Detection of *Salmonella* Gallinarum and *S.* Typhimurium DNA in Experimentally Infected Chicks by Polymerase Chain Reaction

Lawrence M. TUCHILI*, Hiroshi KODAMA**, Yoko IZUMOTO, Masafumi MUKAMOTO, Tsuneo FUKATA[1], and Tsuyoshi BABA

Laboratory of Veterinary Immunology and [1]Laboratory of Veterinary Internal Medicine, Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, Sakai 593, Osaka, Japan

(Recieved 12 July 1994/Accepted 12 October 1994)

ABSTRACT. DNA detection with polymerase chain reaction (PCR) as a mean of identifying *Salmonella* infection in chickens was compared with the conventional culture procedure. DNA was extracted from organs of experimentally infected chicks with either *S.* Gallinarum or *S.* Typhimurium. The pair of primers used were those directed at the *InvA* gene. Bacteria isolation was done by inoculating the pre-enrichment media with samples. As was expected a 284 bp fragment DNA was amplified from extracted DNA of infected organs by PCR. The results of our studies indicate that the PCR method is more sensitive than the conventional culture procedure since we were able to detect both *S.* Gallinarum and *S.* Typhimurium DNA not only in samples positive for bacteria isolation but also in negative samples. It was possible to detect *Salmonella* DNA in 15 out of 20 organ samples from chicks infected with *S.* Gallinarum 21 hr after infection, but, only five were positive for bacteria isolation. *Salmonella* DNA was detected throughout the entire test period. The results of this study confirm that PCR is a useful tool for the detection of *Salmonella* infection in poultry.—KEY WORDS: DNA, *InvA* gene, polymerase chain reaction (PCR), *Salmonella* Gallinarum, *Salmonella* Typhimurium.

J. Vet. Med. Sci. 57(1): 59–63, 1995

For many years the test of choice in serological diagnosis of pullorum disease and fowl typhoid has been the slide agglutination which was originally developed by Runnels *et al.* [17] for use with serum and adapted by Schaffer *et al.* [19] for whole blood by using stained antigen. This test can, however, sometimes yield erratic results which may be dependent on the pseudo-positive reaction with *S.* Javiana or *S.* Typhimurium infection. The agglutination test has also been found not to be applicable for the detection of the bacteria when used with eggs [1]. In recent years there has been an increasing application of other tests such as latex agglutination [14], enzyme-linked immunosorbent assay [1, 6] and DNA hybridization test [9]. Many of these tests are very rapid but have also been found to suffer from a lack of specificity, a factor which has limited their acceptance as reported by Rahn *et al.* [16]. Detection of *Salmonella* from poultry has also been made difficult by the indiscriminate use of antibiotics by some farmers, especially in many developing countries [8]. Immune response to infection with *Salmonella* in young birds has also been reported to be low [21].

Polymerase chain reaction (PCR) has been shown to offer a new strategy in the detection of *Salmonella* [5, 16, 22]. Rahn *et al.* [16] used the PCR method to detect all but four of 630 *Salmonella* strains of 112 serovars with 99.4% sensitivity by using the *Inv* gene sequence as a primer. The PCR could therefore be used to detect all *Salmonella* species in chickens even though the agglutination test cannot be applied to paratyphoid infection. The PCR has also been used to detect low numbers of enterotoxigenic *Escherichia coli* (3 colony-forming units in 25 g of minced meat) [20] and *Campylobacter* (30 to 60 bacteria per PCR assay) [15]. Experimentally, *Salmonella* DNA could be amplified when one colony forming unit of bacteria was contained in 1 g of fecal sample [5]. PCR, however, has not been used to detect *Salmonella* from poultry clinical specimens. It has been reported that it was not successful in amplifying *Salmonella* DNA directly from chicken litter and carcasses [16]. It is for this reason that our approach was to detect *S.* Gallinarum and *S.* Typhimurium from organs of experimentally infected chicks by PCR and to compare the results with the isolation method since these *Salmonella*, known as fowl typhoid and paratyphoid, respectively, cause serious problems in the poultry industry throughout the world. To be able to do this, a pair of primers directed at the *InvA* gene [10, 11, 16] were used to amplify the DNA extracted from chick organs.

* PRESENT ADDRESS: TUCHILI, L. M., Department of Disease Control, School of Veterinary Medicine, Zambia University, Lusaka, Zambia.

** CORRESPONDENCE TO: KODAMA, H., Laboratory of Veterinary Immunology, Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, Sakai 593, Osaka, Japan.

MATERIALS AND METHODS

Bacterial strains: A field strain of *S.* Gallinarum (GTZ-3) was isolated from affected chicks in Zambia while that of *S.* Typhimurium (L-55) came from the National Institute of Animal Health, Tsukuba, Ibaraki, Japan. The strains were stored on Dorset's egg slopes (Nissui Pharmaceutical Co., Tokyo, Japan) and maintained at 4°C until needed. The bacterial count was determined by inoculating 20 μl of a 10-fold dilution of bacterial suspension on Heart Infusion agar (Nissui Pharmaceutical Co.). Plates were cultivated at 37°C overnight and colonies were counted.

Experimental design and specimen collection: One-day-old chicks were purchased from a commercial hatchery and were kept in three groups. Feed and water were given *ad libitum*. Experimental infection was done orally with group A (total 15 chicks) receiving 1×10^2 colony-forming units (CFU) and group B (16 chicks) receiving 1×10^4 CFU of *S.* Gallinarum inoculum at two days of age, and group C (11 chicks) receiving 1×10^7 CFU *S.* Typhimurium at four days of age. The amount of inoculum in all cases was 0.5 *ml*. Uninoculated chicks were kept as the control.

Specimens (kidney, spleen, liver, heart and intestine) were aseptically collected in duplicate, a set for DNA extraction and another set for bacteria isolation. One set of collected specimens were placed in sterile petri dishes and frozen at −81°C until required while another set of samples were used to inoculate pre-enrichment media (selenite brilliant green (SBG), Nissui Pharmaceutical Co.) after mincing with scissors and incubated at 41°C overnight. They were later subcultured on brilliant green (BG) agar (Nissui Pharmaceutical Co.) and incubated at 37°C overnight. Identification of colonies was done by biological test and with *Salmonella* antisera (Denka Seiken Co., Tokyo, Japan).

DNA extraction from chicken organs: Grinding frozen chick organ tissues to a fine powder was required before DNA extraction. Briefly, mortars and pestles were kept at −81°C for 30 min before they were used. A frozen sample was then placed in the mortar and quickly ground with a pestle. A 2 *ml* of sterile water was added to the fine powder and then mixed well. The pH which was often low was corrected to 7.8. Then 1.5 *ml* of the organ homogenate was transferred to an Eppendorf tube. Centrifugation was done at 15,000 rpm for 10 min in a high speed micro centrifuge (TM-150; Tomy Seiko Co., Tokyo, Japan). The resultant cell pellets were then washed in TE buffer (10 mM Tris, 2 mM EDTA, pH 8.0) [12]. DNA extraction was done by the method described by Crichton *et al.* [7].

Oligonucleotide primers and PCR procedure: The method was basically the same as that described by Rahn *et al.* [16] except that 5 μl of extracted DNA was used instead of a single colony. The pair of primers 5'-GTG AAA TTA TCG CCA CGT TCG GGC AA-3' and 5'-TCA TCG CAC CGT CAA AGG AAC C-3' were supplied by Nippon Gene Co., Tokyo, Japan. The 25 μl mixture contained 50 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.25 mM $MgCl_2$, 100 μM each of dATP, dGTP, dCTP, dTTP (Wako Pure Chemical Industries, Osaka, Japan), 0.075 μM of each primer and 0.625 units of *Taq* DNA polymerase (Wako Pure Chemical Industries). The reaction mixture were overlaid with two drops of mineral oil (Sigma, St. Louis, MO) to prevent evaporation. A control tube contained only the PCR mixture without DNA. The PCR incubations were performed by the ASTEC System PC-700 (Astec Co., Shimen, Fukuoka, Japan). The cycle times were the same as those described by Rahn *et al.* [16] with initial incubation at 72°C for 7 min followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 53°C for 2 min and primer extension at 72°C for 3 min. Following the last cycle there was 7 min incubation at 72°C. The tubes were then kept at 4°C. A 15 μl aliquot of the PCR reaction mixture was electrophoresed on 2% agarose gel (Wako Pure Chemical Industries) and then DNA bands were stained with ethidium bromide.

For screening of the *InvA* sequence of two *Salmonella* strains, colonies on MacConkey agar (Nissui Pharmaceutical Co.) were picked up and subjected to specific DNA amplification without DNA extraction by a single colony inoculation method described by Saris *et al.* [18].

DNA extraction limit: A 10-fold serial dilution of *S.* Gallinarum was prepared in Luria-Bertani broth (10 g tryptone, 5 g yeast extract, 10 g NaCl in 1*l* water, pH 7.0) to determine the limit of extraction of DNA and isolation of bacteria from experimentally infected chicks. A volume of 0.2 *ml* of each dilution was added to 1.8 *ml* of liver homogenate. Extraction of DNA was done as described before, and subjected to PCR. Equal amounts of the homogenates mixed with serial dilutions of the bacteria were used to inoculate the SBG broth. After incubation at 41°C overnight the growth of the bacteria was determined by inoculating BG agar with samples as described before.

RESULTS

Screening for the InvA gene: With a pair of primers targeted at this gene we were able to amplify both *S.* Gallinarum and *S.* Typhimurium DNA. As expected a 284 bp DNA fragment was amplified when a single colony was applied to PCR (Fig. 1, lane 2). The sizes of the amplified DNA bands from organs (collected from chicks inoculated with 1×10^4 CFU *S.* Gallinarum, lanes 6 to 14) were the same as that obtianed by the single colony method (lane 2). The nucleotide sequence of the amplified DNA confirmed that the DNA fragment was same as that reported by Galan *et al.* [11] to have been successfully amplified (data not shown).

Detection of S. Gallinarum and S. Typhimurium: The study was aimed at detecting both *Salmonella* strains from experimentally infected chicks by means of the PCR. The results in Table 1 were obtained from group A chicks inoculated with 1×10^2 *S.* Gallinarum. The data show that the PCR was sensitive in detecting *Salmonella* since we were able to amplify *Salmonella* DNA from 15 out of 20 samples tested 21 hr after infection. The isolation of *S.* Gallinarum with pre-enriched media, on the other hand, was possible in only five cases out of 20. The PCR still appeared to be more sensitive even in later stages of infection as we could detect *Salmonella* DNA from samples which were negative for bacteria isolation on three, seven and 14 days after infection. *Salmonella* DNA was not detected in two organs even when they were positive on isolation. An increase in the dosage of 1×10^4 with *S.* Gallinarum (group B, total 16 chicks examined, data not shown) resulted in similar results to those obtained in group A, namely, DNA was detected in 16 out of 20 samples by PCR 21 hr after infection whereas five

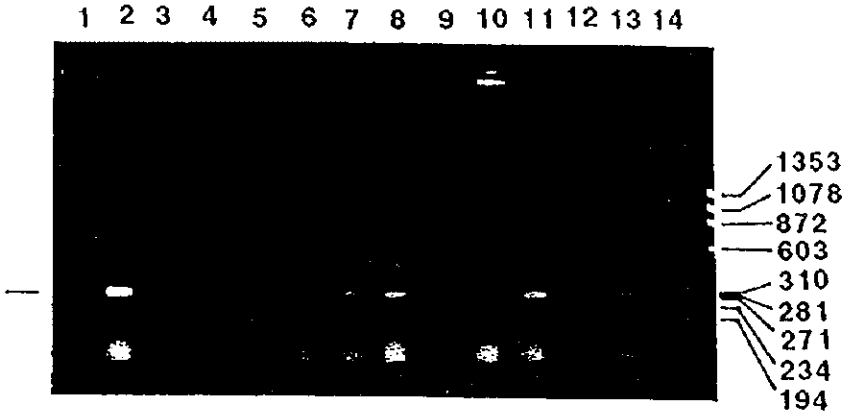

Fig. 1. PCR amplified products from *Salmonella* Gallinarum colony and organs from chicks inoculated with 1×10^4 *S.* Gallinarum (group B). Amplified DNA bands from liver (lane 6), kidney (7), spleen (8) and heart (9), and negative amplification in intestine (lane 5) of chick No. 33 three days after inoculation. Lanes 10 to 14 show DNA bands (chick No. 31) from the same organs as No. 33. Negative amplification in kidney (lane 3) and intesitne (4) of chick No. 34 is also shown. Lanes 2 and 1 show positive (DNA amplified from single colony) and negative (PCR mixture without extracted DNA) controls, respectively.

Table 1. Isolation of *Salmonella* Gallinarum and detection of *Salmonella* DNA from organs of experimentally infected chicks

Chick No.	Time after infection[a)]	Heart		Kidney		Liver		Spleen		Intestine	
		Isol	PCR	Isol	PCR	Isol	PCR	Isol	PCR	Isol	PCR
1	21 hr	+	−	−	+	−	+	+	+	−	+
2		+	+	−	+	−	−	−	+	+	+
3		−	+	+	+	−	+	−	−	−	+
4		−	+	−	+	−	−	−	+	−	−
5	3 d	−	+	−	+	−	−	−	+	+	+
6		−	+	−	−	+	+	−	+	−	−
7		−	+	−	−	+	+	−	−	−	−
8		−	−	−	+	−	+	−	−	−	−
9	7 d	+	+	+	+	+	+	+	+	+	+
10		+	+	+	+	+	+	+	+	+	−
11		−	+	−	+	+	+	+	+	−	+
12		−	−	−	−	−	−	−	−	−	−
13	14 d	−	+	−	+	−	+	+	+	−	+
14		−	−	−	+	−	−	−	+	−	+
15		−	−	−	+	−	+	−	−	−	−

a) Two-day-old chicks were inoculated orally with 1×10^2 CFU of *S.* Gallinarum GTZ-3 strain.

positives were obtained on bacterial isolation. None of the organs from uninoculated control chicks resulted in specific DNA amplification.

In the case of infection with *S.* Typhimurium in a total of 11 chicks, once again the PCR was more sensitive, as we could detect *Salmonella* DNA from 17 samples out of 20 organs tested just 21 hr after infection (Table 2). Detection of the bacteria by isolation during the same period was positive in 11 samples. The large number of isolations appeared to be due to the large numbers of bacteria with which the chicks were inoculated.

DNA extraction limit: Extraction of *S.* Gallinarum DNA was only possible when the number of bacteria exceeded 1×10^3 CFU/*ml* in the organ homogenate. At this concentration of bacteria, it was possible to extract *Salmonella* DNA which could be amplified by PCR and visualized in 2% agarose. On the other hand, the limit of bacteria isolation was found to be 1×10^2 CFU/*ml*.

