの3カ月　一（1、2）	1997	岩獣会報、23, 4-9, 52-58	紹介記事
鯉江洋	ザンビア通信　1－5	1997	獣医畜産新報 Vol. 50, p511,614,699,756,858	紹介記事
石谷類造・茉莉	ザンビア日記			書籍
藤本幹・俊子	ザンビア動物記	1991	共同文化社	書籍
佐藤良彦	住んでみたザンビア			書籍
北海道新聞	ザンビアの獣医師育成に道産子奮闘	1986	北海道新聞、1986.2.17	紹介記事
北海道新聞	アフリカに根付く本道畜産学	1987	北海道新聞、1987.11.6 夕刊	紹介記事
北海道新聞	アフリカに実る北方の英知、ザンビア大学支える道産子スタッフ	1987	北海道新聞、1987.11.5	紹介記事
朝日新聞	牛の伝染病原菌見分け方を開発、ザンビアの獣医師チタンボさん	1992	朝日新聞、1992.4.3	紹介記事
北海道新聞	ザンビアの明日担う獣医さんら、北大に留学中	1992	北海道新聞、1992.3.25	紹介記事
室蘭民報	獣医育成を支援、北大とザンビア大ユニーク交流協定	1992	室蘭民報、1992.1.4	紹介記事

国際協力事業団、その他の関連報告書

報告書名	年
ザンビア共和国ザンビア大学獣医学部建設計画基本設計調査報告書	1983
ザンビア共和国ザンビア大学獣医学部建設計画建設事情資料集	1983
ザンビア共和国ザンビア大学獣医学部技術協力計画長期調査員報告書	1984
ザンビア共和国ザンビア大学獣医学部技術協力事前調査報告書	1984
ザンビア共和国ザンビア大学獣医学部技術協力計画実施協議報告書	1985
ザンビア共和国ザンビア大学獣医学部技術協力計画打ち合わせ調査団報告書	1986
ザンビア大学獣医学部技術協力計画の現状と効率的効果的運営のための私見：見上彪	1987
ザンビア大学獣医学部技術協力計画巡回指導調査団報告書	1988
ザンビア大学獣医学部技術協力計画巡回指導調査団報告書	1989
ザンビア大学獣医学部技術協力計画評価調査団報告書	1989
ザンビア大学獣医学部技術協力計画計画打ち合わせ調査団報告書	1990
ザンビア大学獣医学部技術協力計画フェーズII事前調査団報告書	1991
ザンビア大学獣医学部技術協力計画フェーズII実施協議調査団報告書	1992
ザンビア共和国ザンビア大学獣医学部技術協力計画：ザンビア大学獣医学部	1992
文部省科学研究費補助金、国際学術研究研究成果報告書、発展途上国における家畜放牧の広域化が野生動物に及ぼす影響に関する共同研究、鈴木明（研究代表者）	1992
ザンビア共和国ザンビア大学獣医学部技術協力計画フェーズII計画打ち合わせ調査団報告書	1993
ザンビア大学獣医学部（フェーズI）（ザンビア）：国際協力総合研修所	1993
ザンビア大学獣医学部技術協力計画フェーズII巡回指導中間エバ調査報告書	1995
ザンビア大学獣医学部技術協力計画フェーズII巡回指導調査団報告書	1996
ザンビア大学獣医学部技術協力計画フェーズII評価調査団報告書	1997

Bacteriology

SEROVARS AND ANTIBIOTIC SENSITIVITY PATTERNS OF *SALMONELLA* STRAINS ISOLATED FROM DOMESTIC ANIMALS IN ZAMBIA

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Summary

Thirty-seven strains of Salmonella isolated from cattle, goats, pigs and fowls in Zambia were typed serologically and their antibiotic sensitivity patterns were determined. Eight serovars were identified, five, namely *makoma*, *livingstone*, *newington*, *anatum* and *Schwarzengrund*, being reported from Zambia for the first time.

Introduction

Except for the published works of Gaspar and Hrabeta (1977) and Gaspar (1978), there is no information on *Salmonella* in domestic animals from the Republic of Zambia. We present here the antibiotic sensitivity patterns of serovars isolated from cattle, goats, pigs and chickens.

Materials and Methods

Faecal and intestinal samples from animals with gastro-enteritis were received from private farmers at the University Veterinary Diagnostic Laboratory over a period of nine months. They were cultured and examined for *Salmonella* by conventional methods using selenite broth, desoxycholate hydrogen sulphide lactose (DHL) and MacConkey agar. Isolates provisionally identified as *Salmonella* by biochemical methods (Cowan, 1979) and with the use of polyvalent 'O' and 'H' antisera were serotyped at the Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan. Pure trypticase broth cultures were seeded on to Oxoid sensitivity test agar and examined for antibiotic sensitivity with eleven monodisks (Eiken & Co., Japan) (Table 2). An isolate was considered sensitive if there was a clearing around it after incubation at 37°C overnight.

Results

Salmonella was isolated from 29 cattle, two goats, one pig and five fowls.

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Twenty-eight strains belonging to eight serovars were identified (Table 1). Five serovars, namely, *makoma*, *livingstone*, *newington*, *anatum* and *Schwarzengrund*, are reported from Zambia for the first time. The biotyping of the nine other strains was not attempted. The antibiotic sensitivity patterns are shown in Table 2.

Table 1: Distribution and Sources of *Salmonella* Serovars

Serovar	*No. of strains*	*Cattle*	*Goats*	*Pigs*	*Fowls*
dublin	11	11	–	–	–
livingstone	5	5	–	–	–
agona	5	3	–	–	2
newington	2	2	–	–	–
anatum	1	1	–	–	–
Schwarzengrund	2	–	–	–	2
bovis morbificans	1	1	–	–	–
makoma	1	1	–	–	–
untyped	9	5	2	1	1
Total	37	29	2	1	5

Discussion

Cattle were the species most commonly affected with twenty-nine isolates (78,3 per cent of the total isolates), ten (27,0 per cent) being from calves less than one year old. The predominant serovar, *dublin* (29,7 per cent) accounted for 70 per cent of the serovars in calves. This finding is similar to the observations of Sojka and Field (1970) and Gaspar and Hrabeta (1977) who found *dublin* to be the predominant serovar in cattle in England and Wales and Zambia respectively. It is worth noting the isolation of serovar *newington* from a two-year-old ox which died of acute gastro-enteritis and of one of the untyped serovars in association with severe haemorrhagic enteritis with striking haemorrhagic longitudinal stripes in the small intestine. The other bovine strains and the two untyped caprine strains were from adult animals presented with clinical diarrhoea. The only strain from pigs was isolated from the uterus of a dead sow. Serovars *gallinarum* and *pullorum*, reported previously in fowls in Zambia by Gaspar and Hrabeta (1977), were not encountered in this investigation, only *agona* (2 strains), *Schwarzengrund* (2 strains) and one untyped strain being isolated from fowls. This could be a result of the small number of samples of avian origin that were examined.

It was shown that 35 (94,6 per cent) and 33 (89,2 per cent) of the total strains

Table 2: Antibiotic Sensitivity Patterns of *Salmonella* Serovars

	dublin	*livingstone*	*agona*	*newington*	*Schwarzen-grund*	*anatum*	*bovis morbificans*	*makoma*	untyped
Gentamicin 2 µg	11(100)*	3(60)	5(100)	2(100)	2(100)	1(100)	1(100)	1(100)	9(100)
Kanamycin 5 µg	9(81,8)	4(80)	5(100)	2(100)	2(100)	1(100)	1(100)	1(100)	8(88,9)
Cephazolin 5 µg	8(72,7)	4(80)	5(100)	2(100)	2(100)	1(100)	1(100)	1(100)	8(88,9)
Ampicillin 5 µg	10(90,9)	3(60)	3(60)	2(100)	2(100)	1(100)	1(100)	1(100)	5(55,6)
Sulbennicillin 5 µg	10(90,9)	2(40)	2(40)	0(0)	1(50)	1(100)	1(100)	0(0)	5(55,6)
Nalidixic acid 2 µg	7(63,6)	1(20)	2(40)	0(0)	2(100)	0(0)	0(0)	0(0)	2(22,2)
Benzylpenicillin 1 unit	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Cloxacillin 1 µg	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Lincomycin 1 µg	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Erythromycin 0,5 µg	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Kitasamycin 1 µg	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

figures in brackets indicate percentage sensitivities

were sensitive to gentamicin and kanamycin respectively. It would therefore appear that a chemotherapeutic agent with either of these drugs or both in combination would be of value for field use.

References

Cowan, S.T. (1979) *"Cowan and Steel's Manual for the Identification of Medical Bacteria"* 2nd Edition, Cambridge University Press, Cambridge. pp 237.

Gaspar, P. (1978) Bull. anim. Hlth Prod. Afric., 26, 230–231.

Gaspar, P. and Hrabeta, P. (1977) Bull. anim. Hlth Prod. Afric., 25, 61–64.

Sojka, W.J. and Field, H.I. (1970) Vet. Bull. 40, 515–531.

Communication

Preliminary observations on ovine paratuberculosis (Johne's disease) in Zambia

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PANDEY (G. S.), SHIMIZU (K.), ORINO (K.), SCHNEEBELI (M.). Observations préliminaires sur la paratuberculose ovine (maladie de Johne) en Zambie. *Revue Élev. Méd. vét. Pays trop.*, 1989, 42 (4) : 515-516.

Dans un troupeau de moutons importés d'Afrique du Sud, une brebis est devenue partiellement anorexique et a perdu régulièrement du poids et sa bonne condition physique. Après examens clinique, coprologique et histologique, la paratuberculose (maladie de Johne) a été confirmée. Cependant, le résultat des cultures est resté douteux. Une recherche sérologique sur d'autres moutons du même troupeau et sur ceux d'une autre ferme a mis en évidence des anticorps anti-*Mycobacterium paratuberculosis* par un test de fixation du complément. L'étude suggère que la maladie est dans sa phase d'extension. C'est la première fois, en République de Zambie, qu'un cas de paratuberculose ovine est rapporté. *Mots clés* : Ovin - Paratuberculose - Histopathologie - Culture - Sérologie - Diagnostic - Zambie.

Johne's disease is a chronic enteritis of ruminants caused by *Mycobacterium johnei*. Although there are numerous reports on paratuberculosis (Johne's disease) in cattle, there is little published information of the naturally occurring disease in sheep. The disease is economically important not only as a cause of death but also due to losses which result from reduced productive capacity during the lengthy preclinical stage of the disease. Johne's disease in sheep was initially described by HOWARTH (1) in the USA and since then has been reported from Britain, Iceland, New Zealand, Germany, Spain, Italy, Yugoslavia, Israel, Iraq and India. From African continent it has been reported from South Africa (7), Egypt (2) and recently from Libya (3). The occurrence of paratuberculosis in sheep in Zambia is being reported for the first time in the present communication. In this report a natural case of paratuberculosis in a 3-year old ewe is described and brief pathogenesis, diagnosis and some serological incidence and prevalence of the disease are presented.

A three-year old Dorper ewe belonging to Galaunia Farm initially in good condition started becoming partially anorexic and gradually losing weight and condition for about four months. Other sheep in the flock were not similarly affected. This flock was imported from South Africa about two years before. The ewe was given anthelmintic, vitamins, and antibiotics during the course of sickness but without any response. The ewe became cachectic with conspicous bottle jaw. Faeces were soft but there was no diarrhoea. Faecal examination did not reveal any helminthic ova and blood smear examination was found negative for any blood parasite. Finally the ewe was sacrified and at necropsy revealed superficial lymph glands specially delate submaxillary and prescapular swollen and oedematous and intermandibular space had serofibrinous oedema. The organs of the thoracic cavity were normal. In the abdominal cavity the mucous membrane of the ileum showed generalised thickening with clear transverse ridges. The mesenteric lymph glands were enlarged and oedematous. Smears made from the faecal material and ileum scrapings, heat fixed and stained with Ziehl-Neelsen's showed huge number of typical acid fast bacilli with characteristic clusture arrangement suggesting *Mycobacterium johnei*. Sections made from the ileum and lymph glands fixed in 10 percent formol saline and stained with haemotoxylin and eosin and Ziehl-Neelsen showed characteristic tissue reaction as observed by RAJYA and SINGH (5) but without any caseation or calcification as reported by MARTIN (2). Typical numerous acid fast bacilli in clustures and singles were observed in mucosal epithelium and within the cytoplasm of the epithelioid macrophages in lamina propria. Mesenteric lymph node was oedematous and contained few epithelioid macrophages in their sinuses but acid fast organisms were not seen in lymph node sections.

Cultural attempt was done on Lowensteins Jensen media containing 10 percent Mycobactin and also without Mycobactin. Bacterial growth was observed after 10 weeks in slants and when smear from growth of bacteria was stained, numerous acid fast bacilli in clumps and singles were observed in both type of slants however the *Mycobacterium paratuberculosis* should not grow in media without Mycobactin. After two months sera samples were collected from 50 aged sheep of same flock, 16 sera samples gave positive reaction to the antibodies of *Mycobacterium paratuberculosis* on complement fixation test (CFT). Smears made from faecal samples of 50 sheep, 4 revealed acid fast bacteria of which three were out of 16 seropositive on CFT while 1 was out of negative samples. One hundred sera samples were collected from another sheep farm about 120 km away from the first farm having suspected history of paratuberculosis. Sheep at this farm were imported long time back from Zimbabwe. Forty-five samples gave positive reaction to CFT for antibodies of *Mycobacterium paratuberculosis* at different titre. Faecal samples collected did not reveal any clumps of acid fast bacteria.

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Clinical and pathological features of this case are similar to those reported previously for ovine paratuberculosis (5). Progressive emaciation without diarrhoea, gross thickening of the wall of ileum and colon, granulomatous enteritis and involvement of the mesenteric lymph node are described as feature of disease in sheep (1, 5, 6). The presence of numerous acid fast bacilli in the faeces, ileum scraping and within macrophages in lamina propria adds further support of ovine paratuberculosis. Cultural attempt did not confirm the bacteria but remains doubtful.

Serological evidence indicates the presence of disease and demonstrates that infection is actively spreading. Similar situation could be attributed to other farm indicating the serological presence of the disease. Absence of typical acid fast bacilli in feaces could be possibly due to preclinical sheep unable to shed the bacilli.

These observations and findings are reported to direct attention to the first occurrence of ovine paratuberculosis in Zambia. Field veterinarians and progressive farmers should be aware that diarrhoea is not a constant feature and should include this disease in their differential diagnosis when facing chronic emaciation in adult sheep. Though the disease has been reported recently in Zambia in cattle (4), it is very difficult to suggest the introduction of this disease in this country where there is no restriction on importation. Authors believe to have more work done to establish the presence and extent of disease.