Table 2. Isolation of *Salmonella* Typhimurium and detection of *Salmonella* DNA from organs of experimentally infected chicks

Chick No.	Time after infection[a)]	Heart		Kidney		Liver		Spleen		Intestine	
		Isol	PCR	Isol	PCR	Isol	PCR	Isol	PCR	Isol	PCR
16	21 hr	+	+	+	+	+	−	+	+	−	+
17		−	+	+	+	+	+	−	+	−	+
18		−	+	+	+	+	+	+	+	−	−
19		−	+	+	+	+	+	−	−	−	+
20	4 d	+	+	+	+	−	+	+	+	+	+
21		−	+	−	+	−	+	−	+	+	+
22		+	+	−	+	+	+	−	−	−	−
23		−	−	−	+	−	+	−	−	−	−
24	7 d	−	−	+	−	+	+	+	+	+	+
25		−	+	−	−	+	ND	−	−	−	+
26		−	−	+	−	+	−	−	−	−	+

a) Four-day-old chicks were inoculated orally with 1×10^7 CFU of *S.* Typhimurium L55 strain.

DISCUSSION

The emphasis in this study was to apply the PCR with the *InvA* sequence gene to detect *S.* Gallinarum and *S.* Typhimurium DNA from experimentally infected chicks. With a pair of primers directed at the *InvA* gene we were able to detect the DNA of both strains in about 70% of the tested samples. The size and nucleotide sequence of the amplified DNA were in agreement with those reported by Rahn *et al.* [16] and Galan *et al.* [11], respectively, as we were able to amplify a 284 bp fragment from the extracted DNA. The specificity of the test has been demonstrated as none of the non-*Salmonella* strains including total 49 species, 142 strains of gram negative and positive bacteria yielded the specific amplification product [16]. Our experience in this study showed that DNA extraction improved when the pH of the organ homogenate was corrected to 7.8. There were a few exceptions in which some positive cases on bacteria isolation were negative in PCR amplification. This could possibly be attributed to the loss of DNA during extraction procedures.

The PCR was more sensitive than bacteria isolation with pre-enrichment media both in the early and late stages of infection as we could detect *Salmonella* DNA from samples in which a substantial number of bacteria could have been reduced by the defense mechanisms of the chick during the course of infection. The low level of bacteria isolation in the late stages of infection possibly indicates that isolation of *Salmonella* from chronically infected chickens even when pre-enrichment media is used may not be very reliable especially in antibiotics-treated chickens. PCR is therefore a very useful method to detect latent infection by microorganisms. Boyle and Blackwell [4] reported that herpesvirus DNA was successfully amplified in fish with latent infection.

The *Salmonella* DNA amplified in the present study was derived from *Salmonella* bacterial cells present in the experimentally infected organs. The discrepancy between the DNA amplification and bacteria isolation detection limits is still difficult to explain, but it could possibly be due to the fact that a number of bacteria are killed by the host defense during the course of infection but dead cells remained in tissues. DNA could also be amplified from the dead cells before it is rapidly degraded in host tissues by DNAse. Since it has been reported that less than 0.1 pg of DNA was detectable in fish specimen when the PCR was used [4], this would lead to higher frequency of the detection of *Salmonella* DNA but poor isolation of bacteria from specimens in the present study.

We also tried to amplify *Salmonella* DNA directly from chick organ homogenate but no specific DNA was detected. This result is also supported by earlier results of Rahn *et al.* [16] who failed to amplify *Salmonella* DNA directly from chicken litter and chicken carcass rinse samples, though these results are not in agreement with that of Cohen *et al.* [5]. PCR has been used to detect many infectious agents [2, 4, 5, 15, 16, 18, 20, 22], but its application detecting infectious agents from clinical specimens has not been widely used, and this is partly because of the supposedly presence of certain inhibitors that have been found to affect the performance of the PCR. Haemoglobin [13] and heparin [3] have been cited as inhibitors of the PCR reaction. Extractin of DNA from organs therefore seemed to be essential.

Our results confirm that the *InvA* gene of *Salmonella* could be used to detect *Salmonella* from clinical specimens if the DNA extraction procedure is used. The PCR method has been found to be valuable in detecting *S.* Enteritidis from feces [5]. Probably what is significant about this study is the fact that *Salmonella* DNA was detected from organs of experimentally infected chicks 21 hr after the infection even when bacteria could not be isolated. It now remains to be determined whether the same results could be obtained from naturally infected chicks, especially infections due to *S.* Enteritidis which is of great public concern throughout the world.

ACKNOWLEDGEMENTS. We sincerely thank the Japan International Co-operation Agency (JICA) which financially supported this research work. Thanks also go to Dr. M. Komori for his valuable suggestions and discussions in performing PCR, and Dr. K. Kuroda and Dr. H. Tsuruoka for nucleotide sequencing of amplified DNA.

REFERENCES

1. Barrow, P. A., Bechieri Jr., A., and Haddad, O. A. 1992. Serological response of chickens to infection with *Salmonella gallinarum - S. pullorum* detected by enzyme-linked immunosorbent assay. *Avian Dis.* 36: 227–236.
2. Bej, A. K., Mahbubani, M. H., Miller, R., Dicesare, J. L., Haff, L., and Atlas, R. M. 1990. Multiplex PCR amplification and immobilized capture probes for detection of bacterial pathogens and indicators in water. *Molecular Cellular Probes* 4: 353–365.
3. Beutler, E., Gelbart, T., and Kuhl, W. 1990. Interference of heparin with polymerase chain reaction. *Bio Techniques* 9: 166.
4. Boyle, J. and Blackwell, J. 1991. Use of polymerase chain reaction to detect latent channel catfish virus. *Am. J. Vet. Res.* 52: 1965–1968.
5. Cohen, N. D., Mcgruder, E. D., Neibergs, H. L., Behle, R. W., Wallis, D. E., and Hargis, B. M. 1994. Detection of *Salmonella enteritidis* in feces from poultry using booster polymerase chain reaction and oligonucleotide primers specific for all members of the genus *Salmonella*. *Poult. Sci.* 73: 354–357.
6. Cooper, G. L., Nicholas, R. A. J., and Bracewell, C. D. 1989. Serological and bacteriological investigation of chickens from flocks naturally infected with *Salmonella enteritidis*. *Vet. Rec.* 125: 567–572.
7. Crichton, P. B., Yakubu, D. E., Old, D. C., and Clegg, S. 1989. Immunological and genetical relatedness of type-1 and type-2 fimbriae in salmonellas of serotypes Gallinarum, Pullorum and Typhimurium. *J. Appl. Bacteriol.* 67: 283–291.
8. Falade, S., Sato, G., Ulaya, W., and Mwanza, L. 1989. Serovars and antibiotic sensitivity patterns of *Salmonella* strains isolated from domestic animals in Zambia. *Zimbabwe Vet. J.* 20: 19–22.
9. Flowers, R. S., Mozola, M. A., Curiale, M. S., Gabis, D. A., and Silliker, J. H. 1987. Comparative study of a DNA hybridization method and conventional culture procedure for detection of *Salmonella* in foods. *J. Food Sci.* 52: 781–785.
10. Galan, J. E. and Curtis, R. III. 1989. Cloning and molecular characterization of genes whose products allow *Salmonella typhimurium* to penetrate tissue culture cells. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6383–6387.
11. Galan, J. E., Ginocchio, C., and Costeas, P. 1992. Molecular and functional characterization of the *Salmonella* invasion gene *InvA*: Homology of InvA to members of a new protein family. *J. Bacteriol.* 174: 4338–4349.
12. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1989. Analysis and cloning of eukaryotic genomic DNA. pp. 9.1–9.62. *In*: Molecular Cloning. A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
13. Mercier, B., Gaucher, C., Feugeas, O., and Mazurier, C. 1990. Direct PCR from whole blood without DNA extraction. *Nucleic Acids Res.* 18: 5908.
14. Metzler, J. and Nachamkin, I. 1988. Evaluation of a latex agglutination test for the detection of *Salmonella* and *Shigella* spp. by using broth enrichment. *J. Clin. Microbiol.* 26: 2501–2504.
15. Oyofo, B. A., Thornton, S. A., Burr, D. H., Trust, T. J., Pavlovskis, O. R., and Guerry, P. 1992. Specific detection of *Campylobacter jejuni* and *Campylobacter coli* by using polymerase chain reaction. *J. Clin. Microbiol.* 30: 2613–2619.
16. Rahn, K., De Grandis, S. A., Clarke, R. C., McEwen, S. A., Galan, J. E., Ginocchio, C., Curtiss, R., and Gyles, C. L. 1992. Amplification of an *InvA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular Cellular Probes* 6: 271–279.
17. Runnels, R. A., Coon, C. J., Farley, H., and Thorp, F. 1927. An application of the rapid-method agglutination test to the diagnosis of bacillary white diarrhea infection. *J. Am. Vet. Med. Assoc.* 70: 660–667.
18. Saris, P. E. J., Paulin, L. G., and Uhlen, M. 1990. Direct amplification of DNA from colonies of *Bacillus subtilis* and *Escherichia coli* by the polymerase chain reaction. *J. Microbiol. Methods* 11: 121–126.
19. Schaffer, J. M., MacDonald, A. D., Hall, W. J., and Bunye, H. 1931. A stained antigen for the rapid whole blood test for pullorum disease. *J. Am. Vet. Med. Assoc.* 79: 236–240.
20. Wernars, K., Delfgou, E., Soentoro, P. S., and Notermans, S. 1991. Successful approach for detection of low numbers of enterotoxigenic *Escherichia coli* in minced meat by using the polymerase chain reaction. *Appl. Env. Microbiol.* 7: 1914–1915.
21. Williams, J. E. and Whittemore, A. D. 1975. Influence of age on the serological response of chickens to *Salmonella typhimurium* infection. *Avian Dis.* 19: 745–760.
22. Widjojoatmodjo, M. N., Fluit, A. C., Torensma, R., Keller, B. H. I., and Verhoef, J. 1991. Evaluation of the magnetic immuno PCR assay for rapid detection of *Salmonella*. *Eur. J. Clin. Microbiol. Infect. Dis.* 10: 935–938.

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 avian infectious diseases in a small village in Zambia

M. Hasegawa (Japanese Overseas Co-operation Volunteer), Department of Disease Control , School of Veterinary Medicine, University of Zambia, PO Box 32379, Lusaka

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

each other. It is important to understand the disease status of village chickens for the development of the poultry industry in Zambia. Further investigations on village chickens should be carried out in other areas of Zambia.

TABLE 1 : The antibody rates to avian disease agents in Shingoma village.

ND	IBD	*S. pullorum*	*M. gallisepticum*	*M. synoviae*
10/10	17/18	0/18	18/18	18/18
(100.0)	(94.4)	(0.0)	(100.0)	(100.0)

* Number of positive samples / number of samples examined. (percentage)

A five-year summary of diseases of chickens diag-nosed in the School of Veterinary Medicine at the University of Zambia, Lusaka.

M. Hirai[1], E. Baba[2], G. S. Pandey[3]

[1]M. Hirai (Japan Overseas Cooperative Volunteer), [2]E. Baba (Japan International Cooperation Agency),
[3]G. S. Pandey, Department of Disease Control, School of Veterinary Medicine, The University of Zambia, PO Box 32379, Lusaka, Zambia

Few reports on occurrences of poultry diseases from developing countries are available. The School of Veterinary Medicine in the University of Zambia which was founded in 1985 has been providing diagnostic services to veterinarians and farmers who submit broiler and layer chicken carcases for necropsy. The diagnoses during the last five years (1989-93) have been summarised.

Live or dead chickens of both broiler and layer types were submitted for diagnosis in 1989 to 1993. The diagnosis records were retrieved according to the numbers of outbreaks regardless of the number of chickens submitted at each outbreak (Table 1). Many submissions included two to five chickens as specimens. The presenting history of the poultry farm was recorded, and the chickens were examined pathologically, microbiologically, and parasitologically.

A normal scale poultry farm rears several hundred birds for broilers, breeders or layers in the Lusaka area. Only a small number of big commercial farms keep several thousands of birds. Transportation is not always convenient even for big farms, such that chickens must have been submitted for diagnosis to the Diagnostic Laboratory only when farmers were suffering from severe problems such as high mortality. Many latent cases of small outbreaks might not have been considered.

Colibacillosis has been described as one of the opportunistic infections which cause few large-scale outbreaks, nevertheless an increasing number of colibacillosis cases is a sign that the management and environment of farms in this area might not have been improved. In the present survey, concurrent infection with *E. coli* and *Mycoplasma spp.* was not obvious. Colibacillosis cases did not always accompany respiratory disorders or omphalitis. Since the relationship between serotype and pathogenicity have been described (Gross 1984), an investigation on serotyping *E. coli* isolates is suggested in order to understand the epidemiological situation in this country.

Cases of Salmonella infections have tended to increase in number. In a serological survey of parent stocks in local hatcheries it was noticed that a number were contaminated with *Salmonella gallinarum* (unpublished). *S gallinarum* rather than *S pullorum* was isolated and classified according to its biological properties. Despite the importance of agricultural policy against fowl typhoid the incidence of the disease tends to increase. The initial approach to prevent an outbreak should be education of farmers to understand that economic losses due to fowl typhoid are greater than the expense of disease control. Even in cases where half of introduced chicks were killed by salmonellosis, the local farmers try to treat them with antibiotics to attempt to recover financial losses.

Large outbreaks of Mycoplasma infections in 1991 brought severe disaster to farmers. The serum samples collected were immunologically positive for *M. gallisepticum*. Good management might have minimized the outbreaks.

No severe outbreaks of viral infections were observed during the 5 year period. Almost all commercial farms have been using good vaccination programmes against the important viral diseases. The inconvenience of communicating between farms helps to minimize epidemics of lethal viral diseases.

Other sporadic diseases such as coccidiosis, parasitic diseases, or fungal infections are rarely submitted from farms where low levels of the outbreaks exist.

AVIAN DISEASES 38:201-202, 1994

Case Report—

Bilateral Duplication of the Ceca in a Domestic Chicken

G. S. Pandey, K. Kobayashi, H. Hirai, and M. M. Musonda

School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia

Received 30 June 1993

SUMMARY. Bilateral duplication of the ceca in an adult chicken was observed. One characteristic in addition to the duplication was excessive enlargement of one cecum, which contained 336 grams of cecal contents. Undeveloped and developed eggs were also retained in the oviduct because of oviductal atresia.

There are few reports of anomalies in domestic fowl (1,2,3,5). Most studies of the incidence of abnormalities in domestic fowl have been assessments of case collections. This paper reports a rare congenital abnormality of the ceca in a domestic chicken, unreported before in the literature, together with atresia of the oviduct and retention of undeveloped and developed eggs in the oviduct.

RESUMEN. *Reporte de Caso*—Duplicación bilateral del ciego en un ave doméstica.

Se observó la duplicación bilateral del ciego en un pollo adulto. Además de la duplicación, se observó un aumento excesivo del ciego que contenía 336 gramos de contenido cecal. Debido a la atresia del oviducto, hubo retención de huevos formados y en proceso de formación.

CASE REPORT

On March 2, 1993, four white leghorn layers aged 26 weeks were submitted to our laboratory with mild symptoms of swollen head and eyes. Other than exhibiting the above symptoms, the birds apparently looked healthy and would eat and drink normally. Infectious coryza was suspected. These birds originated from a commercial broiler breeder farm which supplies day-old chicks to the broiler industry. Blood was collected to test for antibodies against salmonella and mycoplasma; results were negative.

The birds were sacrificed for detailed postmortem examination. One bird had cecal abnormality and retention of eggs. In addition to complete bilateral duplication of the ceca with full functional development, the right cecum was excessively enlarged to a small ball size containing 336 grams of cecal contents (Fig. 1). Histologic examination of this enlarged cecum revealed that the outer layers of the tunica muscularis were much thicker than normal. This was probably to accommodate the excessive cecal content, which was rougher and granular. On gross and histologic examination, the three other ceca resembled a normal cecum in cecal content, development, size, and function.

Aplasia of the left cecum of the chicken has been reported (2). Grewal *et al.* (4) reported a single cecal tube, but the apex was divided into two equal blind parts. A unilateral cecum has also been observed in a 12-day-old chick (5). We want to put on record this unusual congenital finding, not reported in the literature to our knowledge. Many undeveloped and three fully developed eggs were retained: one was found in the abdomen, and two others were in the oviduct. It was not possible to ascertain whether the egg escaped during necropsy because of cutting or rupture of the oviduct. The wall of the oviduct at the posterior end was very thin. Atresia of the oviduct has been reported before, and it is attributed to sub-lethal factors in hens (1). The authors surmise that the two anomalies could be coincidental. However, the possible constant pressure exerted by the additional and excessively enlarged cecum on the posterior part of oviduct, contributing to retention of eggs and atresia of the oviduct, is difficult to rule out.

REFERENCES

1. Finne, I., and N. Vike. A new sub-lethal factor in hens. Poult. Sci. 30:455-465. 1951.

2. Grewal, G. S., B. Singh, and P. S. Sahota. Con-

Fig. 1. Bilateral cecal duplication. One of the ceca (C) is excessively enlarged, and three eggs (E) were removed from the oviduct.

genital anomalies of domestic fowl: seven cases. Avian Dis. 20:581–586. 1976.

3. Grewal, G. S., B. Singh, and R. Kumar. Cystic pancreas in a broiler chick. Avian Dis. 21:710–711. 1977.

4. Grewal, G. S., G. S. Khatra, and B. C. Deka. Two congenital abnormalities in domestic chickens. Avian Dis. 24:504–506. 1980.

5. Tripathy, D. N., A. R. Smith, and L. E. Hanson. Bifunctional oviduct in an adult hen and unilateral cecum in a young chicken. Avian Dis. 25:758–760. 1981.

ACKNOWLEDGMENTS

The authors are grateful to Prof. C. E. A. Lovelace, Dean of the School, for her encouragement and facilities provided.

An Occurrence of Stomach Impaction in Ostriches (*Struthio camelus*) on a Farm in Zambia Associated with High Mortality

Yoshihiko SATO, Jun YASUDA[1], Henry SINSUNGWE[1], Henry CHIMANA[1], and Gihei SATO[1]
Nagano Livestock Hygiene Service Center, 1993 Amori, Nagano 380, Japan and [1]Department of Disease Control, School of Veterinary Medicine, University of Zambia, P. O. Box 32379, Lusaka, Zambia
(Received 24 August 1993/Accepted 26 March 1994)

ABSTRACT. Fifty-one ostriches (*Struthio camelus*), 6 weeks old, were imported from Namibia and introduced onto a farm in Zambia. Soon after introduction, most of the birds manifested clinical symptoms such as anorexia and diarrhea and 19 birds died within 1 week. The proventriculus and gizzard in the 4 dead birds were full of solid masses of lucerne hay mixed with maize and consequently the gizzard was extremely impacted by them. Neither pathogenic bacteria nor parasites were detected from the autopsied birds and 8 fecal samples. After diagnosis, the ostrich feed was improved and a demulcent was given immediately; then all ostriches that remained on the farm recovered. In conclusion, these data confirmed that the present case was stomach impaction in ostriches.—KEY WORDS: impaction, ostrich, Zambia.

J. Vet. Med. Sci. 56(4): 783–784, 1994

The ostrich (*Struthio camelus*) is one of the biggest birds in the world and is naturally distributed in some African countries. Recently, many ostrich farms have been established in the southern area of Africa because of huge international demand for ostrich products such as fine hide, low-cholesterol meat, large eggs, and beautiful feathers [10]. In Zambia, 4 commercial ostrich farms have been established since 1990. In spite of the rapid expansion of ostrich farms, there are few reports concerning diseases of the birds [10]. The authors encountered an occurrence of stomach impaction in ostriches associated with high mortality, that occurred on a newly established ostrich farm in Zambia.

The farm involved was in Lusaka and imported 51 ostriches, 6 weeks old, from Namibia for breeding in 1991. Soon after arrival, these birds were introduced into a pen with a small open paddock on the farm, and they were fed on a mixture of lucerne hay and uncracked yellow maize. However, most of the birds manifested clinical symptoms such as loss of appetite, diarrhea, ruffled feathers, and general weakness, and 19 of the 51 birds died within 1 week after arrival.

Four female dead birds, 7 weeks old and around 6 kg in body weight, were submitted to the University of Zambia for diagnosis. The autopsy revealed conspicuous dilation of the proventriculus (Fig. 1), which was twice the normal size [2, 3]. The proventriculus and gizzard were completely full of solid masses of interwoven lucerne hay and full-sized yellow maize (Fig. 2), and consequently the pylorus of the gizzard was extremely impacted by them.

Fig. 1. Conspicuous dilation of the proventriculus (P) seen in an autopsied bird. G: gizzard. E: esophagus. D: duodenum. L: liver.

Fig. 2. The proventriculus (P) and gizzard (G) were full of solid masses of interwoven lucerne hay and maize.

Furthermore, the intestinal contents were severely watery because of the impaction. No other specific lesions were seen.

Pieces of the heart, liver, spleen and cerebrum from the 4 autopsied birds were cultured on plates of blood agar, MacConkey agar, and heart-infusion agar, and incubated at 37°C for 24 hr. The intestinal contents of the 4 autopsied birds and 8 fecal samples obtained from birds remaining on the farm were also cultured into selenite broth. Subcultures from the incubated selenite broth cultures were made on MacConkey agar plates. No pathogenic bacteria, including *Salmonella*, were isolated from any postmortem materials or fecal samples examined.

Intestinal contents of the postmortem materials and the 8 fecal samples were examined parasitologically by the flotation method. Neither parasitic ova nor coccidial oocysts were detected from any samples examined.

As the birds manifested severe diarrhea in the present case, salmonellosis or coccidiosis could be suspected. However, neither pathogenic bacteria nor parasites were detected as mentioned above. Therefore it seems that diarrhea was caused by mechanical blockage of the feeds in the stomach, except water which was seen in the intestines.

Through the above findings and examinations, the present case was tentatively diagnosed as stomach impaction. Soon after diagnosis, the feed was improved, properly replacing lucerne hay with fresh lucerne leaves. Furthermore, mineral oil as a demulcent [1] was given immediately to the remaining ostriches to induce smooth movement of the blockage in the stomach. After this, clinical symptoms seen among the birds that remained on the farm were gradually reduced and all birds recovered within 2 weeks. These data confirmed that the birds died of stomach impaction.

For rearing ostriches, it is reported that special attention must be given to the young ostrich bedding, because they will eat any kind of debris on the floor, which will block the proventriculus and gizzard and consequently kill them [1, 4]. In young turkey flocks as well, gizzard impaction can cause high mortality during the first 3 weeks of life, because they tend to eat litter [9]. As the case described above occurred among young ostriches on a newly established farm, the owner had no experience in rearing ostriches. Therefore, the birds were fed an inadequate feed including stalks of lucerne hay for a week, which caused gizzard impaction.

Conspicuous dilation of the proventriculus was also seen in the present case. There are some studies concerning dilation of the proventriculus in chicks fed on a finely ground diet lacking in fiber [7–9]. According to these reports, the gizzards were poorly developed due to the diet and were unable to handle the feed, so that the proventriculus was dilated considerably by the feed. However, the gizzard in the present case was properly developed macroscopically, thus the sick birds remaining on the farm recovered after treatment and improvement of the feed. Therefore, it seems that the gizzard impaction initially occurred due to the inadequate feed as mentioned above, and that subsequent dilation of the proventriculus occurred because of mechanical blockage by the feed.

There are some reports concerning stomach impaction in ostriches [5, 6]. Honnas *et al.* [5] reported 7 cases of the disease seen on the same farm and mentioned that stress such as transport appears to play an important role in indiscriminate eating of foreign materials. As the present case was seen soon after transport, the birds showed indiscriminate eating of lucerne hay and maize because of stress, though they are not foreign materials. Therefore, it seems that stress is one of the most important reasons why the present case was associated with high mortality.

REFERENCES

1. Burning, D. F. and Dolensek, E. P. 1986. pp. 277–291. *In*: Zoo and Wild Animal Medicine, 2nd ed. (Fowler, M. E. ed.), WB Saunders Co., Philadelphia.
2. Fowler, M. E. 1991. *J. Zoo Wildl. Med.* 22: 204–227.
3. Fowler, M. E. 1993. pp. 194–198. *In*: Zoo and Wild Animal Medicine, 3rd ed. (Fowler, M. E. ed.), WB Saunders Co., Philadelphia.
4. Gonzalez, V. 1992. *Misset-World Poultry* 8 (No. 7): 8–9.
5. Honnas, C. M., Jensen, J., Cornick, J. L., Hicks, K., and Kuesis, B. S. 1991. *J. Am. Vet. Med. Assoc.* 199: 461–465.
6. Jacobson, E. R., Ellison, G. W., McMurphy, R., Heard, D. J., and Ackerman, N. 1986. *J. Am. Vet. Med. Assoc.* 189: 1117–1119.
7. Newberne, P. M., Muhrer, M. E., Craghead, R., and O'Dell, B. L. 1956. *J. Am. Vet. Med. Assoc.* 128: 553–555.
8. Riddell, C. 1976. *Avian Dis.* 20: 442–445.
9. Riddell, C. 1991. pp. 845–847. *In*: Diseases of Poultry, 9th ed. (Calnek, B. W. ed.), Iowa State University Press, Ames.
10. Sato, Y. 1992. *Animal Husbandry* 46: 849–855 (in Japanese).

Epidemiological survey of poultry diseases in commercial breeding farms in Zambia

M. Hasegawa, L. M. Tuchili, G. S. Pandey, Department of Disease Control, School of Veterinary Medicine,University of Zambia, PO Box 32379, Lusaka

Abstract Two hundred and twenty-eight serum samples were obtained from 7 commercial farms, rearing grandparent and parent stocks, in order to understand the disease situation of poultry in Zambia. The antibody positive rates to Infectious Bursal Disease (IBD) virus were 100% in 5 farms that were vaccinated against IBD. Only one serum sample was found positive to Egg Drop Syndrome-1976 (EDS-76) virus. A variety of antibody positive rates to *Salmonella pullorum* and *S. gallinarum* was observed. At one of the farms, 92.3% of chickens were positive for antibody to the organism. Antibodies to *Mycoplasma gallisepticum* were recovered from 100% of samples at 4 farms and *M synoviae* antibody positive rates ranged between 8.3-100% in all seven farms. The results indicate that vaccination against IBD was successful, although particular attention must be paid to EDS-76 in future, and Pullorum Disease (Fowl Typhoid) and Mycoplasmosis.

In Zambia the poultry industry has been introduced and has developed steadily in the last few decades. The steady development of the poultry industry has, however, been hindered by the increase in poultry diseases. In the present investigation, chicken blood samples were collected from seven out of the existing nine commercial breeding farms rearing grandparent and parent stocks for a sero-epidemiological study of Infectious Bursal Disease (IBD), Egg drop syndrome-1976 (EDS-76) virus, *Salmonella pullorum/gallinarum*, *Myco-plasma gallisepticum* and *M synoviae*. The samples were collected from different areas of the country and analysed in the School of Veterinary Medicine.

Materials and methods

Two hundred and twenty-eight serum samples were obtained from parent stocks and grandparent stocks in 7 commercial breeding farms (Fig. 1) between September 1993 and August 1994. The serum samples were tested for antibodies against IBD virus by the agar-gel precipitation test, using 1 per cent Difco bacto agar in phosphate buffer (pH 7.2) with 8.5 per cent NaCl and 0.1 per cent NaN 3 . Gel was prepared on slide glasses by pouring 5ml of the molten agar on each slide. After solidifying, a pattern consisting of a central well and 6 peripheral wells, was made. The plates were incubated at room temperature in a humid chamber and examined daily over a period 3 days. IBD virus was isolated from the bursae of chickens and the antigen was prepared from positive cases of IBD for the agar-gel precipitation test. As a control, standard antiserum supplied by the National Institute of Animal Health, Tsukuba, Japan, was used.

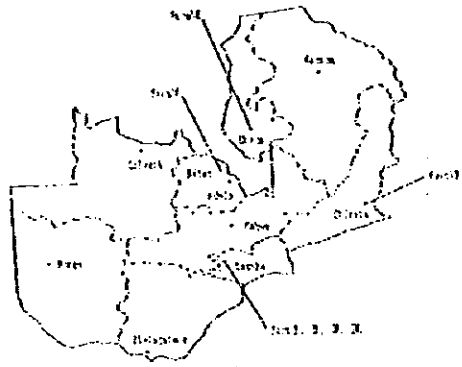

Fig. 1: Location of Farms used in the study

Another set of samples were tested for antibodies against EDS-76 virus by the haem-agglutination inhibition test. Two-fold serial dilution of each serum in phosphate buffer solution (pH 7.2) was added to the equal volumes of 8 HA units antigen using U-type microplate. After leaving samples at room temperature for 30 minutes, a 2 times volume of 0.8 per cent suspension of chicken red blood cells was added to each well. The plates were then incubated at room temperature for 1 hour. Samples whose titer was more than 1:4 was determined as positive. EDS-76 antigen (JRA-1) was supplied by the National Institute of Animal Health (Tsukuba, Japan) and propagated in chicken kidney cells. The diagnostic antigen was prepared from the viral suspension for the haemagglutination inhibition test in the Laboratory. Standard antiserum (anti-C14) was supplied by the National Institute of Animal Health, Tsukuba, Japan, and used as positive control.

Antibodies against *S pullorum/gallinarum* were determined by the rapid slide agglutination test using the diagnostic antigen (Chiba Prefecture Serum Institute, Chiba, Japan). The serum sample which was obtained from a field positive case of *S pullorum/gallinarum* was used as the positive control. Many *S pullorum/gallinarum* strains isolated in Zambia have been classified into the biovar of *S gallinarum*.

Antibodies against *M gallisepticum* and *M synoviae* were determined by the rapid slide agglutination test using the diagnostic antigen (Nihon Pharmacy, Tochigi, Japan and Kitasato Institute, Tokyo, Japan). The serum samples from a field case strongly positive for *M gallisepticum or M synoviae* were referred to as the positive control.