Acknowledgements

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PANDEY (G. S.), SHIMIZU (K.), ORINO (K.), SCHNEEBELI (M.). Preliminary observations on ovine paratuberculosis (Johne's disease) in Zambia. *Revue Élev. Méd. vét. Pays trop.*, 1989, 42 (4) : 515-516.

In an imported flock of sheep from South Africa, an ewe became partially anorexic and gradually losing weight and conditions. The paratuberculosis (Johne's disease) was confirmed on clinical, feacal and histopathological examination. Cultural examination remained doubtful. Serological investigation of other sheep in the flock and at other farm reacted to antibodies of *Mycobacterium paratuberculosis* on complement fixation test. The study suggests that the disease is actively spreading. This is the first report of ovine paratuberculosis in the Republic of Zambia. *Key words* : Sheep - Paratuberculosis - Histopathology - Culture - Serology - Diagnosis - Zambia.

References

1. HOWARTH (J. A.). Paratuberculosis enteritis in sheep caused by an acid fast organism. *J. Am. vet. Med. Ass.*, 1932, 81 : 383-387.
2. MARTIN (W. B.). Diseases of sheep. Edinburgh, Blackwell Scientific Publication. P. 52.
3. MUSTAFA (A. A.), MUGADMI (K. E.). First report of paratuberculosis (Johne's disease) in Libya. *Vet. Rec.*, 1986, 118 : 729.
4. PANDEY (G. S.), MUSONDA (T. L.), CHIZYUKA (H. G. B.), SCHNEEBELI (M.). Paratuberculosis in herd of Friesian cattle in Zambia. *Vet. Rec.*, 1987, 120 : 369.
5. RAJYA (B. S.), SINGH (C. M.). Pathologic changes in sheep with naturally occurring infection. *Am. J. vet. Res.*, 1961, 22 : 189-202.
6. STAMP (J. T.), WATT (J. A.). Johne's disease in sheep. *J. comp. Path.*, 1954, 64 : 26-40.
7. VAN NIEKERK (O. T.), VAN DER WALT (K.). Paratuberculosis (Johne's disease) in an imported German Merino ram. *J. S. Afr. vet. Med. Ass.*, 1967, 38 : 23-24.

Tuberculin Test Results in Cattle in Southern Province of Zambia

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Summary

Surveys of bovine tuberculosis in Southern Province of Zambia were performed using the tuberculin test. The six year study revealed a high prevalence of positive reactors to tuberculin in some areas and an eradication program of "test and slaughter policy" was recommended.

Introduction

The existence of bovine tuberculosis was suspected because a high rate of tuberculin test reactors had been reported previously (Moorhouse,1986) and the disease is also of great importance for public health in Zambia.

The present paper presents data from the investigation and some success from the control measures carried out during the period from 1983 to 1988 on commercial and traditional farms. There are differences in management and usage between the two types of farming system. Commercial farmers regularly sell milk and meat in the market to earn money. Traditional farmers, however, do not want to slaughter their cattle solely to earn money because they primarily keep the cattle for draught power, payment of bride price, consumption, funerals and other ceremonies, prestige and income in hand.

Materials and methods

Experimental animals were of various ages, sex and breeds from traditional and commercial farms. The breeds of cattle from traditional farms were Zebu, Zebu X Holstein, Boran and other cross-breeds; and from commercial farms were Holstein, Brahman, Simmental, and Boran. Cattle in the traditional farms are kept in small kraals during the night and put on grazing in open communal pastures with movement over a long distance during the day. Particularly during the dry season they drink water from communal water points. Cattle in commercial farms were put on enclosed pastures all year round.

The areas used for investigation were located in the Southern Province of Zambia, and the investigation was performed during the period from 1983 to 1988.

Types of tuberculin test: The single intradermal test was used in 1983 and 1984, and the single comparative intradermal test (Blood *et al.*,1985) was used in 1985, 1986 and 1988. The reaction of the animal to tuberculin was read 3 days after injection by measuring the skin thickness (in millimetres) at the non-injection and injection sites of avian and bovine tuberculin. Positive skin thicknesses at bovine and avian tuberculin sites were determined as B and A. Interpretation was made as follows:-

Increase in thickness (mm.)	Interpretation
$B \leq 2.0$ or $B \leq A$	negative
$B = 2.0$~3.9 and $B-A \leq 2.0$	doubtful
$B \geq 4.0$ and $B-A \geq 2.0$	positive

If the results were doubtful, the test was repeated at an interval of 30-60 days.

Table 1 shows the results of the tuberculin test. The overall prevalence of reactors in traditional and commercial farms were 4.6% and 0.2%, respectively during the observed period. In the traditional farms the prevalence showed a great variation from 1.0% to 34.6%. However, in commercial farms the prevalence remained very low. There were no significant differences in the doubtful percent between the two types of farms.

Table 1a. Results of tuberculin testing of cattle: traditional farms

Year	Province/ district	Town/ village	No. animals	No. positive (%)	No. doubtful (%)
1983*	Southern/ Namwala	Namwala	103	14 (13.6)	0 (0.0)
1984*	Namwala	Namwala	265	27 (10.2)	15 (5.7)
1985*	Southern/ Mazabuka		325	12 (3.7)	0 (0.0)
1986**	Southern/	Kalambabakali	886	12 (1.4)	0 (0.0)
	Mazabuka	Kabanje	60	14 (23.3)	0 (0.0)
1988**	Southern/	Lubombo	248	4 (1.6)	5 (2.0)
	Mazabuka	Mukuyu	229	6 (2.6)	4 (1.7)
		Ngwezi	291	3 (1.0)	3 (1.0)
		Kabanje	81	28 (34.6)	3 (3.7)
Subtotal			2,488	120 (4.8)	30 (1.2)

Table 1b. Results of tuberculin testing of cattle: commercial farms

Year	Province/ district	Town/ village	No. animals	No. positive (%)	No. doubtful (%)
1983*	Southern	Mazabuka	1,664	0 (0.0)	0 (0.0)
		Monze	277	0 (0.0)	5 (1.8)
		Choma	547	4 (0.7)	6 (1.1)
		Kalomo	53	1 (1.9)	1 (1.9)
		Livingstone	834	2 (0.2)	22 (2.6)
Subtotal			3,375	7 (0.2)	34 (1.0)
Total	1983-1988		5,863	127 (2.2)	64 (1.0)

Type of tuberculin test: * = single intradermal test; ** = single comparative intradermal test

Table 2 shows the tuberculin reactors in different age groups of cattle in traditional farms. Cattle of older than 5 years in Namwala and Kabanje towns showed the highest prevalences. On the other hand, prevalence of reactors in Lubombo, Mukuyu and Ngwezi remained at a low level. The traditional farmers in these areas were selected from members of the Project Management Unit Holder Dairy Development Project (PMU-SHDDP) who sell milk regularly in the market and are educated to cull the tuberculin positive cattle for the prevention of zoonotic transfer. The overall reactor rate in individual age groups varied between 3.3% and 7.2% with a mean of 5.8%. There was a trend towards the proportion of reactors in aged cattle to be higher than that in the young ones.

traditional farms around towns of Southern Province

Age in years	Namwala 1983 No. (%) positive	Lubombo, Mukuyu, Ngwezi 1988 No. (%) positive	Kabanje 1988 No. (%) positive	Total No. (%) positive
2	1/26 (3.8)	4/123 (3.3)	5/24 (20.8)	10/173 (5.8)
3-4	1/26 (3.8)	0/187 (0)	7/28 (25)	8/241 (3.3)
5-6	5/24 (20.8)	5/219 (2.3)	7/17 (41.2)	17/260 (6.5)
7+	7/27 (25.9)	4/239 (1.7)	9/12 (75)	20/278 (7.2)
Total	14/103 (13.6)	13/768 (1.7)	28/81 (34.6)	55/952 (5.8)

Table 3 shows the prevalence of reactors from 1983 to 1988 in Batoka Dairy Cross Breeding Ranch in Choma in the Southern Province. This ranch is a Government farm which was established to supply the dairy cross cattle to traditional farmers. The Government carried out a test and slaughter policy for bovine tuberculosis. The ratio of the positive reactors decreased during the observed period.

Table 3. Changes of positive reactors in Batoka Dairy Cross Breeding Ranch in Choma

Year	No. cattle inspected	No. (%) positive
1983	1,835	15 (0.82)
1984	247	0
1985	304	2 (0.66)
1986	217	0
1988	2,122	1 (0.05)
Total	4,725	18 (0.38)

Discussion and conclusion

The positive ratios of cattle in traditional farms were extremely high with a variation from 1.0% to 34.6%. The ratios in Lubombo, Mukyu, and Ngwezi were very low in comparison with those of other town/villages. Especially, the positive ratios of cattle in the traditional farms who do not bring their cattle to Kafue Flats were lower than those of others in the areas.

Kafue Flats is surrounded by Blue Lagoon National Park, Lochinvar National Park and Kafue National Park where cattle have close contact with wild animals infected with tuberculosis (Moorhouse,1986). Kafue Flats seems to provide good conditions not only for pasture and water, but also for the survival of mycobacterial bacilli (Blood *et al.*, 1985). This would explain why cattle in Namwala District and Kabanje town on Kafue Flats have a high rate of positive reactors to the tuberculin test.

The positive ratios of cattle in the commercial farms were low with a variation from 0.0% to 1.9% because cattle were kept in a separate area from wild animals as well as from Kafue Flats.

comparison with that of traditional farms because of the different socio-economical backgrounds and lifestyles. However, traditional farmers even keep the positive reactor without culling according to their social background. Therefore, it can be said that such a practice helped raise the positive ratio up to 75% in cattle of more than seven years old in Kabanje and Namwala towns in the Southern Province.

In Batoka Dairy Cross Breeding Ranch the ratio of positive reactors decreased gradually from 0.82 in 1983 to 0.05% in 5 years after the test and slaughter policy was introduced. Therfore, it seems that a continuous test and slaughter policy may be effective in a confined area.

The rate of tuberculin test positive reactors out of 9,149 head of cattle in 1975 was 0.5% to 8.8% in Zambia (Moorhouse,1986), and is still very high. These positive rates are extremely high in comparison with that of Japan where the low positive rates have been brought about by a "test and slaughter policy" over the years.

It is concluded that the ratio of tuberculin test positive reactors is still high in cattle of many parts of Zambia and that the test and slaughter system also seems to be effective for eradication of bovine tuberculosis in a confined area and that some types of farm management may contribute to lowering the number of tuberculin test reactors.

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References

1. Blood, D.C., Radostitis, O.M.and Henderson, J.A.(1985) "Veterinary Medicine", Bailliere Tindal, Eastboume, UK.
2. Moorhouse, P. (1986) in "Selected diseases of livestock in Zambia - Brief epidemiological details". Epidemiology Unit FAO/DVCTS, Lusaka, Zambia.
3. Worthington, R.W. and Kleeberg, H.H. (1966) J. S. Afr.med.Ass., 37 (2), 213-225.

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ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* AND *SALMONELLA* FROM APPARENTLY HEALTHY SLAUGHTERED CATTLE AND PIGS, AND DISEASED ANIMALS IN ZAMBIA

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ABSTRACT

Escherichia (E.) coli and *Salmonella (S.) choleraesuis* (subsp. *choleraesuis* and subsp. *salamae*) from apparently healthy slaughtered cattle and pigs in 1989 in Zambia, were examined for antibiotic resistance and the presence of conjugative R plasmid. *Salmonella* strains from diseased animals (cattle, chickens, leopards, lions and warthogs) were similarly tested. The majority of the cattle had been nomadically kept in so-called "traditional farms" while all the pigs were from commercial farms. More pigs (39 %; 41/105) harboured drug-resistant *E. coli* than cattle (6.7 %; 7/105). Moreover, the number of drug-resistant *E. coli* was higher among strains from pigs (31.2 %; 49/157) than cattle (4.2 %; 7/167). For both cattle and pigs, drug resistance was more frequently observed against tetracycline, streptomycin, sulfadimethoxine and ampicillin than other antibiotics and the single resistance pattern occurred most frequency, especially among pig *E. coli* strains. Drug-resistant *Salmonella* was recorded in 3.6 % (1/28) of strains from slaughtered cattle and 31.3 % (10/32) of those from diseased animals. Drug-resistant *E. coli* from pigs and cattle carried R plasmid at high frequency.

Key words: *Escherichia coli*, *Salmonella*, R plasmids, antibiotics, animals in Zambia

INTRODUCTION

Contamination of edible meat by drug-resistant *E. coli* and *Salmonella* is a problem of public health importance[9]. Generally speaking, in many countries the use

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of antibiotics to promote growth in domestic animals, especially in fattening calves, pigs and chickens, has greatly contributed to the appearance of drug-resistant bacteria[1,3,6,7,12,17]. In most developing countries, the bacteria drug resistance status is not clearly defined. Such is true in Zambia concerning the *E. coli* and *Salmonella* drug resistance situation in slaughtered animals. In Zambia, about 80 % of the cattle and the majority of the pigs are traditionally maintained depending on natural pasture with little or no supplementation[13].

In this paper, the drug resistance of *E. coli* and *Salmonella* isolates from abattoir-slaughtered cattle and pigs and *Salmonella* from sick animals in Zambia, was surveyed. The presence of transferrable R plasmid in drug-resistant strains was also examined.

Materials and Methods

Tested strains and sampled animals: Examination of caecal contents and mesenteric lymph nodes from 105 cattle and 105 pigs, slaughtered at abattoirs in Lusaka, Zambia, in 1989, yielded isolation of different serovars of *S. choleraesuis* (subsp. *choleraesuis* and subsp. *salamae*)[8] (Table 1) and *E. coli* strains. Twenty-eight *Salmonella* strains from cattle and 39 from pigs were tested in this investigation.

Table 1. *Salmonella* serovars[a] of tested strains

Strains from slaughtered cattle		Strains from slaughtered pigs		Strains from diseased animals		
Serovar	No. of strains	Serovar	No. of strains	Serovar	Animal	No. of strains
Typhimurium	14	Bredeney	22	Dublin	Cattle	11
Heidelberg	2	Braenderup	5	Livingstone	Cattle	5
Othmarschen	2	Infantis	2	Agona	Cattle	3
Bonn	8	Muenchen	4	Agona	Chiken	2
Weltevreden	2	Newport	3	Agona	Lion	2
		Bovismorbificans	1	Agona	Warthog	1
		Elisabethville	2	Newington	Cattle	2
				Anatum	Cattle	1
				Schwarzengrund	Chicken	2
				Bovismorbificans	Cattle	1
				Makoma	Cattle	1
				Virchow	Leopard	1
Total	28		39			32

a) Except for *S. choleraesuis* subsp. *salamae* serovar Makoma (*S.* Makoma), the rest are various serovars under *S. choleraesuis* subsp. *choleraesuis*.