Results and Discussion

Infectious Bursal Disease antibody positive rates were 100% in all farms surveyed, except for farm W (60.0%) and farm Y (87.5%)(Table 1). It is recognized that the economic losses are a result of the immunosuppressive effect of IBD infections in the 1st or 2nd week of life. Current emphasis is on control of early infection via maternal immunity (Lukert 1984). The IBD outbreaks in Zambia were observed in broiler farms situated on the Copperbelt, Central and Southern provinces in 1976 (Sharma *et al.*, 1977). Vaccination against IBD has increased and all the 7 commercial breeding farms which were investigated had used a vaccine against IBD. This result possibly indicates that vaccination against IBD was successful as there was a 100% immune response in the 5 farms that were vaccinated aginst IBD.

The antibody positive rates against EDS-76 virus was 5.6% (1/18) in Indian River flocks of farm X. In other farms, no significant antibody was recovered (Table 1). Only one serum sample was found positive (HI titer 1:32), and the origin of this particular bird was Zimbabwe. Reconfirmation of this result was not carried out as all breeding chickens in farm X which were investigated had been slaughtered. The origin of this positive result could not be confirmed as subsequent samples from the same source (Zimbabwe) were negative. EDS-76 is an infectious disease of laying hens characterized by drop in egg production or production of abnormal eggshells without any other clinical signs. Since EDS-76 was first described in Holland in 1976 (Van Eck, 1976), the disease has been recognized in many countries. In Africa the existence of EDS-76 has been reported in South Africa (Bragg 1991) and Nigeria (Durojaiye 1991). This one positive result against EDS-76 would require further investigation to ascertain the presence or absence of the disease in Zambia.

Commercial breeding farms in Zambia purchase chicks or eggs from farms in European countries where EDS-76 virus exists. EDS-76 virus could possibly be brought into Zambia by importation of chickens. It is suggested that particular attention must be paid to this disease in future.

Salmonella pullorum/gallinarum positive rates are shown in Table 1. The highest positive rates were observed at farm T (92.3%) in the Arbor Acres breed, farm W (19%) and farm V (15.6%). There were no positive cases at farms U, X and Z. Although farm T and farm X had purchased parent stocks from farm U, there was no indication to show that the infection at the two farms could have originated from farm U as there was no positive reaction found at farm U to suggest any possibility of farm U infecting the other two farms (T, W). It is therefore possible that the two farms (T, W) could have been contaminated from other sources. In the same table (Table 1), there was a difference in susceptibility to infection *with S pullorum/gallinarum* by chicken breed, as a higher antibody positive rate was observed in the Arbor acres breed (92.3%) despite the fact that all these breeds (Ross, Arbor Acres and Crest) were kept in the same vicinity. In Zambia, Pullorum Disease (Fowl Typhoid) was recorded in imported chicks in 1935, 1936 and 1938, and has been permanently present, showing a rising trend and constituting an economic problem in the poultry industry (Gaspar and Hrabeta, 1977).

S pullorum/gallinarum has been controlled in developed countries. But the situation has been unknown in many deve-loping countries and this is mainly due to lack of proper investigations and laboratory facilities. In Zambia, *S gallinarum* isolation from chicks has been on the increase in recent years and the existence of infected carrier-hens has been of major concern. The result of this investigation indicated that many breeding chickens in commercial breeding farms were infected with *S gallinarum*.

Commercial breeding farms have often tried to disinfect fertile eggs before hatching but this has not often been helpful because *S gallinarum* could also be transmitted through eggs. It is expected that the prevention of infection will be based on the establishment and maintenance of *S gallinarum* -free breeding chickens which could be obtained through serological testing and other control measures.

Antibody positive rates against M gallisepticum were highest at farms T, U, X and Y where 100% antibody-positive rates were observed. However, there was no antibody reaction at farm Z. One important observation in this investigation was the result obtained at farms T and W where the antibody positive rates were 100% and these two farms had actually purchased their parent stock from farm U where the antibody positive reaction was found to be 100%. The possibility of the two farms (T, W) having been contaminated by these purchases from farm U is very high. It is likely that the grandparent stock of farm U where the purchases were made were infected *with M gallisepticum*. The antibody-positive reaction against *M synoviae* ranged between 8.30 - 100% in all 7 farms investigated (Table 1). Breed susceptibility to infection between Indian River and Hy-Line was observed at farm X where the antibody positive reaction was 100% in case of Hy-Line and only 6.3% in the case of Indian River although these were kept in the same vicinity. Mycoplasmosis of chickens is often characterized by chronic respiratory diseases or arthritis. In chickens, the organism may be present and cause no disease until triggered by stress e.g. change in housing, management, nutrition, weather, increased levels of dust or ammonia

in the environment, vaccination or infection with other diseases.

Infectious Bronchitis Virus, Newcastle Disease Virus and *E coli* are known to have synergistic effects in the outbreak of the disease. *M gallisepticum* and *M synoviae* are transmitted through eggs laid by inapparent carriers, propagated in an incubator and transmitted horizontally by contact in the contaminated hatcheries. The result of this investigation indicates that the antibody-positive rates for *M gallisepticum* or *M synoviae* in breeding chickens were high at all farms investigated. In Zambia, isolation and identification of Mycoplasma in poultry has not yet been established, and it is only diagnosed by clinical signs, postmortem lesions and serological tests. More investigations should be made in this important poultry disease.

In Zambia, although development of the poultry industry is expected, the epidemiological data of the diseases has not been available. The results of this investigation present us with up to date information on the major poultry disease situation in commercial breeding farms in Zambia. The successful effects of vaccination against IBD is also evident. The presence of EDS-76 in Zambia needs further investigation as the results obtained were not conclusive. On the other hand, Fowl Typhoid and Mycoplasmosis appear to be major problems in the farms rearing parent stock. In conclusion, it is hoped that more emphasis will be placed on the control of these major poultry diseases in Zambia in future.

Acknowledgements. The authors are grateful to Prof. E. Baba, School of Veterinary Medicine, University of Zambia and Dr T. Imada, Tohoku Branch Laboratory, National Institute of Animal Health, Japan for advice, and to the owners of the seven farms investigated for their cooperation.

References

Bragg, R.R. & Allwright, D.M.(1991) Onderstepoort Journal of Veterinary Research 58, 309

Durojaiye, O.A., Ahmed, A.S. & Adene, D.F. (1991) Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux 44, 37

Gospar, P. & Hrabeta, P. (1977) Bulletin of Animal Health and Production in Africa 25, 61

Lukert, P.D. & Hitchner, S.B. (1984) Infectious Bursal Disease. In : Diseases of Poultry. 8th edn. Eds. M.S. Hofstad, H.J. Barnes, B.W. Calnek, W.M. Reid & H.W. Yoder, Jr., Iowa State University Press, p566

Sharma, R N., Benko, L. & D'Cruz, S. (1977) Veterinary Record, 101, 153

Van Eck, J.H.H. & Davelaar, F.G. (1976) Avian Pathology 5, 261

Table 1: Serology results for the seven farms

Farm	Breed	Source of chickens	IBD virus	EDS-76 virus	*S. pullorum/ gallinarum*	*M. gallisepticum*	*M. synoviae*
I	Ross (gp)	UK	22/22 (100)	0/13 (0)	1/22 (4.5)	16/20 (80)	3/18 (16.7)
	Arbor Acres (p)	Farm II	15/15 (100)	0/13 (0)	12/13 (92.3)	13/13 (100)	7/13 (53.0)
	Crest (p)	Zimbabwe	13/13 (100)	0/9 (0)	0/12 (0)	3/12 (25.0)	1/12 (8.3)
II	Arbor Acres (p)	Farm II	25/25 (100)	0/25 (0)	0/25 (0)	24/24 (100)	18/21 (85.0)
III	Cobb (gp)	UK	32/32 (100)	0/25 (0)	5/32 (15.6)	21/32 (65.6)	32/32 (100)
IV	Ross (p)	UK	29/42 (69.0)	0/31 (0)	8/42 (19.0)	23/42 (54.8)	21/42 (50.0)
V	Indian River (p)	Zimbabwe	19/19 (100)	1/18 (5.6)	0/16 (0)	1/16 (6.3)	10/16 (62.5)
	Hy-Line (p)	Zimbabwe	20/20 (100)	0/17 (0)	2/20 (10.0)	19/19 (100)	17/18 (94.4)
VI	Arbor Acres (p)	Farm II	21/24 (87.5)	0/14 (0)	8/24 (33.3)	24/24 (100)	24/24 (100)
VII	Cobb (p)	Farm II	25/25 (100)	0/10 (0)	0/25 (0)	0/25 (0)	23/23 (100)

gp = grandparent stock; p = parent stock; * Number of positive samples/number of samples examined (percentage

RECENT SERO-CHARACTERIZATION OF SALMONELLA STRAINS ISOLATED FROM CHICKENS IN ZAMBIA.

L. M. Tuchili[A], C. Kaneuchi[B], W. Ulaya[A],
[A]University of Zambia, School of Veterinary Medicine,
Department of Disease Control, Box 32379, Lusaka, Zambia.

[B]Department of Veterinary Public Health II
Azabu University, 1-17-71 Fuchinobe,
Sagamihara, Kanagawa 229, Japan.

Summary

Except for published work of Sharma et al (1991), there has been no further attempt to sero classify strains of *Salmonella* isolated from chickens in Zambia.
In this paper, we present the recent sero-characterization of twenty six randomly selected strains of Salmonella from a stock of thirty strains isolated from chickens between July, 1992 and March, 1993. Seven serotypes were identified and these were *S. gallinarum, S haifa, S alamo, S. virginia, S dublin, S infantis* and *S. agona. S. haifa, S. alamo,* and *S. virginia* were encountered for the first time in Zambia Three strains DC 529-1, DC 529-2 and DC 529-3 which were initially thought to belong to Salmonella genus were infact *Proteus mirabilis.*

INTRODUCTION

Poultry salmonellosis has been reported all over the world. In Zambia however, there are only a few existing reports on avian salmonellosis (Gasper 1977; Sharma et al 1991). So far there are fourteen serotypes of Salmonella recognized from poultry in Zambia. These are *S. bonn, S. dublin, S. enteritidis, S. gallinarum, S heidelberg, S. infantis, S. kisarawe, S. oranienburg, S. rubislaw, S. typhimurium, S. weltverden, S. worthington, S. agona and S. schwarzengrund* (Falade et al 1989; Sharma et al 1991) The present paper gives the recent serotypes of Salmonella existing in chickens in Zambia and discusses the public health implications of these serotypes.

MATERIALS AND METHODS

The twenty six strains of *Salmonella* which were used for this study were a part of the thirty strains isolated from chickens between July 1992 and March 1993. Isolation of bacteria was done fron clinical specimens submitted to the School of Veterinary Medicine of the University of Zambia for *Salmonellosis* diagnosis. The clinical history in the majority of these samples submited was of high mortality especially in 7-21 days old chicks and diarrhoea, dullness, hudling, pasty vents, somnolence and death in case of adult chickens. Specimens were obtained from the liver, spleen, heart and embryonated eggs and then were innoculated into the enriched media (selenite brilliant green medium, Nissui Pharmaceutical co. Tokyo, Japan) and incubated at 37 C overnight. Suspected *Salmonella* single colonies were then picked and later subjected to biochemical and serological identification using salmonella antisera supplied by Denka Seika Co Tokyo-Japan.

RESULTS

All the twenty six strains randomly selected from a stock of *Salmonella* strains isolated from this study were serotyped except for three strains which were later identified as *Proteus mirabilis*. The identified serotypes were *S. gallinarum, S. haifa, S. alamo, S. virginia, S. dublin, S. infantis* and *S. agona*. The commonest serotype was *S. gallinarum* (47.8%). Six out of seven *S. agona* strain interestingly enough were all isolated from the same localtiy near Lusaka. On the other hand *S. gallinarum* appeared to be evenly distributed as this was isolated from all sampled localities around Lusaka. Probably for the first time in Zambia, *S. haifa S. alamo, and S. virginia* were isolated from chickens. Equally there is no record either from animals or poultry studies of these serotypes having been isolated before in Zambia. *S. dublin* though known to be a strain mainly affecting cattle was also isolated from the chicken yolk sac.

DISCUSSION

In Zambia, the poultry industry is currently developing very rapidly with small scale farmers being in the fore-front. Probably with this development, salmonella infection in poultry farms and hatcheries also appears to be on the increase as evidenced by the appearance of new serotypes *(S. alamo, S. virginia and S. haifa)* which have not been encountered before. Zambia poultry farmers import alot of breeding parents stocks from many parts of the world and the appearance of these new strains could possibly be linked to these importations. Previously, it was generally accepted that there was only *S. gallinarum pullorum* affecting poultry in Zambia but this result probably indicates the existence of more other serotypes. It is probably now correct to state that there exists many serotypes of Salmonella in Zambian chicken population. It now remains to be investigated whether all these serotypes would be clinically significant. One locality was found to be predominantly contaminated with *S. agona*. There is a likelyhood that the *S. agona* serotype had a common source of contamination, but elaborate investigations need to be done to determine whether these strains have the same genetic origin. DNA finger printing would offer such a possibility. The possible existence of these new serotypes (*S. alamo, S. virginia, S. haifa*) in the animal population needs to be explored. The public health significance of serotypes also requires urgent investigation as some of the diarrhea cases often reported in humans could be attributed to some of these serotypes if consumed through contaminated poultry products.

References

1. Falade, S., Sato, G., Ulaya, W., Mwanza, L. (1989). Serovars and antibiotic sensitivity patterns of *Salmonella* strains isolated from domestic animals in Zambia. Zimbabwe Veterinary Journal.

2. Gasper, P., Hrabeta. (1977). A survey of *salmonellosis* in domestic animals in Zambia. Bulletin of Animal Health and Production in Africa 25 (1) 61.

3. Sharma R. N. , Pandey, G. S., Khan A. Q., Francis B. K. T. (1991). *Salmonella* isolation from poultry in the republic of Zambia. Bulletin of Animal Health and Production in Africa. 39, 173-175.

Recent Characterisation of *Salmonella* Strains Isolated from Chickens in Zambia

Lawrence TUCHILI*, William ULAYA, Yukio KATO[1], and Choji KANEUCHI[1]

School of Veterinary Medicine, Department of Disease Control, University of Zambia, Box 32379, Lusaka, Zambia and [1]Department of Veterinary Public Health II, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 229, Japan

(Received 4 July 1995/Accepted 1 September 1995)

ABSTRACT. Among the 23 *Salmonella* strains isolated from 21 diseased chickens and 2 embryonated eggs in 6 poultry farms near Lusaka City in Zambia, serovars identified were *S.* Gallinarum (11 strains), *S.* Agona (7 strains), *S.* Alamo (1 strain), *S.* Infantis (1 strain), *S.* Virginia (1 strain), *S.* Haifa (1 strain), and *S.* Dublin (1 strain). *S.* Gallinarum was detected at the highest incidence and from all the poultry farms. Fourteen serovars have been reported for the chickens in Zambia so far. Three serovars (*S.* Alamo, *S.* Haifa, *S.* Virginia) were newly identified in this study. — KEY WORDS: poultry, *Salmonella*, serovar.

J. Vet. Med. Sci. 58(1): 77-78, 1996

Poultry salmonellosis has almost been reported all over the world. In Zambia, however, there are only a few existing reports on avian salmonellosis [2, 3]. So far there are 14 serovars of *Salmonella* recognized from poultry in Zambia. These are *S.* Bonn, *S.* Dublin, *S.* Enteritidis, *S.* Gallinarum, *S.* Heidelberg, *S.* Infantis, *S.* Kisarawe, *S.* Oranienburg, *S.* Rubislaw, *S.* Typhimurium, *S.* Weltevreden, *S.* Worthington, *S.* Agona, and *S.* Schwarzengrund [1, 3]. The present paper gives the recent serovars of *Salmonella* existing in chickens in Zambia and discusses the public health implications of these serovars.

Between July, 1992 and March, 1993, 23 strains of *Salmonella* were isolated from 23 out of 250 chickens submitted to the School of Veterinary Medicine, University of Zambia for diagnosis of salmonellosis and other diseases from 6 poultry farms around Lusaka City. The clinical history of the majority of these samples submitted was of high mortality especially in 7-21 days old chicks and diarrhea, dullness, huddling, pasty vents, somnolence, and death in case of adult chickens. The liver, spleen, heart, or embryonated egg were inoculated into the enrichment media (selenite brilliant green medium, Nissui Pharmaceutical Co., Tokyo, Japan) and incubated at 37°C overnight. A loopful of the enrichment broth was then streaked on blood agar (Oxoid Ltd., Basingstoke, Hants, England) and MacConkey agar (Oxoid Ltd., Basingstoke, Hants, England) and incubated at 37°C overnight. Suspected *Salmonella* single colonies were then picked and later subjected to conventional biochemical and serological tests. Identification of *Salmonella* serovars was done by the use of *Salmonella* antisera supplied by Denka Seiken Co., Tokyo, Japan.

The identified serovars were *S.* Gallinarum (11 strains), *S.* Agona (7 strains), *S.* Haifa (1 strain), *S.* Alamo (1 strain), *S.* Virginia (1 strain), *S.* Dublin (1 strain), and *S.* Infantis (1 strain) (Table 1). The commonest serovar was *S.* Gallinarum (47.8%) followed by *S.* Agona (30.4%). Six of 7 *S.* Agona serovars, interestingly enough, were isolated from the same farm near Lusaka City. On the other hand, *S.* Gallinarum appeared to be evenly distributed as this was isolated from all sampled farms. Probably for the first time in Zambia, *S.* Haifa, *S.* Alamo, and *S.* Virginia were isolated from chickens. Equally there is no record either from animals or poultry of these serovars having been isolated before in Zambia. *S.* Dublin though known to be a serovar mainly affecting cattle was also isolated from the chicken embryonated eggs.

In Zambia, the poultry industry is currently developing very rapidly with small scale farmers being in the forefront. Probably with this development, *Salmonella* infection in poultry farms and hatcheries also appears to be on the increase as evidenced by the appearance of new serovars (*S.* Alamo, *S.* Virginia, and *S.* Haifa) in the present paper, which have not been encountered before. Zambian poultry farmers import a lot of breeding parent stocks from many parts of the world and the appearance of these new strains could possibly be linked to these importations. Previously it was generally accepted that there was only *S.* Gallinarum/Pullorum affecting poultry in Zambia but the present result probably indicates the existence of more other serovars. It is probably now correct to state that there exist many serovars of *Salmonella* in the Zambian chicken population. It now remains to be investigated whether all these serovars would be clinically significant. One farm was found to be predominantly contaminated with *S.* Agona (Table 1). There is a likelihood that this contamination could probably have had a common source, but elaborate investigations need to be done to determine whether these serovars have the same genetic origin. DNA fingerprinting would offer such a possibility. The possible existence of these new serovars (*S.* Alamo, *S.* Virginia, and *S.* Haifa) in the animal population needs to be explored. The public health significance of these serovars also requires urgent investigation as some of the diarrhea cases often reported in humans could be attributed to some of these serovars if consumed through contaminated poultry products.

* CORRESPONDENCE TO: TUCHILI, L., School of Veterinary Medicine, Department of Disease Control, University of Zambia, Box 32379, Lusaka, Zambia.

Table 1. *Salmonella* serovars isolated from Chickens in Zambia

Farm[a)]	Chick no.	Specimen type	O antigen	H antigen (phase 1)	H antigen (phase 2)	Serovar
Hybrid	DC 196–1	Culture[b)]	6,7	g,z_{51}	1,5	*S.* Alamo
Hybrid	DC 273	Culture	8	d	1,2	*S.* Virginia
Hybrid	DC 354	Culture	6,7	r	1,5	*S.* Infantis
Hybrid	DC 529–1	Culture	4,12	f,g,s	–	*S.* Agona
Hybrid	DC 529–4	Culture	4,12	f,g,s	–	*S.* Agona
Hybrid	DC 529–5	Culture	4,12	f,g,s	–	*S.* Agona
Hybrid	DC 529–6	Culture	4,12	f,g,s	–	*S.* Agona
Hybrid	DC 529–8	Culture	4,12	f,g,s	–	*S.* Agona
Hybrid	DC 529–9	Culture	4,12	f,g,s	–	*S.* Agona
Hybrid	DC K4	Culture	4,12	f,g,s	–	*S.* Agona
Hybrid	DC K10	Culture	1,9,12	–	–	*S.* Gallinarum
Bera	DC 130	Liver	1,9,12	–	–	*S.* Gallinarum
Bera	DC 139	Heart	1,9,12	–	–	*S.* Gallinarum
Bera	DC 186–2	Heart	1,4,12	z_{10}	1,2	*S.* Haifa
Tamba	DC 299–1	Embryonated egg	1,9,12	g,p	–	*S.* Dublin
Tamba	DC 299–2	Liver	1,9,12	–	–	*S.* Gallinarum
Tamba	DC 344	Embryonated egg	1,9,12	–	–	*S.* Gallinarum
CC	DC 408	Liver	4,12	f,g,s	–	*S.* Agona
CC	DC K12	Liver	1,9,12	–	–	*S.* Gallinarum
Poultry Breeders	DC 420–1	Spleen	1,9,12	–	–	*S.* Gallinarum
Poultry Breeders	DC 420–2	Spleen	1,9,12	–	–	*S.* Gallinarum
Poultry Breeders	DC 420–3	Spleen	1,9,12	–	–	*S.* Gallinarum
Makeni	DC 420–4	Spleen	1,9,12	–	–	*S.* Gallinarum
Makeni	DC 486	Spleen	1,9,12	–	–	*S.* Gallinarum

a) All the farms are located around Lusaka City.
b) *Salmonella* cultures were obtained from Hybrid farm.

REFERENCES

1. Falade, S., Sato, G., Ulaya, W., and Mwanza, L.(1989). *Zimbabwe Vet. J.* 20: 19–22.
2. Gasper, P. and Hrabeta, P. 1977. *Bull. Anim. Health Prod. Africa* 25: 61–64.
3. Sharma, R. N., Pandey, G. S., Khan, A. Q., and Francis, B. K. T. 1991. *Bull. Anim. Health Prod. Africa* 39: 173–175.

Detection of *Salmonella* DNA in Chicken Embryos and Environmental Samples by Polymerase Chain Reaction

Lawrence M. TUCHILI, Hiroshi KODAMA[1]*, Ravindra N. SHARMA, Ichiro TAKATORI, Girja S. PANDEY, Swithin KABILIKA, Masafumi MUKAMOTO[1], Sachiyo TSUJI[1], and Tsuyoshi BABA[1]

The School of Veterinary Medicine, University of Zambia, Box 32379 Lusaka, Zambia and [1]Laboratory of Veterinary Immunology, Department of Veterinary Science, College of Agriculture, Osaka Prefecture University, Sakai, Osaka 593, Japan

(Received 14 March 1996/Accepted 7 May 1996)

ABSTRACT. By polymerase chain reaction (PCR) using a pair of primers specific for *Salmonella phoE* gene a 365-bp specific gene fragment could be amplified from yolk of infertile eggs and dead-in-shell chicken embryos, and from environmental samples. Out of 45 dead-in-shell embryo samples, 20 (44.4%) were found positive for *Salmonella* DNA by PCR compared to 11 (24.4%) by bacteria isolation. *Salmonella* DNA could also be detected from infertile eggs, chicken faeces, floor litter and chick fluff, which incidence was higher than that by bacteria isolation. — KEY WORDS: chicken embryo, DNA detection, polymerase chain reaction, *Salmonella*.

J. Vet. Med. Sci. 58(9): 881–884, 1996

Salmonellosis is a major constraint in the poultry industry. Development of rapid and sensitive methods is very important because control of salmonellosis in poultry largely depends on the identification of infection in the early stages. The polymerase chain reaction (PCR) whose principle is based on the enzymatic amplification of a DNA fragment that is flanked by a pair of primers that hybridize to opposite strands of the target sequence has been used to detect many infectious agents [1, 4, 6, 8, 12] and is steadily being accepted as a very useful tool in the clinical diagnosis of various infections. The PCR has also been shown to offer a new strategy in the detection of *Salmonella* [3, 7, 10, 11]. However, these PCR amplifications were performed on either purified DNA using the phenol-chloroform method from samples of experimental infection or using single bacterial colonies isolated by conventional methods. PCR has therefore, not been successfully used to detect *Salmonella* from chicken embryos and environmental samples in the field. In a previous report we showed that PCR was more sensitive than conventional *Salmonella* isolation in chickens experimentally infected with the bacteria [10]. In this study, therefore, we applied the PCR for amplification of *Salmonella* DNA directly from yolk of infertile eggs and chicken dead-in-shell embryos and from environmental samples after DNA extraction.

MATERIALS AND METHODS

Specimen and bacterial culture: A total of 870 chicken egg samples comprising of 450 dead-in-shell embryos, 210 infertile eggs and 210 table eggs, and 80 environmental samples (48 faecal, 1 rat liver, 17 chick hatcher fluff, and 14 chicken floor litter) were obtained from a hatchery near Lusaka City, Zambia. Eggs were pooled in small sample sizes of 10 eggs each. Yolk pooled from 10 eggs made one samples: 1 *ml* of the egg yolk was used to inoculate 10 *ml* pre-enrichment media (Selenite Brilliant Green, Nissui Pharmaceutical Co., Tokyo, Japan) and incubated at 41°C overnight. These were later subcultured on Brilliant Green agar and incubated at 37°C overnight. Direct culture of the yolk was done on blood and MacConkey agar (Oxoid Ltd., Basingstoke, Hants, England) and incubated at 37°C overnight. Bacteria isolation from environmental samples was done by suspending 2 g of sample into the 10 *ml* pre-enrichment broth and subcultured on Brilliant Green agar. Identification of colonies was done by conventional bacteriological tests and by using *Salmonella* antisera (Denka Seiken Co., Tokyo, Japan).

DNA extraction from faeces, litter and fluff: DNA extraction was done as described by Cohen *et al.* [3]. Briefly, 2 g of the sample was ground to fine powder and resuspended in 10 ml of a solution containing 50 mM EDTA, 50 mM Tris-HCl and 0.5% Tritone X and was allowed to settle overnight at room temperature. Three *ml* of supernatant was then centrifuged at 15,000 rpm for 15 min using a micro centrifuge (TM-150, Tomy Seiko Co., Tokyo, Japan). Pellets were resuspended in 150 *μl* of solution containing 50 mM EDTA, 50 mM Tris-HCl, 20% sucrose and 1 mg of lysozyme. The suspension was incubated at 37°C for 30 min. One *ml* of lysing buffer containing 100 mM NaCl, 25 mM EDTA, 50 mM Tris-HCl, 0.5% SDS, and 1 mg proteinase K was added to the sample and the solution was incubated at 60°C for 1 hr. DNA extraction was done by using TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0)-equilibrated phenol (2 times) followed by one extraction with 24:1 chloroform-isoamyl alcohol and precipitated with ethanol. Resultant DNA was resuspended in 50 *μl* TE buffer. DNA extraction from liver of the rat was done as described by Tuchili *et al.* [10].

PCR procedure and oligonucleotide primers: The egg yolk, culture broth of bacteria and extracted DNA from

* CORRESPONDENCE TO: KODAMA, H., Laboratory of Veterinary Immunology, Department of Veterinary Science, College of Agriculture, Osaka Prefecture University, Sakai, Osaka 593, Japan.

Table 1. Isolation of *Salmonella* and detection of *Salmonella* DNA from egg yolks

Specimen type	No. of samples tested	Bacteria isolation		Detection by PCR	
		direct	after enrichment	direct	after enrichment
Chicken embryo (dead-in-shells)	45	6 (13.3%)	11 (24.4%)	20 (44.4%)	23 (51.1%)
Infertile eggs (yolk)	21	2 (9.5%)	5 (23.8%)	6 (28.6%)	6 (28.6)
Table eggs (yolk)	21	0	0	0	0

environmental samples formed the material for DNA amplification. The method was a slight modification of that described by Rahn *et al.* [7]. A 5 μl of egg yolk, extracted DNA or enriched broth culture were used as a template instead of a single colony. A pair of primers specific for *Salmonella* based on *S.* Typhimurium *phoE* (5' AGC GCC GCG GTA CGG GCG ATA AA-3' (Oligo ST5) and 5'-ATC ATC GTC ATT AAT GCC TAA CGT-3' (Oligo ST8C)) [9] were used in this study. The 100 μl PCR mixture contained 2.5 U/0.5 μl of recombinant Taq DNA polymerase, 10 μl of 10 × PCR buffer, 8 μl dNTP mixture (2.5 mM), 5 μl of the sample and 5 μl (0.075 μM) of each primer. The PCR reaction mixture was overlaid with two drops of mineral oil (Sigma, St. Louis, MO). Control tubes contained *Salmonella* cells picked up from single colony and PCR mixture, or only the PCR mixture without the sample. The PCR incubations were performed by the ASTEC System PC-700 (Astec Co., Shimen, Fukuoka, Japan). The cycle time and temperature were exactly as those described by Rahn *et al.* [7]. A 10 μl aliquot of the PCR reaction mixture was analyzed on agarose gel (Wako Pure Chemical, Tokyo, Japan) electrophoresis.