Thirty-two *Salmonella* strains from sick animals (cattle, chickens, leopards, lions and warthogs)[4] were also similarly tested. Tested *E. coli* strains were isolated from 105 cattle (167 strains) and 105 pigs (157 strains). Cattle were from 4 provinces and the majority had been traditionally reared while all the pigs had been commercially kept in 7 piggeries in Lusaka (Table 2). All the tested strains had been stored on Dorset egg slope media at 4 °C in our laboratory. *E. coli* strain K12 ML1410 (nalidixic acid-resistant and methionine-requiring F^- derivative of K-12), used as a recipient in detection of transferrable R plasmid, was kindly supplied by Dr. N. Ishiguro (Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan).

Drugs and drug concentrations for drug sensitivity test: Ten antibiotics were used at the following concentrations: 25 μg/ml for ampicillin (Ap), chloramphenicol (Cp), gentamicin (Gm), kanamycin (Km), nalidixic acid (NA) and tetracycline (Tc); 12.5 μg/ml for streptomycin (Sm); 6.3 μg/ml for colistin (Cl) and furazolidon (Fz); and 800 μg/ml for sulfadimethoxine (Su). The drug concentrations were according to a previous report[16] with some modifications. The agar dilution method was used for detection of drug resistance and heart infusion agar (Eiken Chemical Co., Ltd, Tokyo, Japan) was used except in the case of Su, where Mueller Hinton agar (Eiken Chemical Co., Ltd, Tokyo, Japan) was used. A strain was recorded as resistant when its growth was not inhibited by the drug concentration mentioned above.

Table 2. Origin of cattle and pigs, and number harbouring drug-resistant *E. coli* strains

Animal	Source	Farming system	Positive animal/total no. of animals (%) [b]
	province/piggery[a]		
Cattle	Southern	Traditional	1/59 (1.8)
	Western	Traditional	5/25 (20)
	Central	Traditional	0/4 (0)
	Lusaka	Commercial	1/17 (5.9)
Total			7/105 (6.7)
Pigs	A		21/40 (52.5)
	B		3/10 (30)
	C	All	1/10 (10)
	D	commercial	4/10 (40)
	E		6/10 (60)
	F		4/10 (40)
	G		2/15 (13.3)
Total			41/105 (39)

a) A to G = Piggeries, all in Lusaka Province.
b) Positive animals yielding drug-resistant *E. coli* strains.

Detection of conjugative R plasmid: A test for detection of the R plasmid was conducted on all *E. coli* and *Salmonella* strains (donor) showing drug resistance. The *E. coli* strain K12 ML1410 was used as a recipient strain. The test was done according to the method described by Sato *et al.*[16]. Each of the donor isolates was cultivated in brain heart infusion broth (Eiken Chemical Co., Ltd, Tokyo, Japan) at 37 °C for 18 hours. Broth (2 ml) in a test tube was inoculated with 0.2 ml of each donor broth culture and an equal amount of the recipient culture similarly cultivated. The mixure was incubated at 37 °C for 18 hours. A loopful of the mixed culture was streaked on a selective agar plate containing NA (50 μg/ml) and appropriate concentrations of drugs to which the donor was resistant. Desoxycholate hydrogen sulfide lactose (DHL) agar (Nissui, Tokyo, Japan) was used as a basal medium for Cp, Sm, Km and Ap; heart infusion agar for Tc; and Mueller Hinton agar (Eiken Chemical Co., Ltd, Tokyo, Japan) for Su. To the latter 2 media had been added 1.5 g of lactose and 4 ml of 0.2 % bromothymol blue per 100 ml. The drug concentrations used for selective media were the same as for the drug sensitivity test. The selective media were incubated at 37 °C for 24 hours. To determine transconjugant recipients and their resistance patterns, colonies of transconjugants on each selective medium were purified by successive single-colony isolations on the same selective medium and were examined for drug resistance pattern. When a transconjugant recipient was not found on the selective medium, the mixed culture of donor and recipient, which had been left overnight at 25 °C was reinoculated on the selective medium to detect temperature-sensitive R plasmid.

RESULTS

Drug resistance of tested strains: The sources of the slaughtered cattle and pigs are shown in Table 2. The percentage of cattle heads from which drug resistant *E.coli* was isolated was 6.7 % (7/105) while that of pigs was 39 % (41/105) and there was a statistically significant difference in isolation rates between cattle and pigs ($P < 0.01$). Heads of cattle, from each of the 4 provinces, harbouring drug-resistant *E. coli* ranged from 0 % (Central) to 20 % (Western). The percentage of pigs carrying drug-resistant *E. coli* was from 10 % (1/10) to 52.5 % (21/40) (Table 2).

The resistance of *E. coli* and *Salmonella* to each of the tested antibiotics is shown in Table 3. Only 4.2 % of the 167 cattle *E. coli* as compared to 31.2 % of the 157 from pigs were resistant to at least one of the following antibiotics; Tc, Sm, Su, Ap, Km and Cl. The rate of resistance of cattle *E. coli* strains was greatest to Tc (3.0 %) and Su (3.0 %) followed by Ap (2.4 %) and Sm (1.8 %), and lastly Km (1.2 %) while pig strains were resistant to Tc (18.5 %), Sm (11.5 %), Ap (10.8 %), Su (9.6 %) and finaily Km and Cl (0.6 %). A higher percentage of drug-resistant *E. coli* was detected in pigs, especially against Tc, Sm, Su and Ap.

Of the 28 cattle *Salmonella* strains, only 1 (3.6 %) serovar was resistant to Ap.

None of the 39 pig *Salmonella* strains were resistant to any of the drugs used. A total of 31.3 % of the 32 *Salmonella* strains from diseased animals showed resistance, mainly against Sm. Seven strains of *S. choleraesuis* subsp. *choleraesuis* serovar Dublin (S. Dublin) and 1 of *S.* Anatum from cattle and 1, *S.* Schwarzengrund, from the chicken were resistant to Sm. One *S.* Agona strain from cattle was resistant to 3 drugs, Tc, Sm and Su (Table 3). *Salmonella* strains from slaughtered cattle and pigs appeared to be sensitive even to commonly used drugs. All the *E. coli* and *Salmonella* strains tested were sensitive to Gm, Cp, Fz and NA (data not shown).

In total, 5 drug resistance patterns, in terms of single or multiple resistance, were observed for *E. coli* and 4 patterns for both cattle and pig *E. coli* (Table 4). In cattle *E. coli* strains, single, double and quintuple patterns occured at the same frequency (29 %) followed by a triple pattern (14 %). In pig *E. coli* strains, the single pattern was more frequent (59 %) than the double (20 %), triple (16 %) and quadruple (4 %) ones. The single pattern was the only pattern observed in *Salmonella* from apparently healthy cattle, and in *Salmonella* from diseased animals it was the most frequent (90 %). At present, the single resistance pattern appears to be more common than multiple drug resistance patterns, especially in pig *E. coli* strains.

Conjugative R plasmid: Of 49 resistant *E. coli* strains from pigs, 20.4 % (10/49) carried R plasmid, of which 60 % (6/10) showed the TcSmSu pattern while in cattle *E. coli* R plasmid was detected in 28.6 % (2/7) of the resistant strains tested. Pig *E. coli* with a triple resistance pattern and that of cattle with a quintuple pattern carried conjugative R plasmid at high frequency. Cattle *E. coli* strains with the quintuple resistance pattern also partially transferred triple and double drug resistance. No R plasmids were detected in the *Salmonella* strains from apparently healthy cattle but triple resistance was transferred from the strain of a sick calf (Table 5). Temperature-sensitive R plasmid was not detected under the present experimental conditions (data not shown). The frequency of transferrable R plasmid appears tn be more common in pig *E. coli* strains carrying the TcSmSu drug resistance pattern.

Table 3. Antibiotic resistance of *E. coli* and *Salmonella* from different sources in Zambia

Bacteria	Source	No. of resistant strains/ total no. of strains isolated (%)	No. (%) of strains resistant to :					
			Tc	Sm	Su	Ap	Km	Cl
E. coli	Cattle	7/167 (4.2)	5 (3.0%)	3 (1.8%)	5 (3.0%)	4 (2.4%)	2 (1.2%)	0
	Pigs	49/157 (31.2)	29 (18.5%)	18 (11.5%)	15 (9.6%)	17 (10.8%)	1 (0.6%)	1 (0.6%)
Salmonella	Cattle	1/28 (3.6)	0	0	0	1 (3.6%)	0	0
	Pigs	0/39 (0)	0	0	0	0	0	0
	Diseased animals[a)]	10/32 (31.3)	1 (3.1%)	10 (31.3%)	1 (3.1%)	0	0	0

a) These include 9/24 of cattle strains, 1/2 of 2 chicken strains and none of 1 leopard, 2 lion and 1 warthog strains.

Table 4. Antibiotic resistance patterns of tested E. coli and *Salmonella* strains

Resistance pattern	Drug	No. of cattle *E. coli* strains	No. of pig *E. coli* strains	*Salmonella* from healthy cattle	*Salmonella* from diseased animals
Single	Ap	2	11	1	—
	Tc	—	13	—	—
	Sm	—	3	—	9
	Su	—	1	—	—
	Cl	—	1	—	—
Subtotal (%)		2(29)	29(59)	1(100)	9(90)
Double	TcSu	2	2	—	—
	TcSm	—	3	—	—
	SmSu	—	2	—	—
	ApSu	—	1	—	—
	TcAp	—	1	—	—
	SmAp	—	1	—	—
Subtotal (%)		2(29)	10(20)	—	—
Triple	TcSmSu	1	6	—	1
	TcSmAp	—	1	—	—
	TcSuAp	—	1	—	—
Subtotal (%)		1(14)	8(16)	—	1(10)
Quadruple	TcSmSuAp	—	1	—	—
	TcSmSuKm	—	1	—	—
Subtotal (%)		—	2(4)	—	—
Quintuple	TcSmSuApKm	2	—	—	—
Subtotal (%)		2(29)	—	—	—
Total		7	49	1	10

Table 5. Transferred antibiotic resistance patterns from *E. coli* and *Salmonella* strains

Bacteria	Source	Donor strains:		Transconjugant:		%(No. of strains with R plasmid/total no. of resistant strains tested)
		resistance pattern	no. of strains	resistance pattern	no. of strains[a]	
E. coli	Pig	Sm	1	Sm	1	
	Pig	Su	1	Su	1	
	Pig	ApSu	1	Su	1	
	Pig	TcSmSu	6	TcSmSu	6[b]	
	Pig	TcSuAp	1	TcSu	1	
	Subtotal		10		10	20.4(10/49)
	Cattle	TcSmSuApKm	2	TcSmSuApKm TcSmSu, SmSu	2	28.6(2/7)
Salmonella (*S. Agona*)	Sick calf	TcSmSu	1	TcSmSu	1	10.0(1/10)

a) No. of strains carrying conjugative R plasmids.
b) This pattern constituted 60% (6/10) of pig *E. coli* carrying R plasmid.

Discussion

Although drug resistance in *Salmonella* from sick animals has been reported in Zambia[4], this is the first report on the drug resistance and conjugative R plasmid of *E. coli* and *Salmonella* from abattoir-slaughtered cattle and pigs.

The investigation revealed more pigs harbouring drug resistant *E. coli* than cattle. This was confirmed by different isolation rates, both of animals examined and of strains isolated between pigs and cattle. In Zambia, most of the cattle are nomadically and "traditionally" reared with limited administration of drugs, while in commercially reared pigs drugs are administered more frequently. This difference in rearing pattern between cattle and pigs is likely to be an important factor. Similar findings were made in Indonesia where animal rearing conditions are almost comparable to those in Zambia[10].

For pig *E. coli*, resistance was higher against Tc, Su, Ap and Sm, and not detected against Gm, Cp, Fz and NA. High incidence of *E. coli* resistance against Tc, Sm and Su is a common finding world-wide where these antibiotics are included in animal feeds[1,3,6,15]. The presence of antibiotic-resistant bacteria in domestic animals, especially those under intensive management, due to consumption of feeds containing antibiotics has been pointed out by many authors[1,6,7,9,1,17]. In Zambia, the use of

antibiotics is regulated but some farmers use the drugs indiscriminately. Drugs such as Tc, Sm and Su are more commonly used, especially on commercial farms, for various purposes.

Salmonella strains from both healthy cattle and pigs appear to be sensitive even to the commonly used drugs but the strains from diseased animals were more resistant. In healthy cattle, the limited administration of drugs could account for this. In Indonesia, Nakamura *et al.*[11] could not detect resistance in *Salmonella* against any of the antibiotics used. However, continuous usage of antibiotics may easily results in drug-resistant *Salmonella* as is likely to have occurred in the strains from the diseased animals. The situation in pigs in Zambia needs to be carefully evaluated although *Salmonella* strains from pigs are not resistant at present. Because of the high number of drug resistant *E. coli* and the presence of R plasmids in pigs, transfer of resistance from *E. coli* to *Salmonella* could possibly occur[14].

In total, 5 drug resistance patterns in terms of single or multiple resistance, were observed, four for both cattle and pig *E. coli*, 1 for *Salmonella* from apparently healthy cattle and 2 for *Salmonella* from diseased animals. The single resistance pattern was significantly more often detected among pig *E. coli* than others. However, despite being under intensive management, administration of drugs in pigs is likely to be infrequent in Zambia. Results obtained in Nigeria in domestic animals being fed antibiotic-containing feeds and regularly given antibiotics as prophylactic or chemotherapeutic agents, showed higher degrees of drug resistance[1].

The majority of the detected conjugative R plasmids were in pig *E. coli*. This might be attributed to the differences in managemental care of pigs and cattle in Zambia already mentioned. Intensively reared pigs (also calves and chickens) have been observed to be carriers of large numbers of bacteria with R plasmids[1,5,15]. In this study, similar results were obtained. The transfer of R plasmids carrying multiple resistance to *S.* Typhimurium and the problems associated with it are well known in Great Britain[2]. The high frequency of transferrable resistance observed in pig *E. coli* with TcSmSu resistance and those of cattle with TcSmSuApKm resistance suggests the necessity of further work to monitor possible transfer from *E. coli* to *Salmonella* in both commercially and traditionally kept domestic animals in Zambia, since 1 of the 2 *E. coli* strains carrying R plasmid was from tradionally reared cattle. Although temperature-sensitive R plasmid was not detected, more work needs to be done to substantiate this finding. Temperature-sensitive R plasmid has been widely reported and is known to transfer Tc resistance[5].

In general, the frequency of resistant bacteria is low in both cattle and pigs in Zambia. That is, at present contamination of meats by antibiotic-resistant *E. coli* and *Salmonella* is likely not to be a big problem. Especially for *Salmonella*, at present the commonly used drugs seem to be useful for the treatment of salmonellosis.