Specificity of primers was tested on 10 non-*Salmonella* strains belonging to the Enterobacterecea: *Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae, Shigella flexneri, Proteus mirabilis, Edwardsiella tarda, Morganella* sp., *Yersinia enterocolitica, Serratia marcescens* and *Enterobacter cloacae.*

RESULTS

The results are presented in Fig. 1 and Tables 1 and 2. A 365-bp fragment was amplified from the yolk of infertile chicken eggs and embryos (Fig. 1A, lanes 2, 4, 5, 7, 8 and 9). There was no DNA band amplified from negative control tubes (Fig. 1A and 1B, lane 1). Figure 1C shows positive controls in which specific bands are amplified in 10 *Salmonella* species conducted at the same time. BY direct method 20 out of the 45 (44.4%) tested samples from embryo yolk of dead-in-shells could be amplified. *Salmonella* isolation from the yolk of eggs was possible in 6 out of 45 (13.3%) samples by direct culture, compared to isolation from enriched media 11 out of 45 (24.4%) (Table 1). Detection of *Salmonella* DNA by PCR also improved from enriched media samples as *Salmonella* DNA could be detected in 23 out of 45 (51.1%) tested samples. Similar results were obtained from infertile eggs (Table 1). All the samples from fresh table eggs were negative both on bacteriological isolation and by PCR (Table 1 and Fig. 1A, lanes 3 and 6).

Table 2. Isolation of *Salmonella* and detection of *Salmonella* DNA from environmental specimens

Specimen type	No. of samples tested	Bacteria isolation	Detection by PCR
Faeces	48	9 (18.8%)	13 (27.1%)
Hatcher chick fluff	17	1 (5.9%)	6 (35.3%)
Floor litter	14	0	3 (21.4%)
Rat liver	1	0	1 (100%)

Detection of *Salmonella* DNA from environmental samples by PCR was also possible as we were able to detect in 13 out of 48 (27.1%) tested faecal samples, in 3 out of 14 (21.4%) chicken floor litter, and in 6 out of 17 (35.3%) chicken fluff samples (Table 2 and Fig. 1B). One detection by PCR was made from rat liver (Table 2). Bacteria isolation from environmental samples was only possible in faeces (18.8%) and chicken fluff (5.9%). There was no isolation of bacteria either from chicken litter or the rat liver.

DISCUSSION

With a pair of primers directed at the *phoE* gene 28.6% and 44.4% embryo samples were amplified by direct PCR from the yolk of infertile eggs and chicken dead-in-shell embryos, respectively, without need for purification of the DNA. The DNA size of the amplified band and DNA sequencing of 365-bp DNA fragment were in agreement with Spierings *et al.* [9] except for one base (data not shown). Detection of *Salmonella* DNA improved

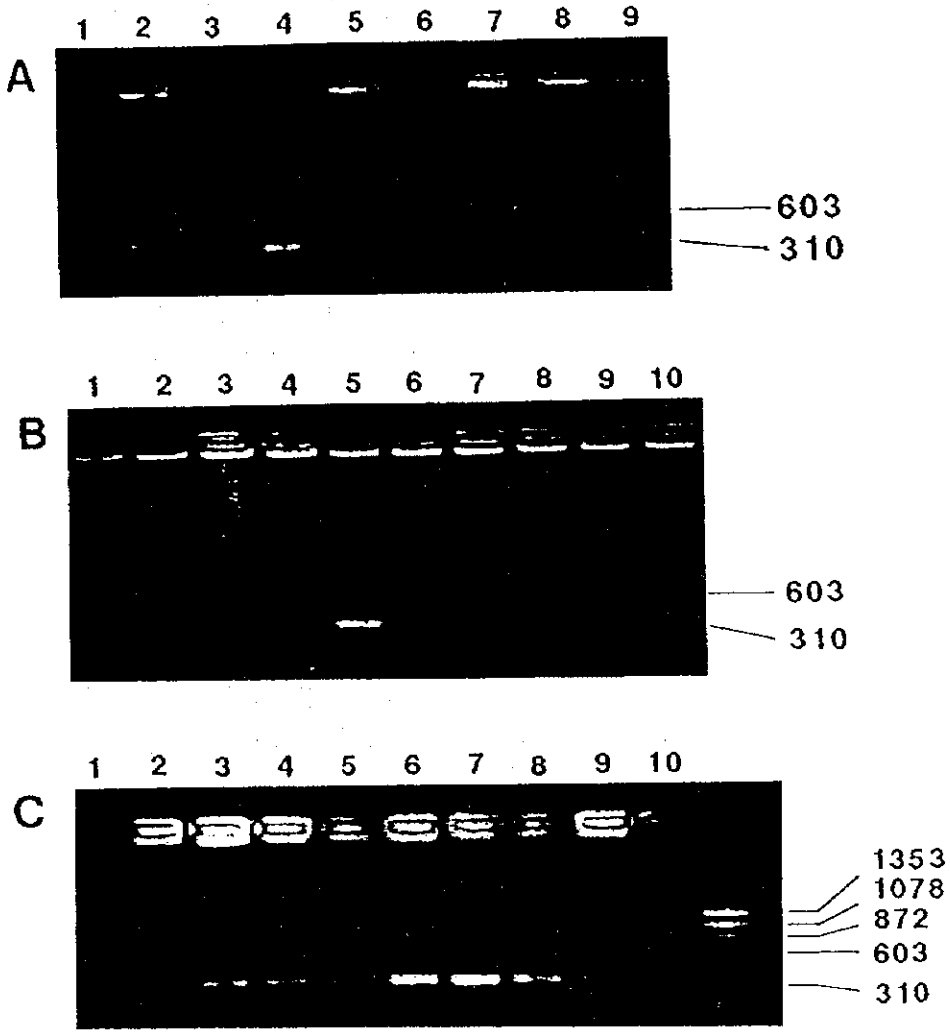

Fig. 1. PCR amplified *Salmonella* DNA from chicken egg yolks (1A) and environmental samples (1B). Amplified DNA bands from yolk of dead-in-shell embryos (1A, lanes 2, 4, and 5), infertile eggs (lanes 7, 8, and 9), and negative samples from fresh table eggs (lanes 3 and 6). Amplified DNA bands from chicken floor litter (1B, lane 2), chicken faeces (lane 5) and hatcher chick fluff (lane 8). Lanes 3, 4, 6, 7, 9 and 10 are negative samples from the floor litter (3 and 4), chicken faeces (6 and 7) and hatcher chick fluff (9 and 10). Lanes 1 of Figure 1A and 1B are negative controls containing only PCR mixture. Lanes 1 to 10 of Figure 1C are positive controls of *S.* Gallinarum, *S.* Agona, *S.* Haifa, *S.* Alamo, *S.* Virginia, *S.* Dublin, *S.* Infantis, *S.* Typhimurium, *S.* Enteritidis and *S.* Brown, respectively.

significantly by enrichment as we could detect 23 out of 45 (51.1%) tested samples compared to conventional bacterial isolation methods 11 out of 45 (24.4%). Certain inhibitors have been cited to be responsible for poor PCR performances [2, 5, 7]. But in this study, there appeared to be no apparent inhibitors in the chicken egg yolk samples to interfere with the PCR reactions for we were able to amplify *Salmonella* DNA directly from chicken yolk without need for DNA purification. There was no detection made from fresh table eggs by both methods. The failure to detect *Salmonella* from these samples could possibly suggest that the bacteria multiplication might have been lowered below detectable levels by the low storage temperature of fresh table eggs practiced at this farm.

Detection of *Salmonella* DNA by PCR from environmental samples was also possible as we detected DNA from faecal samples, chicken litter and fluff. The detection of *Salmonella* DNA from faeces is supported by the work of Cohen *et al.* [3] who detected *S.* Enteritidis DNA by PCR from faeces inoculated with a known number of the bacteria. DNA detection from these samples was done only after DNA extraction because of the presumable

presence of inhibitors in the faeces as reported by Rahn *et al.* [7] who could not amplify *Salmonella* DNA from chicken litter and chicken carcass rinse samples. Other *Salmonella* DNA detection was made from the liver of a rat indicating the possible existence of a reservoir of *Salmonella* infection in the rat population at this particular farm. Isolation of bacteria from environmental samples was poor as we could isolate only one *Salmonella* strain from the litter and chick fluff by using this method. The failure to detect *Salmonella* by isolation method from PCR positive samples probably indicates the difficulties that may exist in tracing *Salmonella* infection when a method of low sensitivity is used.

Direct PCR amplification of *Salmonella* DNA from the yolk of chicken eggs and embryos is probably the first report. The total time required for the detection of *Salmonella* from eggs using PCR is only 6 hr. The results therefore, indicate the superiority of direct amplification of *Salmonella* DNA from clinical specimens over isolation procedures as long as there were no inhibitors present in the samples to interfere with the PCR reactions.

ACKNOWLEDGMENTS. We sincerely thank the Japan International Co-operation Agency (JICA) for their financial support to this research work. Thanks are also due to the School of Veterinary Medicine, The University of Zambia for the support.

REFERENCES

1. Beji, A. K., Mahbulani, M. H., Miller, R., DiCesare, J. L., Haff, L., and Altas, R. M. 1990. Multiple PCR amplification and immobilized capture probes for detection of bacterial pathogens and indicators in water. *Mol. Cell. Probes* 4: 353–365.
2. Beutler, E., Gelbart, T., and Kuhl, W. 1990. Interference of heparin with the polymerase chain reaction. *BioTechniques* 9: 166.
3. Cohen, N. D., McGruder, E. D., Neibergs, H. L., Behle, R. W., Wallis, D. E., and Hargis, B. M. 1994. Detection of *Salmonella enteritidis* in feces from poultry using booster polymerase chain reaction and oligonucleotide primers specific for all members of the Genus *Salmonella*. *Poult. Sci.* 73: 354–357.
4. Jackwood, M. W., Kwon, H. M., and Hilt, D. A. 1992. Infectious bronchitis virus detection in allantoic fluid using the polymerase chain reaction and a DNA probe. *Avian Dis.* 36: 403–409.
5. Mercier, B., Gaucher, C., Feugeas, O., and Mazurier, C. 1990. Direct PCR from whole blood, without DNA extraction. *Nucleic Acids Res.* 18: 5908.
6. Oyofo, B. A., Thornton, S. A., Burr, D. H., Trust, T. J., Pavlovskis, O. R., and Guerry, P. 1992. Specific detection of *Campylobacter jejuni* and *Campylobacter coli* by using polymerase chain reaction. *J. Clin. Microbiol.* 30: 2613–2619.
7. Rahn, K., De Grandis, S. A., Clarke, R. C., McEwen, S. A., Galan, J. E., Ginocchi., C., Curtiss, R., and Gyles, C. L. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Mol. Cell. Probes* 6: 271–279.
8. Saris, P. E. J., Paulin, L. G., and Uhlen, M. 1990. Direct amplification of DNA from colonies of *Bacillus subtilis and Escherichia coli* by the polymerase chain reaction. *J. Microbiol. Methods* 11: 121–126.
9. Spierings, G., Elders, R., van Lith, B., Hofstra, H., and Tommassen, J. 1992. Characterization of the *Salmonella typhimurium phoE* gene and development of *Salmonella*-specific DNA probes. *Gene* 122: 45–52.
10. Tuchili, L. M., Kodama, H., Izumoto, Y., Mukamoto, M., Fukata, T., and Baba, T. 1995. Detection of *Salmonella* Gallinarum and *S.* Typhimurium DNA in experimentally infected chicks by polymerase chain reaction. *J. Vet. Med. Sci.* 57: 59–63.
11. Wernars, K., Delfgou, D., Soentoro, P. S., and Notermans, S. 1991. Successful approach for detection of low numbers of enterotoxigenic *Escherichia coli* in minced meat by using the polymerase chain reaction. *Appl. Env. Microbiol.* 7: 1914–1919.
12. Wu, C. C., Lin, T. L., Zhang, H. G., Davis, V. S., and Boyle, J. A. 1992. Molecular detection of infectious bursal disease by polymerase chain reaction. *Avian Dis.* 36: 221–226.

Serological diagnosis of Newcastle disease between Guinea fowl and chicken in developing country

S.Takahashi[a], A.K.Suzuki[a], G.S.Pandy[b] and T.kaji[b]

[a]*National Institute for Environmental Studies*
Tsukuba-shi 305, Japan
[b]*University of Zambia, School of Veterinary Medicine*
P.O.Box 32379, Lusaka, Zambia

Introduction

In Japan, the Newcastle disease has been eradicated by vaccination of inactivated or lentogenic Newcastle disease virus (NDV). However, in Asia and Africa, the NDV is inducing a serious problem for poultry industry.

We performed serological diagnosis of the NDV in Zambia, a part of Fund was supported by the International Joint Research, the Ministry of Education, Japan at 1991. In developing countries, there are a lot of infectious disease. However, the infectious route is not defined even in the NDV. This study shows that the NDV might be common disease between Guinea fowl and chicken.

Materials and Methods

Chicken: The chickens were supplied from the markets and University in Lusaka, capital of Zambia as follows. ① Control chickens originated in the farm in the University of Zambia (UNZA) (NDV vaccine injecting chicken flock) : 30 samples , ② Chickens originated in the farm in UNZA (NDV outbreaking chicken flock) : 12 , ③ Chickens originated in Northmid market (chicken flock on the market) : 8 , ④ Chickens originated in farm around Lusaka (poultry farming flock) : 11. (total : 61 samples).

Guinea fowl: Total 16 blood samples of Guinea fowls were collected in Lutale village located near by Kafue national park, central part of Zambia.

Measurement of antibody titer: The NDV haemoagglutinin inhibition antibody titer (NDV-HI titer) was examined by the micro-titer plate method. The maximum dilution value was converted into $\log_2$ and the titer was displayed as an integer.

Results and Discussion

The blood samples from the chicken were centrifuged and total 61 sera were examined for the antibody titer to NDV. Table shows the result of the NDV-HI titer of the chicken. The flocks of NDV vaccine no injecting group (③and④) were also positive of 3.50 and 3.45($\log_2$)

on the average, respectively. It means that NDV spread on the site. Actually, 12 chickens among 42 samples (① and ②) infected by the NDV in spite of the NDV vaccination and all chickens of farming flock(④) were dead within 1 week. Sharma et al (1985) reported that the Newcastle disease was decreased by lentogenic NDV vaccination from 1974 to 1984. However, the present study shows that the NDV might spread widely on some districts in Zambia.

By the way, Guinea fowls are being bred in the village around the national park, and chickens are co-existing in Zambia. Because Guinea fowls are inhabiting common to wild and domestic areas, they might be the carrier of infectious disease between wild birds and chickens. From the micro-titer analysis of Guinea fowls, the NDV-HI titer showed only 3 $\log_2$ ($\times 8$). And the symptom to the NDV infection was not so serious as that of chicken. Therefore, the Guinea fowl might have more higher resistant to NDV than that of the chicken. This shows that the NDV will spread to the chicken through the Guinea fowls. Then, increasing the number of examples and detecting the NDV positive in Guinea fowl become very important in the future. Moreover, it needs to clarify the infections route of various pathogens between domestic and wild animals.

Table. Comparison of NDV-HI titre in Zambian chickens

Lot No. of Chicken	n	NDV-HI Titre ($\log_2$) ME ± SD	
		NDV Vaccination	
Univ. Zambia(①)	30	3.60 ± 1.16	Done
(Infected) (②)	12	3.83 ± 1.11	
Northmid Mar.(③)	8	3.50 ± 0.92	No
Lusaka Farm(④)	11	3.45 ± 4.03	No

ME: mean value SD: standard deviation

References

R.N.Sharma, N.A.Hussein, G.S.Pandy and M.N.Shandomo: A Study on Newcastle disease outbreaks in Zambia, 1975-1984, Rev. Sci. Tech Off. Int. Epiz., Vol.5, p.5-14, 1985

AVIAN DISEASES 41:490–495, 1997

Status of Salmonella gallinarum-pullorum Infections in Poultry in Zambia

Yoshihiko Sato,[A] Gihei Sato,[B] Lawrence Tuchili,[B]
Girja Shanker Pandey,[B] Atsuko Nakajima,[B]
Henry Chimana,[B] and Henry Sinsungwe[B]

[A]Nagano Livestock Hygiene Service Center, 1993 Amori, Nagano 380, Japan
[B]Department of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia

Received 17 July 1996

SUMMARY. Ten outbreaks of *Salmonella gallinarum-pullorum* infections on poultry farms in Zambia were investigated. Three cases were seen in day-old broiler chickens and were diagnosed by culture as *S. gallinarum-pullorum* and characterized as pullorum disease because the mortality was only in the first few weeks. Another case was diagnosed by culture from broiler parent stock. Day-old chicks from two of the three cases were supplied by a hatchery. Five cases in 5-to-18-month-old layer chickens were diagnosed by culture as *S. gallinarum-pullorum* and characterized as fowl typhoid because of the clinical disease appearing after 5 months of age and the typical lesions of fowl typhoid. The last case was in 5-month-old village-bred fowls and was diagnosed by culture and clinical manifestation as fowl typhoid. Outbreaks of *S. gallinarum-pullorum* are still manifest in Zambia. Clinically, both pullorum disease and fowl typhoid were observed, and it was indicated that hatchery infection plays an important role in the transmission of *S. gallinarum-pullorum.*

RESUMEN. *Reporte de Caso*—Estado de las infecciones por Salmonella gallinarum-pullorum en la avicultura de Zambia.

Se investigaron 10 brotes de infecciones por *Salmonella gallinarum-pullorum* en granjas avícolas de Zambia. Se observaron tres casos en pollos de engorde al día de edad que fueron diagnosticados por cultivo como *S. gallinarum-pullorum* y caracterizados como pullorosis debido a que la mortalidad ocurrió sólo durante las primeras semanas. Otro caso fue diagnosticado por cultivo a partir de reproductoras pesadas. En dos de los tres casos, los pollitos de un día de edad procedían de la misma planta incubadora. En ponedoras de 5 a 18 meses de edad, por cultivo se diagnosticaron cinco casos como *S. gallinarum-pullorum* que fueron caracterizados como tifoidea aviar debido que la enfermedad clínica apareció después de los 5 meses de edad con lesiones típicas de la tifoidea. El último caso ocurrió en aves de traspatio de 5 meses de edad, diagnosticado come tifoidea aviar tanto por cultivo como por las manifestaciones clínicas. En conclusión, en Zambia todavía se observan brotes de *S. gallinarum-pullorum.* Clinicamente, se observaron tanto la pullorosis come la tifoidea aviar. Se in dica que la infección a nivel de la planta de incubación juega un papel importante en la transmisión de la *S. gallinarum-pullorum.*

Key words: fowl typhoid, poultry, pullorum disease, salmonellosis, Zambia

Salmonella choleraesuis subspecies *choleraesuis* serovar *gallinarum-pullorum* (*Salmonella gallinarum-pullorum*) (4) infection is one of the most well known *Salmonella* infections (5,9). Pullorum disease, caused by *Salmonella gallinarum-pullorum* biovar *pullorum* (*S. pullorum*), was once enzootic in many areas of the world but decreased to the point of eradication in the most advanced poultry-producing areas. The incidence of fowl typhoid, caused by *Salmonella*

gallinarum-pullorum biovar *gallinarum* (*S. gallinarum*), is also quite rare in developed countries. However, in some areas such as Africa, South America, the Middle East and the Indian subcontinent, *S. gallinarum-pullorum* is still the most common *Salmonella* serovar in poultry, causing major economic losses in the poultry industry (1,5,9).

According to brief reviews of *S. gallinarum-pullorum* infections in Zambia (3,8), the first outbreak of fowl typhoid was recorded in village poultry in 1931, and pullorum disease was recorded in imported chickens in 1935. Then, fowl typhoid continued with a fluctuating incidence of records from 1940 through 1973. *Salmonella gallinarum-pullorum* was isolated frequently from poultry during the 1970s and 1980s (5,8). Tuchili *et al.* (10) recently detected *S. gallinarum-pullorum* at the highest incidence from commercial poultry farms. However, the agents in those reports were considered as one, *S. gallinarum-pullorum*, and the isolates were not classified into two biovars. In recent years, we have encountered 10 outbreaks of *S. gallinarum-pullorum* infections on poultry farms and attempted to classify them into pullorum disease and fowl typhoid epizootiologically and pathologically.

CASE REPORT

Materials were obtained from specimens submitted for routine diagnostic examinations during the period January 1990–June 1991 (7). Samples of the heart, liver, spleen, intestinal contents, ovary, and retained egg yolk of the autopsied chickens were cultured on plates of desoxycholate hydrogen sulfide lactose agar, heart-infusion agar, and blood agar and also selenite broth and were incubated at 37 C for 24 hr. Isolates from these materials were examined biochemically for *Salmonella* and were serotyped using diagnostic *Salmonella* antisera for O and H (Denka, Tokyo). When the isolates were nonmotile, were negative for hydrogen sulfide, indole, and urease production, showed no growth on citrate agar, and were positive for group 9 of *Salmonella* O antigens, they were identified as *S. gallinarum-pullorum*.

There were 10 cases of *S. gallinarum-pullorum* cultured out of 168 poultry specimens. Diagnoses of fowl typhoid or pullorum disease in the following 10 cultured cases were made on the basis of epizootiologic findings and gross lesions suggestive of either disease (5,9). The outline of these cases is summarized in Table 1.

Case 1 occurred among 1500 1-day-old broiler chicks that were introduced from hatchery A (Case 4) in Lusaka to a commercial poultry farm in Lusaka. Despite being dosed with oxytetracycline and furazolidone, 1000 chicks died by 2 wk of age. The incidence was diagnosed as pullorum disease.

Case 2 occurred among 100 1-day-old broiler chicks that were introduced from the same hatchery A to a farm in Lusaka. Fifty percent of them died during the first week. The disease was diagnosed as pullorum disease.

Case 3 occurred among 2000 1-day-old broiler chicks that were introduced from hatchery B in Lusaka to a poultry farm in Kabwe, 110 km north of Lusaka. Despite being dosed with oxytetracycline and furazolidone, 932 birds died by 30 days of age and the number of deaths were confined to the first few weeks (Fig. 1). This case was diagnosed as pullorum disease.

Case 4 was observed at hatchery A in Lusaka, where 2000 broiler parent stock chickens were kept. These chickens had symptoms, and 13 died or were culled by 4 weeks of age. *Salmonella gallinarum-pullorum* was cultured from these birds, and the case was diagnosed as pullorum disease. Chicks with pullorum disease from cases 1 and 2 also came from hatchery A.

Case 5 occurred on a farm in Lusaka that kept five layer flocks of 600–1000 birds each. The chickens in two of the five flocks, 31- and 42-wk-old each, had clinical symptoms. Despite medication with furazolidone, 303 out of 680 chickens of 31-wk-old flock and 120 out of 660 chickens of the 42-wk-old flock died within 1 mo. At necropsy, white toci of miliary type in the liver with perihepatitis was observed (Fig. 2). The isolates of *S. gallinarum-pullorum* in this case fermented maltose and dulcitol. The disease was diagnosed as fowl typhoid. The chickens involved originated from hatchery C in Lusaka.

Case 6 occurred on a farm in Kitwe, 359 km north of Lusaka, where 10,000 layer chickens were kept in four flocks. The chickens in those flocks, 7-, 9-, 12-, and 18-mo-old each, had clinical signs. Although they were treated with oxytetracycline and furazolidone, 2400 chickens died. The incidence was diagnosed as fowl

Table 1. Outbreaks of *S. gallinarum-pullorum* infections in Zambia during the period 1990–91.

Case	Date of outbreak	Breed[A]	Age of infected birds	Number of birds		Clinical findings	Pathologic findings	Diagnosis
				In flock	Died			
1	August 1990	Broiler (A)	1 day old	1500	1000	Diarrhea, weakness, survivors culled	White nodules in the heart, gizzard, and intestines	Pullorum disease
2	August 1990	Broiler (A)	1 day old	100	50	Diarrhea, drooping of wings, survivors culled	Omphalitis, enlarged liver and spleen, retained egg yolk	Pullorum disease
3	January 1991	Broiler (B)	1 day old	2000	932	Diarrhea, weakness, survivors culled	Omphalitis, retained egg yolk, yellowish liver	Pullorum disease
4	March 1991	Parent stock[B]	4 wk	2000	13	Weakness, emaciation, survivors culled	Enlarged liver with white foci, enlarged spleen, enteritis	Pullorum disease
5	February 1990	Layer (C)	31 & 42 wk	1340	423	Diarrhea, somnolence, reduced egg production	White foci in the liver, enlarged spleen, enteritis, misshapen ovary	Fowl typhoid
6	April 1990	Layer (D)	7, 9, 12, & 18 mo	10,000	2400	General weakness, reduced egg production	Peritonitis, enlarged spleen, enlarged liver with white foci, misshapen ovary	Fowl typhoid
7	July 1990	Layer (C)	30 wk	5000	NT[C]	Diarrhea, ruffled feathers, reduced egg production	Enlarged liver with white foci, enlarged spleen, misshapen ovary	Fowl typhoid
8	December 1990	Layer (C)	20 wk	800	200	Reduced egg production, survivors culled	Enlarged liver and spleen with white foci, misshapen ovary	Fowl typhoid
9	February 1991	Layer (unknown)	50 wk	2500	250	Diarrhea, reduced egg production	Enlarged liver and spleen with white foci	Fowl typhoid
10	January 1991	Native (home)	5 mo	20	2	Emaciation, weakness	Greenish liver, enlarged spleen	Fowl typhoid

[A]Letter designation of hatchery from which birds originated is included in parentheses.
[B]The farm involved was hatchery A for broiler.
[C]Definite number was not available but mortality was high.

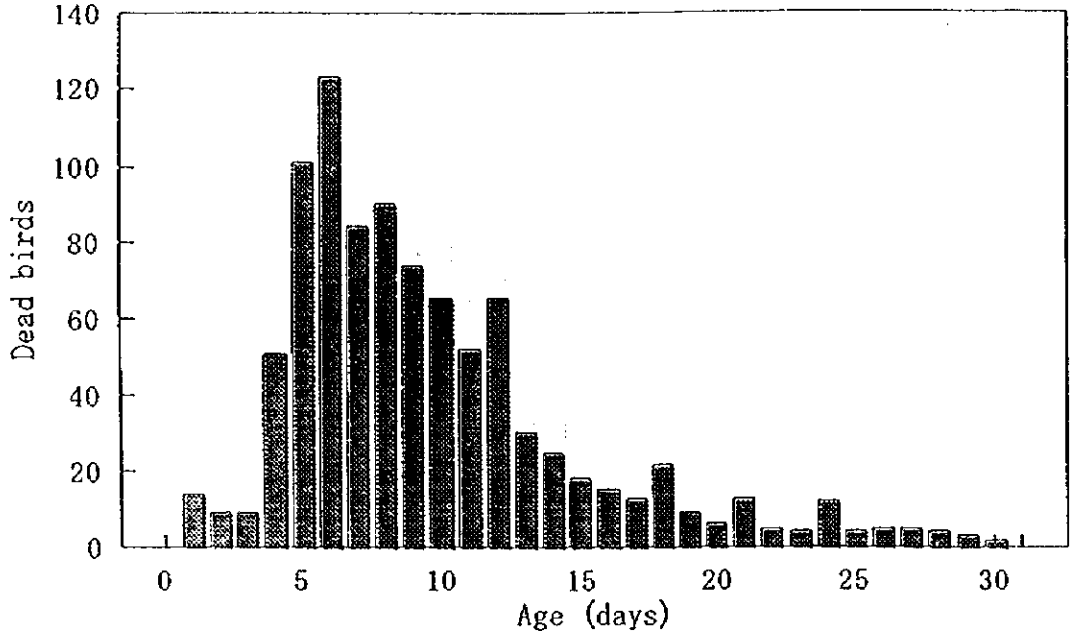

Fig. 1. The number of dead chickens in Case 3 was confined to the first few weeks, indicating that the incidence was pullorum disease.

typhoid. The chickens involved originated from hatchery D in Kitwe.

Case 7 was observed on a farm in Luanshya, 331 km north of Lusaka, keeping 5000 layers. Initially, 10,000 1-day-old chicks originating from hatchery C in Lusaka were reared to 16 wk of age on the egg production unit in Lusaka; half of them were then transferred to the farm in Luanshya. These birds appeared healthy and egg production increased toward 70%. Then many birds displayed symptoms such as reduced egg production (25%) and high mortality at the age of 30 wk. After a few weeks, the chickens partially recovered and egg production increased, but only to 50%–55%. The incidence was diagnosed as fowl typhoid.

The other half of the 10,000 chickens that originated from the same batch of 1-day-old chicks and were retained on the egg production unit in Lusaka were quite normal. Moreover, the farm owner in Luanshya had been experiencing a similar pattern of the disease with each newly introduced flock that was transferred from Lusaka at the age of laying.

Case 8 occurred on a farm in Kabwe, 110 km north of Lusaka, where 4000 layers were kept in 10 pens. Eight hundred chickens in 2 of the 10 pens were affected after 20 wk of age, and egg production was reduced from 60% to 40%. The chickens were treated with oxytetracycline and furazolidone; however, no efficacy was observed. The disease was diagnosed as fowl typhoid. All the birds that remained in the two pens were culled. The chickens came from hatchery C.

Case 9 occurred on a farm in Lusaka that kept 8000 layer chickens in three flocks. In one of the three flocks, 2500 chickens had clinical symptoms at the age of 50 wk and 250 birds died. The incidence was diagnosed as fowl typhoid.

Case 10 was seen among 20 village chickens, around 5 mo old, reared on the garden of a private house in Lusaka. Ten birds manifested symptoms, and two of them died. This case was diagnosed as fowl typhoid according to morbidity, age, and pathologic lesions.

Transmission of *S. gallinarum*-pullorum infections. As shown in Table 1, pullorum disease was indicated in two cases (Cases 1 and 2) and was apparently transmitted by hatchery A in Lusaka, where parent stock birds were infected (Case 4). The infection in 1-day-old chicks in Case 3 was probably introduced from hatchery B in Lusaka. On the other hand, five fowl typhoid cases (Cases 5–9) were seen in laying chickens, and the most probable source of the infection was considered to be environmental contamination on the farms. Although three farms (Cases 5, 7, and 8) had introduced layer birds directly or indirectly from hatchery C in Lusaka, there were

no sufficient data indicating fowl typhoid prevalent among breeder stock at the hatchery.