REFERENCES

1) ADETOSOYE, I. A. : Infective drug resistance among *Escherichia coli* isolated from clinically healthy domestic livestock. *Vet. Microbiol.* 5 : 333–342, 1980.
2) ANDERSON, S. E. : Drug resistance in *Salmonella typhimurium* and its implications. *Brit. Med. J.* 3 : 333–339, 1968.
3) DUPONT, L. H. & STEELE, H. J. : The human health implication of the use of antimicrobial agents in animal feeds. *Vet. Quart.* 9 : 309–320, 1987.
4) FALADE, S., SATO, G., ULAYA, W. & MWANZA, L. : Serovars and antibiotic sensitivity patterns of *Salmonella* strains isolated from domestic animals in Zambia. *Zimbabwe Vet. J.* 20 : 19–22, 1989.
5) ISHIGURO, N., GOTO, J. & SATO, G. : Genetical relationship between R plasmids derived from *Salmonella* and *Escherichia coli* obtained from a pig farm, and its epidemiological significance. *J. Hyg. Camb.* 84 : 365–379, 1980.
6) KARIUKI, P. D. : Incidence of drug resistance in *Escherichia coli* isolated from scouring calves. *Bull. Epiz. Dis. Afr.* 22 : 115–117, 1974.
7) KELLY, R. W., MOREHOUSE, C. E. & COLLINS, D. J. : The incidence and importance of antibiotic-resistant bacteria in livestock in the Republic of Ireland. *Irish Vet. J.* 26 : 49–57, 1972.
8) LEMINOR, L., VERON, M. & POPOFF, M. : Proposition pour une nomenclature des *Salmonella*. *Ann. Microbiol. (Inst. Pasteur)* 133B : 245–254, 1982.
9) LINTON, H. A. : Antibiotic resistance : The present situation reviewed. *Vet. Rec.* 100 : 354–360, 1977.
10) NAKAMURA, M. : A report on the animal hygiene situation in Indonesia. *J. Miyagi Pref. Vet. Med. Assoc.* 41 : 121–132, 1988 (In Japanese).
11) NAKAMURA, M., ISTIYANINGSIH, NAKASHIMA, N., KAJI, T. & SATO, S. : Isolation of *Salmonellae* from clinically normal cattle and pigs in Indonesia in 1986 and detection of plasmids in the isolates. *Jpn. J. Vet. Sci.* 51 : 1059–1061, 1989.
12) OJENIYI, A. A. : Drug enrichment of commercial poultry feeds and human health in the tropical developing countries. *Acta Vet. Scand.* 30 : 133–139, 1989.
13) PERRY, D. B., MWANAUMO, B., SCHELS, F. H., EICHER, E. & ZAMAN, R. M. : A study of health and productivity of traditionally managed cattle in Zambia. *Prev. Vet. Med.* 2 : 633–653, 1984.
14) POHL, P. & THOMAS, J. : R plasmid reservoirs and circulation. *Ann. Med. Vet.* 123 : 385–396, 1979.
15) SAIDA, K., IKE, Y. & MITSUHASHI, S. : Drug resistance and R plasmids of *Escherichia coli* strains isolated from pigs, slaughterers and breeders of pigs in Japan. *Antimicrob. Agents Chemother.* 19 : 1032–1036, 1981.
16) SATO, G., FURUTA, Y., KODAMA, H., IWAO, T. & OKA, M. : Enzootic occurrence of chloramphenicol-resistant *Salmonella typhimurium* var *copenhagen* in a calf population. *Am. J. Vet. Res.* 36 : 839–841, 1975.
17) SMITH, H. W. : The effect of the use of antibacterial drugs, particularly as feed additives, on the emergence of drug-resistant strains of bacteria in animals. *N. Z. Vet. J.* 15 : 153–166, 1967.

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Antimicrobial Effects of a New Carboxyquinolone Drug, Q-35, on Five Serogroups of *Leptospira interrogans*

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New carboxyquinolone drugs, including the recently developed Q-35, were evaluated for their in vitro potency against five serogroups of *Leptospira interrogans*. Q-35, ofloxacin, ciprofloxacin, and tosufloxacin showed MICs (0.05 to 0.20 μg/ml) comparable to those of tetracycline. However, MBCs of these drugs varied between 10- and 100-fold above the MIC for most strains tested. Q-35 was shown to be active against *L. interrogans* in vitro as judged by the MICs obtained.

Leptospirosis is a zoonosis of worldwide distribution which causes an acute febrile illness in humans and in some domestic animals. *Leptospira* spp. are maintained asymptomatically in the reservoir hosts, including a variety of wild and domestic animals (11). The antibiotics used primarily for the treatment of leptospirosis are penicillin or tetracycline (TC) (3, 6, 11); however, the effectiveness of such treatment is controversial, especially for patients with late-stage disease (9, 10). Other antimicrobial agents have been shown to be active against leptospires in vitro (1, 7), but they have not been used clinically. Recently, several new carboxyquinolones have been developed; these drugs are known to have a broad spectrum of antimicrobial activity and to be well absorbed by the oral route of administration (2). In addition, ciprofloxacin has been shown to be effective against *Leptospira interrogans* in an animal model (8). However, that study was limited to only one serogroup strain, and there have been no studies comparing the antimicrobial effects of these new carboxyquinolones on multiple strains of leptospires. For this paper, several new carboxyquinolones including the recently developed Q-35 (5) were studied for their antimicrobial activity against five serogroups of *L. interrogans*.

The *Leptospira* strains used belong to five serogroups of *L. interrogans*: serogroup Icterohaemorrhagiae serovar *icterohaemorrhagiae* strain RGA, serogroup Hebdomadis serovar *hebdomadis* strain Hebdomadis, serogroup Australis serovar *australis* strain Akiyami C, serogroup Autumnalis serovar *autumnalis* strain Akiyami A, and serogroup Canicola serovar *canicola* strain Hond Utrecht IV. All of the strains were supplied by H. Kida, Department of Veterinary Hygiene and Microbiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan. The leptospires were grown in 0.2% tryptose phosphate broth (TPB) (Difco Laboratories, Detroit, Mich.) containing 10% heat-inactivated rabbit serum (4). Antimicrobial agents tested were Q-35 [1-cycloprophyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylaminopiperdin-1-yl)-4-oxoquinoline-3-carboxylic acid dihydrate] (Chugai Pharmaceutical Co., Tokyo, Japan), norfloxacin (NFLX; Kyorin Pharmaceutical Co., Tokyo, Japan), ofloxacin (OFLX; Daiichi Pharmaceutical Co., Tokyo, Japan), ciprofloxacin (CPFX; Bayer Pharmaceutical Co., Osaka, Japan), tosufloxacin (TFLX; Toyama Chemistry Co., Tokyo, Japan), and tetracycline (Lederle Co., Tokyo, Japan). The activity of each drug against these leptospires was examined by a broth macrodilution technique. Drugs tested were standard powders which were kept at 4°C. Dilutions of drugs were prepared as aqueous solutions in a minimum volume of distilled water and diluted with phosphate-buffered saline to a concentration of 1 mg/ml. On the basis of stability data, drug preparations were freshly prepared before use or were prepared as stock solutions and stored at −40°C. Serial twofold dilutions of each drug starting from 25 μg/ml were prepared in tubes containing 2 ml of TPB. Tubes were inoculated with leptospires (final inoculum, 5×10^6/ml) from logarithmic-phase cells and incubated at 30°C in air for 7 days. To study the effects of Q-35 on the growth of *L. interrogans* serogroup Icterohaemorrhagiae, leptospires were counted with a modified Neubauer chamber under dark-field microscopy on days 2, 5, and 7 of incubation with the drug. The MIC of each drug was defined as the lowest concentration which inhibited visible growth (turbidity) at the seventh day of incubation. The culture tubes were diluted 10-fold on the seventh day of incubation and observed under dark-field microscopy to estimate the number of leptospires. After quantitative determination of the MIC, 10 μl of the drug-exposed culture was

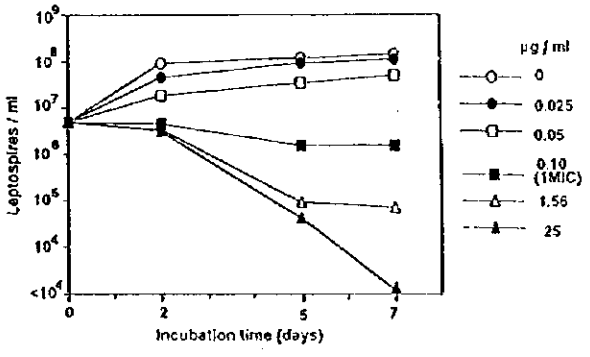

FIG. 1. Growth kinetics of *L. interrogans* serogroup Icterohaemorrhagiae RGA in the presence of Q-35. Leptospires at a concentration of 5×10^5/ml in TPB were inoculated with various concentrations of Q-35. The number of leptospires was determined by dark-field microscopy using a modified Neubauer chamber.

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TABLE 1. MICs and MBCs of antimicrobial drugs for five serogroups of *L. interrogans*

L. interrogans serogroup	MIC (MBC) (μg/ml)					
	Q-35	NFLX	OFLX	CPFX	TFLX	TC
Icterohaemorrhagiae	0.10 (25)	0.39 (>25)	0.20 (>25)	0.10 (>25)	0.05 (>25)	0.10 (6.25)
Hebdomadis	0.05 (1.56)	0.20 (>25)	0.10 (>25)	0.10 (3.13)	0.05 (1.56)	0.10 (3.13)
Australis	0.10 (3.13)	0.78 (>25)	0.20 (>25)	0.10 (3.13)	0.05 (3.13)	0.10 (1.56)
Autumnalis	0.05 (3.13)	0.39 (>25)	0.20 (25)	0.10 (3.13)	0.05 (3.13)	0.10 (3.13)
Canicola	0.10 (3.13)	0.20 (12.5)	0.20 (6.25)	0.10 (3.13)	0.05 (0.78)	0.10 (1.56)

inoculated into 5 ml of drug-free TPB and incubated for an additional 3 weeks at 30°C. The MBC was determined by turbidity measurement and defined as the lowest concentration of drug allowing no growth after 3 weeks at 30°C. The MBC in our study was assumed to reflect >99.99% killing of leptospires since 10 μl of the drug-exposed culture (initial inoculum of 5×10^6/ml) was subcultured for the MBC determination. If the killing of the leptospires was less than 99.99% in the presence of 25 μg of these drugs per ml, MBCs were not determined.

Growth kinetics of *L. interrogans* serogroup Icterohaemorrhagiae were examined in the presence of several drugs. Q-35 inhibited the growth of leptospires at a concentration of ≥0.10 μg/ml after 7 days of incubation as judged by direct cell count and turbidity measurements (Fig. 1). Leptospires at a concentration greater than 5×10^7 cells per ml produced visibly turbid cultures. The optical density at 560 nm at day 7 in drug-free culture was 0.047, which corresponded to 1.4×10^8 leptospires per ml. The MIC of Q-35 determined from this experiment was 0.10 μg/ml for *L. interrogans* serogroup Icterohaemorrhagiae RGA. Growth kinetics of this serogroup were also tested in the presence of TC and OFLX (data not shown); the MICs of TC and OFLX were 0.10 and 0.20 μg/ml, respectively, for this strain.

MICs and MBCs of each drug for five serogroups of *L. interrogans* are shown in Table 1. MICs of Q-35 for five *Leptospira* strains were within the range of 0.05 to 0.10 μg/ml. Q-35, OFLX, CPFX, TFLX, and TC showed similar MICs, while NFLX was less active.

MBCs of each drug for five serogroups of *L. interrogans* are also shown in Table 1. MBCs of each drug varied depending on the strains used. In particular, *L. interrogans* serogroup Icterohaemorrhagiae was relatively resistant to the bactericidal activity of each drug, and the MBC of Q-35 was four times higher than that of TC for that serogroup. MBCs of Q-35 for the other four serogroups of *L. interrogans* ranged from 1.56 to 3.13 μg/ml, and similar values were obtained for CPFX, TFLX, and TC. NFLX and OFLX were found to be significantly less active compared with the other drugs.

Turbidity measurement and microscopic observation were used in this study to define the MIC for multiple *Leptospira* strains. The cell count under dark-field conditions in the presence of a test drug corresponded well with visible turbidity, which verified the method of defining the MIC endpoint. Furthermore, the additional monitoring of diluted cultures under dark-field microscopy made the MIC determination more reliable.

MBCs varied considerably depending on the strains and the drugs. Of the five carboxyquinolones tested, Q-35, CPFX, and TFLX showed similar MBCs. These were comparable to those of TC for *L. interrogans* serogroups, except for serogroup Icterohaemorrhagiae, for which the MBC of TC was considerably higher. NFLX and OFLX had MBCs of >25 μg/ml for most of the serogroups.

L. interrogans serogroup Icterohaemorrhagiae tended to be most resistant to the bactericidal action of these drugs. Our data showed that the MBC of CPFX against *L. interrogans* serogroup Icterohaemorrhagiae was >25 μg/ml, whereas Shalit et al. observed an MBC of CPFX for this serogroup of 0.6 μg/ml (8). This discrepancy might be due to the different experimental procedures employed in the two studies. In our study, a final inoculum of 5×10^6 cells per ml was used, which was 10 times higher than that of Shalit et al. This might produce the observed differences in our MBCs. Another possible explanation might be the difference in strains of serogroup Icterohaemorrhagiae used in the two studies.

The data presented in this paper show that new carboxyquinolone drugs including the newly developed Q-35 are active against *L. interrogans* as evaluated by MICs, although MBCs were usually 10- to 100-fold higher than MICs.

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REFERENCES

1. Alexander, A. D., and P. L. Rule. 1986. Penicillins, cephalosporins, and tetracyclines in treatment of hamsters with fatal leptospirosis. Antimicrob. Agents Chemother. 30:835–839.
2. Bergan, T. 1988. Pharmacokinetics of fluoronated quinolones, p. 119–154. *In* V. T. Andriole (ed.), The quinolones. Academic Press, Inc., New York.
3. Clein, L. 1973. Penicillin in leptospirosis. Br. Med. J. 3:354.
4. Cox, C. D. 1955. Hemolysis of sheep erythrocytes sensitized with leptospiral extracts. Proc. Soc. Exp. Biol. Med. 90:610–615.
5. Ito, T., M. Otsuki, and N. Nishino. 1992. In vitro antibacterial activity of Q-35, a new fluoroquinolone. Antimicrob. Agents Chemother. 36:1708–1714.
6. Koen, R. S. 1962. Leptospirosis; a comparison of symptomatic and penicillin therapy. Br. Med. J. 1:1181–1183.
7. Oie, S., K. Hironaga, A. Koshiro, H. Konishi, and Z. Yoshii. 1983. In vitro susceptibilities of five *Leptospira* strains to 16 antimicrobial agents. Antimicrob. Agents Chemother. 24:905–908.
8. Shalit, I., A. Barnea, and A. Shahar. 1989. Efficacy of ciprofloxacin against *Leptospira interrogans* serogroup Icterohaemorrhagiae. Antimicrob. Agents Chemother. 33:788–789.
9. Stoenner, H. G. 1976. Treatment and control of leptospirosis, p. 375–388. *In* R. C. Johnson (ed.), The biology of parasitic spirochetes. Academic Press, Inc., New York.
10. Torten, M. 1979. Leptospirosis, p. 363–421. *In* J. H. Steele (ed.), CRC handbook series in zoonoses. CRC Press, Inc., Boca Raton, Fla.
11. Turner, L. H. 1969. Leptospirosis. Br. Med. J. 1:231–235.

Outbreaks of *Salmonella* Dublin Infection among Calves on a Dairy Farm Applying *Salmonella* Bacterins in Zambia

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ABSTRACT. In Zambia, a dairy farm keeping about 600 cows and self-contained calves had applied *S.* Dublin and *S.* Typhimurium bacterins to pregnant cows and calves in combination with all-in all-out pen system for rearing calves. Only relatively small scale outbreaks of *S.* Dublin infection occurred repeatedly in these years from 1989 to 1991 among fattening calves on the farm. The results obtained from the epizootiological study suggest that the preventive measures including the vaccination with *Salmonella* bacterins gave insufficient protection against *S.* Dublin infection to the calves, but they might have prevented large scale outbreak of the disease. This is the first report of the epizootiological study on outbreak of bovine *S.* Dublin infection on farm in Zambia.—KEY WORDS : calf, *Salmonella* Dublin, Zambia.

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Bovine salmonellosis including infection of *Salmonella choleraesuis* subspecies *choleraesuis* serovar dublin (*Salmonella* Dublin) is an economically important disease and its wide occurrence has been reported [1, 5, 10]. In Zambia, being located southern area of Africa, salmonellosis is one of the most important diseases in cattle. Since the first isolation from cattle in 1950's (R. N. Sharma, unpublished data), frequent isolations of *S.* Dublin from bovine specimens submitted for laboratory examinations have been reported [3, 4]. However, there has been no report of epizootiological study of outbreak of *S.* Dublin infection on farms. We encountered outbreaks of *S.* Dublin infection among Holstein-Friesian calves on a dairy farm in 1989–1991 in Zambia and carried out an epizootiological study. This report will be useful to better understanding of epizootiological feature of bovine salmonellosis in Zambia.

The farm involved is in Lusaka and keeps about six hundred Holstein-Friesian cattle and only self-contained calves are reared for dairy and for fattening. Groups of these calves are kept separately in a total of 6 pens on pasture (Fig. 1). There is no shelter on the farm. All-in all-out policy had been applied for each pen with some intervals between groups of calves. On the farm, a vaccination program had been applied to all pregnant cows and calves to prevent some bacterial diseases in calves. The vaccine (Bovivac Plus — Hoechst) contained formalinized and aluminium-absorbed cells of selected *Escherichia coli* serotypes of bovine origin, strains of *S.* Dublin, *S.* Typhimurium and Robert's types 1, 2, 3 and 4 of *Pasteurella multocida*. For pregnant cows, 2 subcutaneous injections of 5 *ml* had been made at approximately 6 and 3 weeks prior to calving to reinforce the specific antibodies transferred to the suckling calf via the colostrum. For calves, 2 subcutaneous injections of 2 *ml* at approximately 10 and 24 days of age had been practiced. However, newborn calves in No. 3 pen in 1990 had been vaccinated with the bacterins at 3 and 18 days of age, one week earlier than usual, resulting in possible reduction of immunity.

In December, 1989, the first isolation of *S.* Dublin from diseased fattening calves in No. 3 pen was recorded. Histopathological examination of 2 dead calves revealed acute phase showing centrolobular necrosis in the liver, fibrinous pneumonia and few glanulomatous lesions systemically. During the outbreak, 5 out of 30 calves in the pen died of salmonellosis. Further epizootiological investigations on the outbreak were not carried out on the farm.

In the beginning of October, 1990, 18 of a group of 40 about 3 month old calves kept in No. 3 pen showed severe watery diarrhea, dysentery, loss of appetite and general weakness (Fig. 2). At that time, the farm kept 380 dairy cows and a total of 211 calves (128 heifers and 83 steers of 8 weeks to 6 months of age). The calves had been separated into 6 pens of Nos. 1–6 as indicated in Fig. 1. Except No. 6 pen where 40 heifers were reared for dairy, all 5 pens kept fattening calves. Two of the affected calves died on the 5th and 6th of October and the other one male calf with severe symptoms was submitted for diagnosis to the University of Zambia, School of Veterinary Medicine in Lusaka on the 11th of October.

The autopsied calf was 3 month old male and killed by bleeding at terminal stage. Macroscopically, pinpoint-sized whitish spots were scattered diffusely in the liver, kidney and spleen. General lymphoid tissues were enlarged, especially in mesenteric lymph nodes (Fig. 3) and tonsil. Peritonitis, serofibrinosa and edematous thickening

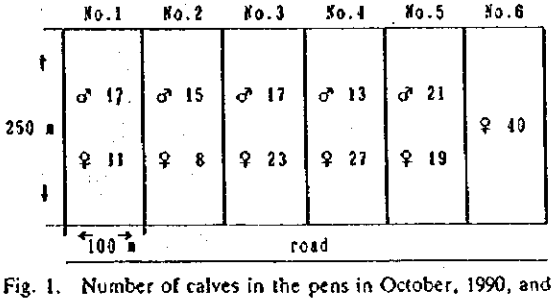

Fig. 1. Number of calves in the pens in October, 1990, and the infection seen in No. 3 pen.

of the intestinal mucosa were found. For histopathological examination, main organs of the autopsied calf were fixed in 10 % formalin and their paraffin sections were prepared and stained with hematoxylin and eosin (HE). The autopsied case revealed more chronic phase showing scattered necrotic granulomatous lesions in the liver, spleen (Fig. 4), kidney and lung. Embolic thromboses were observed generally (Fig. 5).

Pieces of heart, liver and spleen and jejunal and ileal contents of the autopsied calf were cultured on plates of blood agar, MacConkey agar, desoxycholate hydrogen sulphide lactose (DHL) agar and heart-infusion agar and into selenite broth, and incubated at 37°C for 24 hours. The plates were then examined for the growths. Subcultures from the incubated selenite broth cultures were made on MacConkey and DHL agar plates, incubated and subsequently examined. Four fecal samples obtained from the affected calves were also examined in a similar way. The postmortem materials and the 4 fecal samples from affected calves gave *Salmonella* suspect colonies. These isolates were examined biochemically for *Salmonella* and serotyped using Diagnostic *Salmonella* Antisera for O and H (Denka) and *Salmonella* H sera for Phase Induction (Denka) according to the manufacturer's instructions. Finally these isolates were identified as *S.* Dublin. These *S.* Dublin isolates from the postmortem materials were examined for antibiotic sensitivity with 6 Monodisks (Showa) using Oxoid Sensitivity Test agar. They were strongly sensitive to tetracycline, oxytetracycline, kanamycin, ampicillin and gentamicin, and moderately sensitive to streptomycin.

Intestinal contents of the postmortem materials and the 4 fecal samples from affected calves were examined parasitologically by the floatation methods. No parasitic ova or coccidial oocysts were detected from any samples examined.

Results obtained from the pathological, bacteriological and parasitological examinations described above indicated that *S.* Dublin infection broke out on the farm. Then the following measures for treatment and control of salmonellosis were taken on the farm.

All affected calves were soon transferred from No. 3 pen into an isolation pen. Moreover, medication for affected calves with chemotherapeutics started. One bolus of Cotrox (Interchem, Zambia) was given to the calves twice daily for 6 days. Each bolus contained 1.0 g sulfadiazine and 0.2 g trimethoprim which were supposed to have potentiation against *Salmonella* (M. Ngoma, unpublished data). All affected calves medicated recovered within 3 to 4 days after treatment. On the 15th of October, all calves in the pens Nos. 1–6 received one booster vaccination with the bacterins mentioned above. Other control measures such as cleaning and disinfection of pen environment except mangers, and detection of excretors of *S.* Dublin from calves and cows to prevent prolongation of the outbreak of salmonellosis [10] were not practiced. Since this outbreak in 1990, no further outbreak of *S.* Dublin infection had been recorded on the farm until June in 1991 when a small scale outbreak was confirmed among calves (D. S. Misra, unpublished data). Unfortunately no detailed data of the outbreak were available.

No attempt to search the source of *S.* Dublin infection among calves was made at the time of the outbreak in 1990. However, as this farm kept only self-contained calves, possibility of introduction of *S.* Dublin from outside source seemed to have been very low or have been negligible. On the other hand, there seemed to have been 2 possible sources of infection of *S.* Dublin after the outbreak in 1989. One of the possible sources seemed to be environmental contaminations with *S.* Dublin such as soil, water and others in the No.3 pen where affected

Fig. 2. An affected calf in the isolation paddock showing diarrhea, weakness and emeciation.

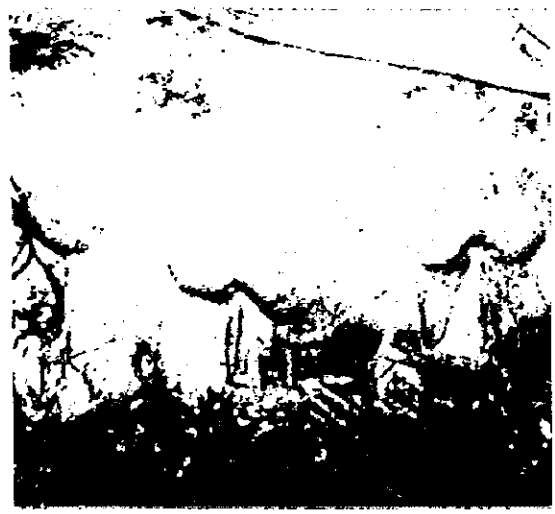

Fig. 3. Conspicuous enlargement of mesenteric lymph nodes.

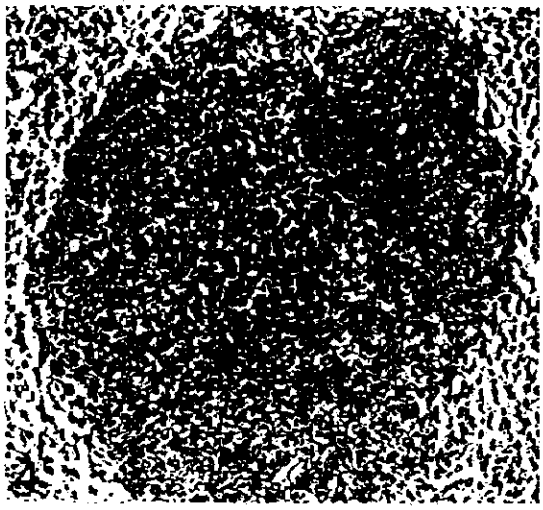

Fig. 4. Granuloma seen in the spleen. HE stain. × 155.

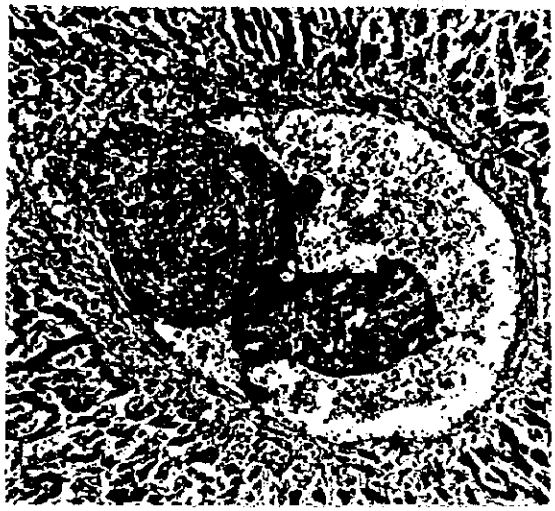

Fig. 5. Thrombosis seen in the central vein in the liver. HE stain. × 155.

calves had been kept during the outbreak in 1989. Although *S.* Dublin survived for at least 1,069 days in artificially contaminated and dried feces and probably died out within 6 months in infected feces out of doors while in feces splashed on walls it might survive up to 10 months [10], no information has been given about the survival of *S.* Dublin in pen environment under weather conditions in Zambia with about 3 months' hot dry season (September to November) with strong sun light and with about 5 months' hot rainy season (December to April) with heavy rainfall [7]. Since *S.* Dublin has higher ability to produce active carrier in cattle than other *Salmonella* serovars [10], another one of the possible infection sources of *S.* Dublin seemed to have been incidence of carrier cattle.

It has been reported that, although vaccines derived from killed organisms do result in increased resistance, it appears that a more solid immunity results from either natural infection or vaccination with living *Salmonella* [8, 10]. Vaccination of the calf and the dam with subsequent colostral passage of antibody to the calf have been studied, using formalin-killed aluminium hydrooxide-precipitated vaccines [2, 6]. *S.* Dublin bacterins were found to protect calves against homologous exposure [2], but Smith *et al.* [9] pointed out the results lacked statistical validity, due to the small number of calves used. Henning [6] in South Africa reported that, when the colostral immunity was challenged with virulent *S.* Dublin cultures given orally the calves exhibited a fair degree of resistance. Thus, he believed that this immunity is sufficient to protect young calves against natural exposure to paratyphoid, and he stated that the immunization of pregnant cows as a means of protecting new-born calves against paratyphoid is recommended an additional method of combating the diseases.

As stated above, only relatively small scale outbreaks of *S.* Dublin infection occurred repeatedly in these years from 1989 to 1991 on the dairy farm conducting the preventive measures such as the vaccination with *Salmonella* bacterins in combination with the all-in all-out pen system. The results obtained from our epizootiological study suggest that the preventive measures including the vaccination with *Salmonella* bacterins gave insufficient protection against *S.* Dublin infection to the calves on the farm, but they might have prevented large scale outbreak of the disease.

REFERENCES

1. Abe, N., Goto, J., Yasui, T., Nakaoka, Y., and Takahashi, T. 1991. *J. Hokkaido Vet. Med. Assoc.* 35: 93–96 (in Japanese).
2. Cameron, C. M. and Fuls, W. J. P. 1976. *Onderstepoort J. Vet. Res.* 43: 31–38.
3. Falade, S., Sato, G., Ulaya, W., and Mwanza, L. 1989. *Zimbabwe Vet. J.* 20: 19–22.
4. Gaspar, P. and Hrabeta, P. 1977. *Bull. Anim. Health Prod. Afr.* 25: 61–64.
5. Hashimoto, K. 1988. pp. 294–299. *In*: Diseases of Cattle, 2nd ed. (Shimizu, T. *et al.* eds.), Kindaishuppan, Tokyo (in Japanese).
6. Henning, M. W. 1953. *Onderstepoort J. Vet. Res.* 26: 45–59.
7. Japan International Cooperation Agency (JICA). 1982. pp. 11–13. *In*: Living in Africa—Republic of Zambia, JICA Service Center, Tokyo.
8. Robinson, R. A. 1970. *N. Z. Vet. J.* 18: 259–277.
9. Smith, B. P., Habacha, F. G., Reina-Guerra, M., and Hardy, A. J. 1980. *Am. J. Vet. Res.* 41: 1947–1951.
10. Wray, C. and Sojka, W. J. 1977. *J. Dairy Res.* 44: 383–425.

Prevalence Of Bovine Brucellosis In Southern Province of Zambia

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Summary

A serological survey on brucellosis was carried out for five years from 1984-88 in cattle and humans mainly in the Southern Province of Zambia. The prevalence of brucellosis on traditional farms was 13.4% and on commercial farms was 2.2%. Some abortions in cows were found to be caused by brucellosis in two traditional farms. Out of 292 human sera collected from residents in stock breeding areas, meat dealers and slaughter-house workers in Mazabuka and Lusaka districts, three sera (1.0%) were *Brucella abortus* antibody-positive, indicating that brucellosis is present in human beings as well as cattle in Zambia.

Introduction

Bovine brucellosis caused by *Brucella abortus* is an important disease of cattle, because it causes abortion, frequent sterility and reduction of milk yield ([1, 2, 3]). Brucellosis occurs in nearly all parts of the world where special care for eradication and prevention of the disease has not been taken ([2, 4])

The disease is also important for human health ([5, 6]). Human brucellosis is transmitted by contact with infected animals or by drinking raw milk from infected animals ([5, 6, 7]). Control and eradication of the disease is most effectively carried out by the segregation and elimination of infected animals (4).

In Zambia, it has been reported that bovine brucellosis is prevalent in all areas except the Northern Province (R. Sovjak, 1978). In this communication, we present the results of a serological survey of brucellosis in cattle and humans from 1984 to 1988 in Southern and Central Provinces in Zambia.

Materials and Methods

There are two types of livestock farms in Zambia, which are referred to as traditional and commercial farms, respectively. The traditional farmers use the communal land tenure system for keeping and feeding cattle, and during the rainy season (November to March) they use the vast wet swamp of the Kafue river for grazing the cattle. On the other hand, the commercial farmers observe the land demarcation system.

5,179 cattle sera were randomly collected from commercial farms and 2,547 from traditional farms in the Southern Province of Zambia in different seasons.

Human sera were collected from 172 residents in Mazabuka area and from 120 residents in Lusaka area. All sera were separated from coagulated blood samples by centrifugation at 2,500rpm. for 5 minutes and kept at - 20°C.

Antibody against *Brucella abortus* was determined by the rapid agglutination test (RAT)([8]), tube agglutination test (TAT)([8]) and complement fixation test (CFT)([9]). The tests were carried out according to the FAO/WHO standard procedure([10]) using *B. abortus* strain 99 Antigen Kits of RAT, TAT and CFT that were purchased from the National Institute of Animal Health, Tsukuba, Japan.

The RAT was performed on a glass slide by rapidly mixing a test serum (20 µl) with 20 µl of RAT Antigen Kit solution. Only RAT-positive serum was next used for TAT. TAT was done by using a micro-titration plate having 96 wells with U-shaped bottoms (Nunc Products, Kastrup, Denmark). Tenfold diluted RAT-positive serum (25 µl) was twofold serially diluted with 0.85% NaCl containing 0.5% phenol solution and mixed with the same volume of TAT Antigen Kit solution diluted tenfold with the same NaCl solution. Fifty percent agglutination was read after 12 hours incubation at 37°C.

Prior to CFT, sera were inactivated at 56°C for 30minutes. Tenfold dilutions of inactivated sera (25 µl) were twofold serially diluted with 0.85% NaCl solution containing 0.01% $MgSO_4$. CFT Antigen Kit solution (25 µl) diluted 100 times with the same NaCl solution and a 60-fold dilution of Guinea-pig serum (50 µl) , which was previously titrated to have 2 haemolytic units, were added to each well and incubated at 4°C for 12 hours. After incubation, 2% sheep red cells sensitised with 2 haemolytic units of haemolysin (Toshiba Chemical Co., Tokyo, Japan) (50 µl) were added as indicator

cells for the detection of complement consumption and incubated at 37°C for 30 min before reading the 50% haemolysis point.

In data from 1984, sera showing 50% agglutination by TAT at more than 40-fold dilution, which is equal to the 100 international units (IU) as proposed by the FAO/WHO, were considered to be antibody-positive (4). Because CFT was not provided at that time, other data including the data shown in Tables 2 and 3, were prepared from the results of both TAT and CFT. Serum showing TAT-positive at more than 80-fold dilution (200 IU) was decided to be antibody-positive regardless of the CFT result. Serum showing both TAT-positive at a 40-fold dilution and CFT-positive at more than 5-fold dilutions was decided to be antibody-positive, but if it was CFT-negative at the same dilution, it was decided to be antibody-negative regardless of the TAT result. Serum that was TAT positive at less than 20-fold dilution was decided to be antibody-negative regardless of the CFT result.

Results

Out of 5,149 sera randomly collected from commercial farms in different districts in the Southern Province 2.2% were positive, whereas 13.4% were positive out of 2,547 sera collected from traditional farms (Table 1).

Twenty-one cows among 200 cows on two traditional farms had histories of abortion for the previous two years and 11 (52.4%) of them were positive against *B. abortus* in comparison to 28 (15.6%) positive cows out of 179 which had normal parturition (Table 2).
Only three sera (1.0%) were positive out of 292 sera from stock-breeders, butchers and slaughterhouse workers in the Mazabuka and Lusaka districts (Table 3).

Discussion

There was a significant difference in the incidence of Brucella-positive animals between commercial and traditional farms (Table 1). This difference could be attributable to the land tenure and grazing systems used by each type of farmer. In the traditional farming systems, it is difficult to segregate positive cows from negative ones in order to prevent contamination of the pastures.

At one commercial farm located in the Livingstone district, 7.1% of cows were positive. The high percentage of positive cows may be a result of brucella vaccination six months before the start of the investigation as reported by the farmers.

Human infection with *B. abortus* was found in 1.0% of the tested population in Mazabuka and Lusaka districts (Table 3). One positive case was a student in Mazabuka who had never come into contact with any cattle, indicating that infection could have occurred by drinking raw milk. There were two positive cases in workers at a cattle slaughterhouse suggesting transmission of brucellosis by contact with infected cattle.

Segregation and elimination of infected animals or vaccination have been carried out in many countries for the control and eradication of brucellosis ([11, 12]). In South Africa, the disease incidence was reduced from 13.4% in 1955 to 7.3% in 1957 by massive vaccination campaigns ([11]).

It would seem that brucellosis is prevalent in the southern Province of Zambia, especially in the traditional farming systems. The improvement of farm sanitation will play an important role in reducing the disease incidence in Zambia. Further surveys on brucellosis should be carried out annually throughout Zambia in attempt to eradicate the disease.

Acknowledgements

The authors wish to thank the Director, Dr. H. G. B. Chizyuka and the staff members of Department of Veterinary and Tsetse Control Services, Ministry of Agriculture and Water Development of Zambia, for supporting this survey. The collaboration of Japan International Cooperation Agency and Japan Overseas Cooperation Volunteers, and the technical guidance from the School of Veterinary Medicine, University of Zambia are greatly appreciated.

References

1. Colton, W.E., and Buck, J.M. (1961). JAVMA, 78, 306
2. Meyer, M.E. (1964). Am. J. Vet. Res., 25, 553.
3. Payne, J.M. (1959). JAVMA, 78, 447.
4. Joint FAO/WHO Expert Committee on Brucellosis (1958). Wld. Hlth. Tech. Rep. Ser., No. 148.
5. Sadler, W.W. (1960). Am. J. Pub. Health, 50, 504.
6. Schnerrenberger, P.J., Martin, R.J., Wactor, P.R., and Jelly, G.G. (1972). Arch. Environ. Health, 24, 337.
7. Renoux, G. (1953). Aspects of human brucellosis. Advances in the control of zoonoses, W.H.O. Monogr., 19, 61.
8. Stableforth, A.W. (1953). Advances in the control of zoonoses W.H.O. Monogr., 19, 89.
9. Hill, W. (1963). Zentr. Veterinary Medizin, 10, 127.
10. Joint FAO/WHO Expert Committee on Brucellosis (1971). Fifth report, WHO Techn. Rep. Ser., No. 464.
11. Stable forth, A.W. (1959). Infection diseases of animals - diseases due to bacteria, London. Butterworths Publication Ltd. p.95.
12. WHO/FAO seminar on zoonoses (1952). W.H.O. Monograph Series No. 19., p.105.

Persistence of Antibody in Cattle after *Brucella abortus* Strain 19 Vaccination in Mazabuka District of Zambia

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The control and eradication of *Brucella abortus* is based on serological tests followed by segregation and elimination of reacting animals ([1,2]). However, widespread vaccination interferes with the use of serological tests as a basis for segregation ([3]) . *B. abortus* strain 19 (S19) live vaccine is frequently used because of its efficacy in prevention of the disease ([1-3]). There is no way of differentiating serological reactors due to natural infection or due to vaccination as *B. abortus* strain 19 (S19) leads to formation of antibodies similar to natural infection. In this communication, we present data on the elevation and disappearance of antibodies in cattle after S19 vaccination in Mazabuka district of Zambia.

Twenty *B. abortus* antibody negative heifers, aged from 6 to 8 months, were selected from a well-managed commercial farm with a total population of 650 cattle (450 cows and 200 heifers) in which no cattle had shown any clinical signs of brucellosis for years. The heifers were bled every month from two months after receiving the S19 vaccine. Collected sera were separated from coagulated blood samples by centrifugation at 2,500rpm for 5 minutes and kept at -20°C. Positive-antibody formation against *B. abortus* was determined by the rapid agglutination test (RAT)([4]), tube agglutination test (TAT)([4]) and complement fixation test (CFT)([5,6]).

All vaccinated heifers became antibody-positive two months after vaccination as shown in Table 1. The antibodies completely disappeared within nine months, but one heifer still showed a agglutinin titre of 1:40 at the seventh month after vaccination.

When a serological survey is carried out, the effects of vaccination on the positive response should be considered. The agglutinin detected by RAT still appeared in 30% of examined sera nine months after vaccination. RAT has been used to screen antibody-positive sera. However, diagnosis should be based on TAT and CFT since they are more accurate than RAT. When a cow which has become negative on TAT and CFT after vaccination, becomes positive again, the cow must be thought to be infected by brucellosis. Therefore, serological surveys for brucellosis should be performed sooner than nine months after S19 vaccination.

Table 1: Antibody retention in 20 cattle after *B. abortus*

Months after vaccination	Positive cattle (%)	Mean and range of TA antibody titre	Mean and range of CF antibody titre
2	20 (100)	95.2 (40-320)	20.0 (10-80)
3	11 (55.0)	54.8 (40-160)	12.3 (5-20)
4	5 (25.0)	40.0 (40-100)	9.4 (5-20)
5	3 (15.0)	40.0 (40)	10.0 (5-20)
6	1 (5.0)	40.0 (40)	5.0 (5)
7	0 (0)	40.0 (40)	0 (0)
8	Not tested		
9	0 (0)	0 (0)	0 (0)

[a] The mean of antibody titres was the geometric mean in antibody-positive individuals.

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The authors wish to thank the Director, Dr. H.G.B. Chizyuka and the staff members of Department of Veterinary and Tsetse Control Services, Ministry of Agriculture and Water Development of Zambia, for supporting this programme. The collaboration of Japan International Co-operation Agency and Japan Overseas Co-operation Volunteers, and the technical guidance from the School of Veterinary Medicine, University of Zambia are greatly appreciated.

References

1. Goode, E.R. Jr., Manthei, C.A., and Amerault, T.E. (1957). Proc. U. S. Livestock Sanitary Assoc., Ann. Meeting, Chicago, p.89.
2. Haring, C.G., Traum, J., and Maderious, W.E. (1947). JAVMA, 110, 103.
3. Buck, J. M. (1930). J. Agric. Res. 41, 667.
4. Stableforth, W.W. (1953). Advances in the control of zoonoses, WHO. Monogr., 19, 89.
5. Hill, W. (1958). Zentr. Veterinary Medizin, B, 10, 127.
6. Joint FAO/WHO Expert Committee on Brucellosis (1958). Wld. Hlth. Tech. Rep. Ser., No. 148.

Table 1: *Prevalence of bovine brucellosis in commercial and traditional farms in southern province of Zambia*

District	Year	No. sera tested	Antibody positive %
Commercial Farms			
Mazabuka	1984	1,587	21 (1.3)
Choma	1984	593	12 (2.5)
Kalamo	1984	64	0 (0)
Livingstone	1984	759	54 (7.1)
Mazabuka[a]	1987	1,372	5 (0.4)
Mazabuka[a]	1988	774	17 (2.2)
Total		**5,149**	**112 (2.2)**
Traditional Farms			
Monze	1984	51	9 (17.6)
Namwala	1984	334	66 (19.8)
Mazabuka[a]	1986	2,162	266 (12.3)
Total		2,547	241 (13.4)

[a] Positive ratios were determined from the results of both the tube agglutination test and the complement fixation test and others were determined from only tube agglutination test results

Table 2: Relation between abortion and *Brucella abortus* antibody elevation in 200 cows bred in two traditional farms of Mazabuka district

Abortion history	No. sera tested	Antibody positive (%)
Normal parturition[b]	179	28 (15.6)
Total	200	39 (19.5)

[a] Cows which had abortion within last two years. [b] Cows which had never aborted.

Table 3: Prevalence of brucellosis in humans in Mazabuka and Lusaka districts

District	No. sera tested	Antibody positive (%)
Mazabuka	172[a]	1[c] (0.6)
Lusaka	120[b]	2[d] (1.7)
Total	292	3 (1.0)

[a] These sera were collected from residents in stock-breeding areas (163), butchers (4) and workers at slaughterhouses (5) in Mazabuka.
[b] These sera were collected from residents (40) and workers at pig (40) and cattle (40) slaughterhouses.
[c] The titre was 1:160 by tube agglutination test and negative by complement fixation test.
[d] Both titres were 1:40 by tube agglutination test and 1:5 by complement fixation test.

An epidemiological survey of av diseases in a small village i

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In the last few decades, many commercial poultry farms have been developing and providing poultry products, nevertheless, numbers of domestic chickens are still reared in every local village and have an important role in supplying animal protein to the inhabitants. These chickens are not vaccinated and rarely medicated even during serious outbreaks of disease. Since each local village is possibly separated from others, it is worth investigating what kinds of disease status such a village would have in their chickens.

In the present study, blood samples were collected from chickens in Shingoma village, Mazabuka District in Southern Province and tested serologically for Newcastle disease (ND), Infectious Bursal Disease (IBD), *Salmonella pullorum, Mycoplasma gallisepticum* and *M. synoviae*. In addition, tracheal and cloacal swabs were taken in an attempt to isolate viruses.

Shingoma village is located 30 kilometres east of Kafue, 100 kilometres north of Mazabuka, along the Kafue river. Seven to 25 chickens of local origin were reared in each household. Most owners reported that chickens had often died, especially in the rainy season. Symptoms such as depression, nasal discharge, coughing and diarrhoea were reported.

Eighteen serum samples, 17 tracheal and 11 cloacal swabs were obtained from chickens from 5 households. The serum samples were tested for antibodies against ND virus by the haem-agglutination inhibition test. Another set of serum samples were tested for antibodies against IBD virus by agar-gel precipitation. Antibodies against *S. pullorum, M. gallisepticum* and *M. synoviae* were tested by the rapid slide agglutination test. Isolation of viruses was attempted by the inoculation of chicken kidney cell monolayers, and identified by cytopathic effect, inclusion bodies and the haemagglutination test.

The antibody positive rates were 100% to ND virus, 94.4% to IBD virus, 0% *to S. pullorum*, 100% to *M. gallisepticum* and *M. synoviae* (Table 1). No virus was recovered. All samples were negative for the haemagglutination reaction. The results indicated an all-or-non phenomenon, that is, outbreaks of the lethal diseases resulted in many dead chickens and a few survivors with a high positive rate for the antibodies. The results of this survey also provided information on the major avian disease situation in village chickens in Zambia. Commercial poultry farms which are located near to local villages could allow diseases to be readily transmitted between

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PREVALENCE OF SALMONELLA IN APPARENTLY HEALTHY SLAUGHTERED CATTLE AND PIGS IN ZAMBIA

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Examination of caecal contents and mesenteric lymph nodes from 105 slaughtered cattle of 18 lots collected from traditional farms of 4 provinces and 105 pigs of 10 lots from 7 piggeries in Zambia, yielded isolation of different Salmonella serovars. Nine cattle (8.5%) of 4 lots from 2 provinces and 12 pigs (11.4%) of 5 lots from 3 piggeries revealed presence of 12 different serovars of Salmonella. Cattle harboured 5 serovars namely, *Salmonella* typhimurium, *Salmonella* heidelberg, *Salmonella* othmarschen, *Salmonella* bonn and *Salmonella* weltevreden, while from pigs, 7 serovars namely S. bredeney, S. braenderup, S. muenchen, S. newport, S. elisabethville, S. infantis and S. bovismorbificans were isolated. From cattle serovar S. othmarschen and from pigs *Salmonella* bredeney, *Salmonella* braenderup, *Salmonella* muenchen, *Salmonella* newport and *Salmonella* elisabethville are being reported for the first time from Zambia.

As food animals are considered to be the main source of Salmonellosis in human and it has been emphasised that domestic animals harbouring Salmonella play an important role in transmission of Salmonellosis from animals to humans (Hobb 1961). Hummel (1979) reported 6.16% infection rate of Salmonellosis in cattle slaughtered at Dar-es-salam in Tanzania.

From Nigeria Collard and Sen (1956) reported 5.5%, carrier rate of Salmonella in healthy cattle slaughtered while McAnulty (1958) showed a carrier rate of 2% among Zibu cattle in Municipal abattoir of Kampala, Uganda. The present paper deals with the isolation of Salmonella from apparently healthy slaughtered cattle and pigs at Lusaka abattoir in Zambia.

MATERIALS AND METHODS

Cattle: A total of 105 beef cattle of 18 lots from Southern, Western, Lusaka and Central provinces taken to a large abattoir were sampled for Salmonella isolation. These cattle originated from traditional farms which allow native breeds to graze under natural conditions with little or no medication.

Pigs: A total of 105 pigs of 10 lots from 7 piggeries of Lusaka province taken to a pig abattoir in Lusaka were sampled similarly.

Sampling: A few mesenteric lymph nodes from small intestinal region and about 10 g of caecal contents were collected by aseptic procedures from slaughtered cattle and pigs. The samples were brought to the laboratory as soon as possible after collection and processed on the day of sampling.

Isolation procedures for Salmonella : The mesenteric lymph nodes from each animal were dissected from the mesenteric fat using sterile instruments, weighed and disinfected with cotton containing 70% alcohol to eliminate the surface contaminants. One gram of the lymph nodes was minced and was directly smeared on to Desoxycholate-Hydrogen-sulfide-Lactose (DHL) agar (Eiken). A portion of the minced tissues were also inoculated aseptically into ten ml of Hajna's Tetrathionate (HT) broth (Eiken) for enrichment. By means of inoculating loop, a loopful of caecal contents was inoculated directly onto DHL agar. About one gram of caecal contents was inoculated into ten ml of HT broth for enrichment. The inoculated agar plates and enrichment broth were incubated at 37°C overnight. Salmonella suspected colonies from the direct cultures were purified onto DHL agar and then examined for biochemical reactions. One loopful each of the enrichment cultures was inoculated onto DHL agar and around five Salmonella suspected colonies were purified into DHL agar used for further tests.

Serotyping of Salmonella Isolates : Isolates identified as Salmonella on the basis of biochemical reactions were serotyped with the use of diagnostic Salmonella antisera (Denka) for O and H and Salmonella H sera for phase induction (Denka) according to the manufacturer's instructions.

Results

Cattle : Results of examination of a total of 105 slaughtered cattle belonging to 18 lots collected from different traditional farms in 4 provinces revealed that 9 cattle (8.5%) of 4 lots from Southern and Western provinces yielded Salmonella. Three of the 9 slaughtered cattle yielded Salmonella only in the mesenteric lymph nodes and 2 of these 3 were positive by direct plating. The other 4 cattle yielded Salmonella only in caecal contents and the remaining two harboured Salmonella in both lymph nodes and caecal contents. Serotyping of bovine isolates revealed five Salmonella servoars, (Table—1).

Pigs : Data presented in Table—1 revealed that a total of 105 slaughtered pigs belonging to 10 lots collected from 7 piggeries (A, B, C, D, E, F and G) were examined for Salmonella and 11 pigs (11.4%) of 5 lots from 3 piggeries (A, B and C) yielded Salmonella. Piggery A gave eight pigs positive for Salmonella in three out of four lots sampled for four successive months. One of the three lots contained five pigs positive for Salmonella, indicating that Piggery A was heavily contaminated with Salmonella. Five of 11 Salmonella positive carcasses yielded Salmonella only in the mesenteric lymph nodes and 2 of them were positive by direct plating, the other 5 were positive only in caecal contents and the remaining 1 positive in lymph nodes and caecal contents. Serotyping results showed that seven serovars were identified in pigs and out of these, four were detected in pigs from piggery A, one from piggery B and two from piggery C.

Discussion

The results of the present study revealed that the isolation rates of Salmonella

Prevalence of Salmonella in apparently healthy slaughtered cattle and pigs

Table—1. Salmonella serovars Isolated from slaughtered cattle and pigs in Lusaka, Zambia.

Serovars	No. of Cattle Positive for Salmonella	No. of Pigs Positive for Salmonella
S. typhimurium	4	—
S. heidelberg	1	—
S. othmaschen	1	—
S. bona	2	—
S. weltevreden	1	—
S. bredeney	—	5
S. braenderup	—	1
S. infantis	—	1
S. muenchen	—	1
S. newport	—	1
S. borismorbificans	—	1
S. elisabethville	—	1
Total	9	11
Percent	8.5	11.4

was 8.5% in slaughtered cattle and 11.4% in pigs. Although isolation rates of Salmonella may vary widely depending upon the influence of animal species, animal age, animal rearing system, pre-slaughter handling practices, hygiene standards, bacteriological methods and in particular the size and kind of the samples examined, the isolation rates obtained in the present study seem not to be high. The prevalence of 8.5% Salmonella in slaughtered cattle in the present study is almost comparable with that 7% in Germany (Stolle and Reuter, 1978), 6.16% in Tanzania (Hummel 1979), 5.5% in Nigeria (Collard and Sen 1956) while in Australia 76% of slaughtered cattle harboured Salmonella when the ruminal contents and the mesenteric lymph nodes sampled from various regions were examined for Salmonella (Samuel *et al* 1980a). It should however be noted that the results in the present study were obtained from cattle collected from traditional farms employing traditional farming methods different from the intensive rearing system. The prevalence of Salmonella (11.4%) in slaughtered pigs in this study was a little higher than the results obtained in similar surveys in United Kingdom (7.3%) (McCaughey *et al* 1973), but much lower than that (27%) recorded in Australia (Riley, 1970). In this study, half of Salmonella positive cattle and half of Salmonella positive pigs yielded Salmonella in the mesenteric lymph nodes and of these, two of each were positive by direct plating, indicating the infected lymph nodes contained large numbers of Salmonella organism.

It is obvious that large numbers of Salmonella in the mesenteric lymph nodes constitute a major source for contamination of the carcass and edible offal in the abattoir (Samuel *et al* 1980b).

Isolation of Salmonella from slaughtered animals is useful guidance for herd health programmes and disease surveys (Morse and Bird, 1984). Salmonella serovars identified in the present work viz. *S. othmarschen* from cattle and *S. bredeney*, *S. braenderup*, *S. infantis*, *S. muenchen*, *S. newport* and *S. elisabethville* from pigs have never been reported from these species in Zambia. Although the number of sample processed in the present investigation seems to be lower for true representation and therefore, more samples from different parts of the carcass and originating from wider area of the country may be necessary to know the more accurate prevalence.

References

Collard, P. and Sen R. (1956). W. Afr. Med. J. *18* : 175-178.
Hobbs, B. C. (1961). J. Appl. Bacteriol. *24* : 340-352.
Hummel, P. H. (1979). Bull. Anim. Hlth. Prod. Afri. *27* : 113-121.
McCaughey, W. J., McClelland, T. G. and Reddy, R. M. (1973). Vet. Rec., *92* : 191-194.
McAulty, B. (1958). Nature, *191* : 576-577.
Morse, J. W. and Bird, D. W. (1984). Am. J. Vet. Res., *45* : 1648-1649.
Riley, M.G.I. (1970). Aust. Vet. J., *46* : 40-43.
Samuel, J. L. ; O'Boyle, D. A. ; Mathers, W. J. and Frost A. J. (1980a). Res. Vet. Sci., *28* : 238-241.
Samuel, J. L. ; O'Boyle, D. A. ; Mathers, W. J. and Frost A. J. (1980b). Res. Vet. Sci. *28* : 368-372.
Stolle, A. and Reufer, O. (1978). Beil. Munch. Tierarztl. Wschr., *91* : 188-193.

Bacteriological Studies of Pneumonia in Zambia

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Summary

Pneumonic specimens were collected mainly at abattoirs of cattle and pigs in Lusaka. In cattle, the microorganisms were isolated from 34 out of 104 cases (33%). In pigs, The microorganisms were isolated from 37 out of 103 cases (36%). Isolated microorganisms were mainly Corynebacterium sp. in cattle but in pigs, they were mainly Streptococcus sp.. Mycoplasmas were not detected through out the survey.

Preface

In Zambia, it is said that the losses of cattle due to Pneumonia are serious, particulary in young animals. The causative agents of pneumonia are usualy Pasteulla, Haemophilus, Streptococci, Corynebacterium, Staphylococci, Chlamydia, Mycoplasmas and Viruses. However, in Zambia, the causative agents of this disease have not yet been identified.

Materials and methods

Specimens of pneumonia were mainly collected at the abattoirs in Lusaka. They were cultured on 5% blood agar plate at 37°C for two days and examined. For Mycoplasmas, specimens were cultured on PPLO agar at 37°C for 14 days.

Results

In cattle, Corynebacterium sp., Streptococcus sp. and Staphylococcus sp. were mainly isolated (Table 1). Other microoganisms were gram negative rods, Pasteulla sp., and Mycobacterium sp. (Table 1). Gram negative rods were identified as E. coli (No1, Table 3) and Klebsiella sp. (No2, Table 3).

In pigs, Streptococcus sp., gram negative rods and Staphylococcus sp. were mainly isolated (Table 2). Other microorganisms were Corynebacterium sp., and Diplococcus sp. etc. Gram negative rods were identified as Klebsiella sp. (No1 and No2, Table 4). 4 out of 6 Staphylococcus sp. were found to be coagulase positive in pigs but no coagulase positive ones from the cattle (Table 1). Mycoplasma were not isolated from lung lesions of cattle (30 specimens from cattle and 40 specimens from pigs).

Further studies are needed on pneumonia cases in Zambia from the virological and Mycoplasmal point of view.

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Table 1. Species of Bacteria isolated from 104 cattle specimens

Species of Bacteria	Numbers isolated
Corynebacterium sp.	8
Streptococcus sp.	6
Staphylococcus sp.*	6
Micrococcus sp.	3
Gram negative rod	3
Diplococcus sp.	2
Pasteulla sp.	2
Mycobacterium sp.	1
Actinomyces sp.	1
Neisseria sp.	1
Total	34

* All Staphylococci were coagluase negative.

Table 2. Species of Bacteria from 103 cases of the pigs specimens

Species of Bacteria	Number isolated
Streptococcus sp.	13
Gram negative rod	7
Staphylococcus sp.*	6
Corynebacterium sp.	5
Diplococcus sp.	3
Micrococcus sp.	2
Actinomyces sp.	1
Total	37

* 4 strains out of 6 were coagulase positive.

Table 3. Results of biochemical tests of gram negative rods from the cattle

No	Catalase	Simons Citrate Media	MR	VP	SIM				TSI			
					M	I	H_2S	G	S	B	M	H_2S
1*	+	-	+	-	+	+	-	+	Y	Y	+	-
2**	+	+	-	+	-	-	-	+	-	-	-	-
3	+	-	-	-	-	-	-	+	-	-	-	-

M; Motility, I; Indole, G; Gas formation, S; Slant surface, B; Butt, Y; Yellow
* No. 1 was identified as E. coli.
** No. 2 was identified as Klebsiella sp..

Table 4. Results of biochemical tests of the 3 strains of gram negative rods from the pigs

No	Catalase	Simons Citrate Media	MR	VP	SIM				TSI			
					M	I	H_2S	G	S	B	M	H_2S
1*	+	+	-	+	-	-	-	+	-	-	-	-
2*	+	+	-	+	-	-	-	+	-	-	-	-
3	+	-	-	-	-	-	-	+	-	-	-	-

M; Motality, I; Indole, G; Gas formation, S; Slant surface, B; Butt

No. 1 and No. 2 were identified as Klebsiella sp..

Serological Survey on Bovine Paratuberculosis in Zambia

Koichi Orino and Kihelji Shimizu

Prevalences of bovine paratuberculosis now indicate worldwide distributions and is becoming a very serious problem. In Zambia, the presence of this disease has been reported histopathologically (Pandey and others 1987). Several diagnostic methods such as Johnin test, FAT, gel-diffusion test, CF test etc are reported, but there are some false positive or negative cases and accurate diagnosis is very difficult. This communication reports a serological survey by CF test on bovine paratuberculosis in Zambia.

A total of 166 bovine sera were tested for antibodies against M. paratuberculosis. All sera were collected at random from all dairy farms in Mazabuka area, which are located in the southern province of Zambia. Sera were inactivated at 56 °C for 30 min and examined by the micro-plate method.

The test antigens were obtained from the National Institute of Animal Health of Japan. The positive reaction was decided by the 100% inhibition of hemolysis and the titer of 1:10 and over was regarded as infected.

Of the 166 sera, 39 (23.5%) gave positive CF reactions, titers ranging from 1:10 to 1:80, mostly 1:10 (Table 1, Table 2) The relations between positive cases and age were not significant (Table 2).

In Lusaka area, more than 50 cows which had diarrhea and were suspected of some chronic infections were examined for paratuberculosis by CF test. The positive ratio was very high (41 positive among 56 ····· positive ratio 73.2%). Several cows showed very high titers such as 1:1280.

Further survey as by Johnin test and cultural method are needed.

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References

PANDEY. G. S., MUSONDA. T .L., CHIZYUKA. H. G. B. & SCHNEEDI. M. (1987)Veterinary Record, 120, p369

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Table 1. Distribution of CF antibodies to M. paratuberculosis by farm

Name of farm	No. of total cow	No. of sera tested	Positive
Kaleya	238	24	7
Syringa	229	23	5
Kavu	168	16	4
Mayfield	145	16	5
Newham	179	20	10
Causeway	131	15	1
Simonga	106	13	3
Coventry	69	9	0
Kushiya	47	10	0
Kirby	19	10	1
Euraff	10	10	3
Total	1,341	166	39

Table 2. Distribution of CF antibodies of the cows grouped by age

Age (year)	Sera tested	CF antibody liter					Positive (%)
		<10	10	20	40	80	
<1	29	23	5	1	0	0	6(20.7)
2	19	14	4	0	1	0	5(26.3)
3	26	22	2	1	0	1	4(15.4)
4	26	22	3	0	0	1	4(15.4)
5	26	19	7	0	0	0	7(26.9)
>5	20	12	5	2	1	0	8(40.0)
Total	146	112	26	4	2	2	34(23.3)

VIABILITY OF *Mycobacterium bovis* IN TRADITIONALLY PROCESSED SOUR MILK AND THE PREVALENCE OF BOVINE TUBERCULOSIS IN NAMWALA DISTRICT OF ZAMBIA

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The viability of *Mycobacterium bovis* (ATCC 35747) and *M. tuberculosis* (H37Ra) in experimentally inoculated milk, which was then traditionally processed to sour milk was investigated. Lowenstein-Jensen

media (enriched with pyruvate/glycerol) was inoculated with processed inoculum made from samples of the inoculated souring milk collected at intervals of 0, 12, 24 and 72 hours after the introduction of the tubercle bacilli into the fresh milk. The pH of the raw milk reduced from pH 7.0 at the start of the experiment to pH 3.0 at the end. Viable *M. bovis* and *M. tuberculosis* were isolated throughout the experiment. A single comparative tuberculin test was carried out in 507 herds of cattle to estimate the prevalence of bovine tuberculosis in Namwala District. The overall prevalence was 12.8% (65/507) with a higher prevalence in cattle older than 25 months (13.3%) compared with the younger ones (6.3%). The results indicated that the traditional method of souring milk does not eliminate the tubercle bacilli at the optimal consumption time. The close association of the villagers and the cattle provide a conducive situation for transmission of the zoonotic tubercle bacilli in Namwala and most probably other areas of Zambia.

AEROBIC INTESTINAL AND FAECAL FLORA AMONG WILD ANIMALS UNDER NATURAL AND RANCHED CONDITIONS

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The intestinal flora is thought to be important in the maintenance of mammalian homoeostasis. The bacterial composition differs little between individuals of same species but varies between animal species and with diet. Enteral samples from wild and domestic mammals were collected during the dry season of 1995/1996. The bacterial composition was determined to obtain preliminary information on gastrointestinal flora of various animal species. Seven lechwe and 1 white rhinoceros were euthanased in Lochinvar and Livingstone National Park respectively. Samples of enteral contents were taken from the large intestines. Freshly dropped faeces of 4 zebra, 3 sable, 3 impala, 3 eland and 3 wildebeest reared in a game ranch in Choma were collected. All samples were transported by swab and cultured aerobically on trypticase soy agar and MacConkey agar. After isolation, colony appearance was recorded and presumptive identification of isolates was carried out using routine characterisation techniques. The ratio of bacterial genera differed in composition in the intestinal and faecal flora between lechwe, white rhinoceros and ranched animals. *Bacillus* spp. and *Staphylococcus* spp. and *Enterobacteriaceae* were isolated from nearly all animal species. In lechwe and white rhinoceros, under natural conditions, the predominant flora were Gram negative bacteria. Although *Providencia* and *Aeromonas* spp. were superior in number in both groups, *Enterobacteriaceae* including *Hafnia* and *Enterobacter* spp. were prevalent in lechwe, and *Alcaligenes*, *Vibrio* and *Acinetobacter* spp. were observed in white rhinoceros in the same ratio as *Enterobacteriaceae*. In ranch farmed animals, the majority of the isolates were Gram positive bacteria, (*Corynebacterium*, *Bacillus* and *Staphylococcus* spp.). Coliform bacteria were isolated from ruminants, e.g. eland, but isolated rarely from zebra.

DISTRIBUTION AND COMPOSITION OF AEROBIC BACTERIAL FLORA IN THE SMALL AND LARGE INTESTINE OF HIPPOPOTAMI (*Hippopotamus amphibious*)

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The intestinal flora of humans and animals is a micro-ecosystem formed and influenced by the characteristics of their diet and habitat. This plays an important role in the natural resistance of the gastrointestinal tract against opportunistic and infectious diseases. In order to examine and compare the distribution of bacterial species, the enteral contents of 55 hippopotami (16 males, 39 females) kept under wild conditions were collected during the dry season in 1995, from Mufuwe in South Luangwa game management area, Zambia. Throughout the culling period, samples were collected from the upper part of the small intestine and the lower part of the large intestine, using sterile swabs. Swabs were kept in Carry-Blair transport medium at room temperature until examination. Contents of the swabs were cultured on trypticase soy agar, MacConkey agar and bovine blood agar, aerobically at 37°C. Five colonies were selected from each agar plate. The isolates were characterised and assorted by a range of biophysical and biochemical tests. The ratio of Gram negative to Gram positive bacteria in the intestinal tract was 57.8% to 42.2% in males and 63.3% to 36.7% in females respectively. Similar proportions of both bacterial groups were observed in the small intestine of males, and in the small and the large intestine of females. The prevalence of Gram negative bacteria was 81.2% in the large intestine of the male. *Enterobacteriaceae* represented 50% of Gram negative isolates and 14 genera out of the known 30 genera were identified. *Providencia*, *Proteus* and *Enterobacter* spp. were the predominant genera. *Salmonella*, *Yersinia* and *Shigella* spp. represented 13.8%, 10.3% and 3.4%, respectively, of isolates in males. *Shigella* and *Yersinia* spp. represented 16.0% and 8.0% of isolates from females. Aquatic bacteria such as *Aeromonas*, *Vibrio* and *Plesiomonas* spp. make up 16.5% of the commensal population in females and 20.5% in males. In the Gram positive group, *Bacillus* spp. represented 58.4% of isolates from males and, 39.3% in females whilst *Streptococcus* spp. represented 14.6% in males and 18.2% in females.

Outbreak of anthrax among the wildlife in South Luangwa National Park, Zambia

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Abnormally numerous numbers of death of the hippopotamus, compared with those in usual dry season, attracted the attentions of the peoples engaged in the wildlife preservation on early June, 1987. Following to the hippopotamus, deaths occurred among the other species of the wild manmmals such as elephant, buffalo, giraffe, puku, kudu and so on.

The first specimen of blood smear from the hippopotamus was examined at The School of Veterinary Medicine, University of Zambia at 22nd, July and was decided as Bacillus anthracis infection.

The South Luangwa National Park is the second largest of Zambia's National Parks created in 1972, occupies 9,050 square kilometres and is one of the best known and most popular parks because of its abundant wild animals such as elephant, buffalo, hippopotamus, impara, kudu, zebra, waterbuck, warthog, baboon, velvet moky, crocodile and so on.

The number of animals died by anthrax is not clear, because ofdifficuly to find out all animals in very wide area and also difficulty of clarification of the causative agents of all died animals, however the number of wild animals died by anthrax is roughly estimated more than one thousand.

It is said that there is no record of incidence of anthrax in this national park until now. Then the infections source is still obscure.

It was reported that one of the earliest record of anthrax in wild animal was Livingstone in 1850 in South Africa where he encountered a disease which affected antelope. It was since been reported from a wide variety of wild animals in several parts of Africa.

In South Africa, sporadic outbreaks were recorded in zebra, sprin?buck, hartebeest, kudu and so on. In 1959 and 1960, over a thousand fatal cases were detected in Kruger National Park. At that time, anthrax also occurred in wildlife in other area of South Africa. The wild animals involved were baboon, civet, lion, cheetah, leopord, ~~animals~~ hyena, elephant, hippopotamus, giraffe, warhog and so on (L.P.E. Chquete and Elir Broughton:Infectious Disease of Wild Life, 2nd Ed., Iowa State University Press, 1981, pp.288).

The present outbreak of anthrax in The South Luangwa National Park is as follows:

4. Number of animals and species which are diagnosed bacteriologically.

As listed in the table, total 26 specimens(10 species and 1 unknown) of the wildlife were brought into the laboratory during from August to October, 1987. Almost all specimens except 3 (1·buffalo, 1 elephant and 1 leopard) were the blood smear. Raw materials were 2 blood specimens from buffalo and elephant and 1 tissue organs from leopard.

Twenty two out of 26 specimens were microscopically diagnosed as anthrax. Cultivation and mice inoculation tests were carried out on the 3 raw materials and Bacillus anthracis was isolated from 1 buffalo and 1 elephant. The tissue organs of the leopard was already decayed and mice inoculation test showed negative result .

5. Isolation experiments of B.anthracis from the environment.

The experiments were carried on to know the contamination of the environments by the spores of B.anthracis in the areas where the outbreak of anthrax had occured .

A. Trial to demonstrate the spore of Bacillus anthracis from the soil samples

a. Specimens

On 29th October, 1987, the authors collected each about hundred grams of the soil samples at 7 points, 2 meters apart each on 4 directions(east, west, north and south) around 3 dead or burnt animals(buffalo, giraffe and elephant) that were suspected anthrax, though they were not examined bacteriologically in The South Luangwa National Park. Thus they collected total 84 specimens of the soil around the dead animals.

b Method of demonstration

About 50 grams of the soil were suspended into the sterile distilled water, mixing well and left it overnight in the cold room(5 C) overnight. Afterwards soil suspensions were subjected to heat treatment to destroy non-sporing bacteria at 80 C for 1 hour.

Then the supernant fluid was centrifuged at 2,500-3,000 for 20-30 minutes and resulting deposits were suspended in small volume of the water, and they were cultivated on nutrient agar at 37 C for 24 hours and also mice inoculation tests were carried out on all soil samples.

C. Result

Detection of Bacillus anthracis resulted in ~~failure~~ negative 84 in all soil samples.

B. Experiment to isolate the spore of B.anthracis from the water.

1. Isolation experiment from the water samples

Water samples were collected from the selected 12 points as indicated in the table.

B.anthracis was isolated from only one sample collected at the place where one baffalo's carcass was found.

Method of isolation of B.anthracis:

Five hundred ml of water sample--Heat treatment at 80 C for 30 minutes--Centrifugation at 3,000 r.p.m. for 30 minutes--Deposit--Suspendd into small volume of physiological saline---Cultivation and mice inoculation tests

2. Isolation experiment from the intestinal content of the catfishes

Four intestinal contents of the catfishes captured at Luangafura, Chinzombo Lagoon, Mufue Lodge River and Luangwa River were examined.

Method of isolation of B.ahthracis.

Intestinal contents---Suspend in physiological saline---Heat treatment at 80 C for 30 minutes---Filtration---Centrifugation at 3,000 r.p.m. for 30 minutes---Sediment---Resuspend in small amount of physiological saline---Cultivation and mice inoculation tests.

Results:

B.anthracis could not be demonstrated by direct cultivation and mice inoculation test.

3. Isolation experiment from the leaves and braches near the carcasses.

Many flies soon crowded on the carcasses. Then, their mouth and legs may be with the organisms if the animals died by anthrax. When the animals ar men gain access to the carcass, these flies move from the carcass to the leaves of the plants and branches of the tree near the carcass, and contaminate them with B. anthracis

Then, isolation experiment was conducted on the specimens from 3 points as described bellow:

1. Specimens collected near the carcass of the hippopotamus(Mufue)
2. Specimens collected near the carcass of giraff(Mufue)
3. Specimens collected near the carcass of elephant(Chimzombo)

Method of Isolation of B.anthracis.

Suspend the leaves and branches in physiological saline---Left overnight at room temperature---Centrifugation at 3,000 r.p.m. for 30 minutes---Deposit---Suspend in small amount of physiological saline---Cultivation and mice inoculation tests.

Results:

B.anthracis was not detected at all.

Despite the such large-scale experiments (cultivation and mice inoculation tests) on soil (84 specimens), water (12 specimens, 4 intestinal contents of the catfish and many leaves and small branches near the carcasses at 3 points (1 ... Hippopotamus, 1 ... giraffe and 1 elephant), Bacillus anthracis was isolated from only one water sample collected near the dead buffalo. However it does not mean that the environments of South Luangwa National Park are not so contaminated by Bacillus anthracis.