DISCUSSION

Serologically, *S. gallinarum-pullorum* has been considered a single *Salmonella* having O antigens of 1, 9, 12 (4,6). However, workers in the U.S.A. have divided its infection into two different diseases caused by two distinct pathogens with significantly different biochemical and epizootiologic characteristics: pullorum disease by *S. pullorum* and fowl typhoid by *S. gallinarum* (9).

Although essential biochemical properties for differentiation of *S. pullorum* from *S. gallinarum* were not examined in the present study, Cases 1–3 were diagnosed as pullorum disease because they manifested clinical symptoms from the age of 1 day and their mortalities were confined to the first 2–3 wk of life, as shown in Fig. 1 (6,9). Moreover, white nodules in the heart, gizzard, and intestines observed in Case 1 are typical of gross lesions seen in pullorum disease (6,9).

On the other hand, Cases 5–9 were diagnosed as fowl typhoid because they manifested clinical symptoms and high mortality later than 20 wk of age. The chickens had white foci in the liver, enlargement of the spleen, and misshapen ovaries. Furthermore, the isolates of Case 5 fermented maltose and dulcitol, indicating partial evidence of essential biochemical properties for *S. gallinarum.*

So far, 17 *Salmonella* serovars have been isolated from chickens and other birds in Zambia (2,3,8,10), and *S. gallinarum-pullorum* is still the most prominent serovar. Sharma *et al.* (8) investigated the prevalence of avian salmonellosis during the period 1976–85 and reported that 781 out of 806 *Salmonella* isolations were *S. gallinarum-pullorum,* although they described the incidence only as fowl typhoid. In the present study, we attempted to divide *S. gallinarum-pullorum* infections into two different groups, pullorum disease and fowl typhoid, based on epizootiologic and pathologic findings.

The present study indicated that some hatcheries play an important role in the transmission of *S. gallinarum-pullorum* infections and confirmed that some layer farms were contaminated by fowl typhoid, especially at the onset of lay. To control these *Salmonella* infections in Zambia, testing of breeder flocks with slaughter of infected flocks, as well as hygienic procedures such as disinfection of contamined farms, should be applied more strictly.

Fig. 2. White foci of the miliary type in the liver with perihepatitis in Case 5 is one of the gross lesions seen in fowl typhoid.

REFERENCES

1. Berchieri, A., Jr., A. M. Iba, and P. A. Barrow. Examination by ELISA of sera obtained from chicken breeder and layer flocks showing evidence of fowl typhoid or pullorum disease. Avian Pathol. 24:411–420. 1995.

2. Falade, S., G. Sato, W. Ulaya, and L. Mwanza. Serovars and antibiotic sensitivity patterns of Salmonella strains isolated from domestic animals in Zambia. Zimbabwe Vet. J. 20:19–22. 1989.

3. Gaspar, P., and P. Hrabeta. A survey of salmonellosis in domestic animals in Zambia. Bull. Anim. Health Prod. Afr. 25:61–64. 1977.

4. Le Minor, L. Genus III. Salmonella Lignieres. In: Bergey's manual of systematic bacteriology, vol. 1, 9th ed. N. Krieg and J. G. Holt, eds. Williams & Wilkin, London. pp. 427–458. 1984.

5. Pomeroy, B. S., and K. V. Nagaraja. Fowl typhoid. In: Diseases of poultry, 9th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr., eds. Iowa State University Press, Ames. pp. 86–99. 1991.

6. Sato, S. Pullorum disease. In: Diagnosis of poultry diseases. T. Horiuchi, ed. Ienohikari Kyoukai, Tokyo (in Japanese). pp. 309–342. 1982.

7. Sato, Y., and I. Phiri. Avian diseases diagnosed at the School of Veterinary Medicine, University of Zambia. UNZA Veterinarian 2:3–4. 1991.

8. Sharma, R. N., G. S. Pandey, A. Q. Khan, and B. K. T. Francis. Salmonella isolation from poultry in the Republic of Zambia. Bull. Anim. Health Prod. Afr. 39:173–175. 1991.

9. Snoeyenbos, G. H. Pullorum disease. In: Diseases in poultry, 9th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr., eds. Iowa State University Press, Ames. pp. 73–86. 1991.

10. Tuchili, L., W. Ulaya, Y. Kato, and C. Kaneuchi. Recent characterization of Salmonella strains isolated from chickens in Zambia. J. Vet. Med. Sci. 58: 77–78. 1996.

SEROLOGICAL SURVEY OF *Mycoplasma gallisepticum* AND *Mycoplasma synoviae* INFECTION IN CHICKENS IN ZAMBIA

Pandey, G. S. and Hasegawa, H.

Samora Machel School of Veterinary Medicine, University of Zambia, P. O. Box 32379, Lusaka, Zambia.

Mycoplasma gallisepticum, which causes chronic respiratory disease (CRD) in chickens and sinusitis in turkeys, and *Mycoplasma synoviae* infection, which causes infectious synovitis, constitute a problem in poultry. The infection has world wide distribution and is of considerable economic importance. Economic losses occur due to mortality, downgrading of carcasses, reduced feed and egg production, poor hatchability and increased medication costs. Avian mycoplasmosis (CRD) has been reported in the Annual Reports of the Department of Veterinary and Tsetse Control Services in the Republic of Zambia since 1970. The disease has been diagnosed on the basis of clinical observations and in most cases on *post mortem* findings. However, systematic planned serological work has been lacking in Zambia and so far no attempt to isolate *M. gallisepticum* and *M. synoviae* has been undertaken in this country. Therefore, an attempt was made to detect the presence of antibodies to pathogenic *Mycoplasma* in sera of chicken from all over Zambia. Two thousand seven hundred fifty-five and 2287 chicken sera were screened serologically for the presence of antibodies to *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, respectively. The serum samples were collected from poultry breeder farms, commercial layer/broiler farms and local village chickens from all over the country during 1993 and 1994. The samples were examined using a rapid serum agglutination test with crystal violet standardised *M. gallisepticum* and *M. synoviae* antigens. Fifty-seven percent of the total samples examined were found positive for *M. gallisepticum*. Breeder farms had the highest (75%) seroprevalence followed by commercial farms (65%) and local chickens (41%). Over all, seroprevalence for *M. synoviae* antibody was found to be 44%. Breeder farms had 66% seroprevalence, followed by 48% in commercial farms and 31% in local village chickens. Eight hundred and eighty samples were seropositive for both antibodies, 689 for *M. gallisepticum* and 128 for *M. synoviae*. However the plate test used in the present study is not very specific and shows cross reaction between *M. gallisepticum* and *M. synoviae*. Also, the sera used were kept frozen and freezing and thawing of chicken sera causes non-specific positive reaction to *M. gallisepticum* and *M. synoviae* in the rapid slide test. Therefore it is possible that many of the positive reactions recorded could have been non-specific. There is need to study more specific serodiagnostic techniques and isolation. The present study reports the seroprevalence of *M. gallisepticum* and *M. synoviae* in wide areas of Zambia. It can be concluded that mycoplasmosis is wide spread in Zambia. Isolation attempts has been suggested and disease prevalence could be reduced through regular testing, elimination of positive breeder chickens, and maintenance of hygiene in the hatcheries and on farms.

DETECTION OF SALMONELLA DNA IN CHICKEN EMBRYOS AND ENVIRONMENTAL SAMPLES BY POLYMERASE CHAIN REACTION

[1]Tuchili, L. M., [2]Kodama, H., [1]Sharma, R. N., [2]Takatori, I., [1]Pandey, G. S., [1]Kabilika, S. [2]Mukamoto, M., [2]Tsuji, S. and [2]Baba, T.

[1]Samora Machel School of Veterinary Medicine, University of Zambia, Box 32379, Lusaka, Zambia; [2]Laboratory of Veterinary Immunology, Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, Sakai 593, Osaka, Japan.

The use of polymerase chain reaction (PCR) as a diagnostic tool for the detection of *Salmonella* in the yolk of infertile eggs, dead-in-shell chicken embryos, and environmental samples was compared with isolation methods. *Salmonella* DNA amplification from the yolk of infertile eggs of dead-in-shell chicken embryos was done directly while that from environmental samples was done after DNA extraction. Bacterial isolation was done by inoculating samples into enrichment media and direct plating on blood agar and MacConkey agar. With a pair of primers specific for *Salmonella pho*E gene a 365-bp specific fragment could be detected directly from the yolk of dead-in-shell embryos. Out of 45 embryo samples, 20 (44.4%) were found positive for *Salmonella* by direct PCR method compared to 11 (24.3%) by isolation in enrichment media. *Salmonella* DNA could also be detected from infertile eggs (28.6%), chicken faeces (27.1%), liner (21.4%) and fluff (35.3%). The amplified DNA was sequenced and showed substantial homology to the *S. typhimurium pho*E gene. The results show that PCR is a more sensitive method than the conventional isolation procedures in *Salmonella* detection.

Public Health, Surgery Reproduction, Wildlife

DOG ECOLOGY SURVEILLANCE AND RESEACH FOR CANINE AND HUMAN RABIES CONTROL IN ZAMBIA

[1]Munang'andu, H., [1]Nakanta, P., [2]Bwalya, J., [2]Ulaya, W. D., [2]Sakala, R. M. and [2]Fujikura, T.

[1]Central Veterinary Research Institute, Balmoral, Lusaka, Zambia;
[2]Samora Machel School of Veterinary Medicine, University of Zambia, P. O. Box 32379, Lusaka, Zambia.

Canine and human rabies is prevailing to date in Zambia. A dog ecology surveillance has been conducted in traditional farming village settings to facilitate development of rabies control strategies in rural areas. A surveillance has been carried out in 21 traditional farming areas in 9 provinces in Zambia including 5702 people in 827 households. Door to door interviews were conducted with a questionnaire paper comprising 50 simple questions. One thousand and fifteen dogs were kept in 452 household (2.2 dogs/household). A dog is shared by 5.6 persons on average. People keep dogs for security reasons (68.4%) and used for hunting and herding their livestock. Those dogs are unrestricted during the night (79.2%) and fed nshima (60.2%) and left overs. However, people are bitten very commonly by dogs (114/590, 19.3%) and 2-6 times more often by neighbouring/stray dogs. Rabies vaccination coverage was as low as 25.0% (109/436). The team was informed of recent human rabies cases (20 lethal cases) in 4 surveyed areas. Dogs also have contact with many wild animals such as jackals. The present surveillance indicates that dog population management/control with improved vaccination coverage is vital in controlling zoonotic rabies in Zambia.

ISOLATION OF *Bacillus anthracis* FROM ENVIRONMENTAL SAMPLES COLLECTED FROM ANTHRAX ENDEMIC AREAS OF WESTERN PROVINCE, ZAMBIA

[1]Tuchili, L. M., [1]Bwalya, J., [1]Ulaya, W. D., [1]Fujikura, T., [2]Bbalo, G. C., [2]Masumbu, A., [2]Tembo, W., [2]Zambara, M., [2]Dyaunka, V. C. and [2]Mkumba, P.

[1]Samora Machel School of Veterinary Medicine, University of Zambia, P. O. Box 32379, Lusaka, Zambia; [2]Department of Animal Health and Production, Mongu, Kaoma, Senanga, Lukulu, Kalabo, Western Province, Zambia.

Anthrax is a zoonosis transmissible from animals to humans through contaminated meat, animal products, and other environmental materials. *Bacillus anthracis* (the causative organism) remains for long periods in these

34

contaminated materials. Research has been conducted to elucidate the transmission cycle of *B. anthracis* in endemic areas in Western Province by collecting samples such as soil, hide, horns, bones, and dried meat which have been potential sources of anthrax infection in the area. Three strains of *B. anthracis* were isolated from the soil and hide samples collected from Muhunguo Village (Kalabo district). PLET medium, a selective medium paralleled with conventional blood agar, is used for isolation. Isolated *B. anthracis* was observed with typical thick capsules and lethal pathogenicity to mice. The present authors are in the process of developing a monitoring and surveillance system of contaminated environments for risk identification and assessment against anthrax.

PRELIMINARY SURVEY OF TRADITIONAL FARMING VILLAGES IN MAZABUKA DISTRICT: A VETERINARY PUBLIC HEALTH APPROACH

[1]Sakala, R. M., [1]Bwalya, J. M., [1]Pandey, G. S., [1]Fujikura, T., [2]Siame, C. M., [2]Mululuma, G., [2]Lubasi, M. M. and [2]Hachitema, D.

[1]Samora Machel School of Veterinary Medicine, UNZA, P. O. Box 32379, Lusaka, Zambia;
[2]District Veterinary Office, Mazabuka, Southern Province, Zambia.

A preliminary survey was conducted with a view to promote primary health care (PHC) of the villagers. It was aimed at looking into various Public Health aspects of traditional farming villages so that appropriate control measures could be implemented. Two areas (Chikankata and Chalimbana; Magoye) were selected without any specifications. The data was collected using a questionnaire with 49 questions. From Chikankata area 29 households were interviewed, and data was collected from 28 households Chalimbana area. It was observed that more people have suffered from tuberculosis in Chikankata (17.3%) than in Chalimbana (11.1%) and that there are more individuals who drink raw milk in the former (26/29) than in the latter (19/36). A wider surveillance could elucidate this. Most of the drinking water comes from either wells or bore holes. This water is known to be relatively safe. However, the villagers have to cover more than 3 km to collect water. In Chikankata some families get water from the river from which most of the animals drink (22/29 herds). Unfortunately not many families in both communities boil water for drinking. People should be encouraged to do so, especially since some individuals still use the bush as a toilet (5/29, 15/36). There are more families with toilets at Chalimbana than Chikankata and more individuals wash hands after using the toilet in the former than in the latter. In both communities, observed causes of diarrhoea associated with food poisoning are varied. However, local drinks (beer included) and meat are some of the usual causes. Eating raw eggs and cadavers are risk factors which could be associated with food poisoning, as well as the transmission and perpetuation of zoonotic diseases. Both communities eat cadavers (15/29, 14/36). Collection of information from rural Health Centres could elucidate the situation concerning the foods commonly associated with food poisoning in the area and how this could be prevented. The information collected in this survey will be used as the basis for wider surveillance.

GROSS REPRODUCTIVE ANOMALIES AND CHROMOSOMAL CHIMERISM IN A FREEMARTIN HEIFER-A CASE REPORT

Makondo, K., Mwanza, A., Zulu, V. C., Patel, O. V. and Hishinuma, H.

Samora Machel School of Veterinary Medicine, University of Zambia, P. O. Box 32379, Lusaka, Zambia

A 330 kg Friesian-Holstein heifer of about 18 months of age was presented for a routine pre-breeding examination. Preliminary external and per rectum examination revealed abnormal development of the genital tract. The length of the vulva from the lower to the upper commissure was 5.5 cm. No significant tuft of vulvar hair was observed at the lower commissure of the vulva and the size of the clitoris appeared normal. The length of the vagina was 8.0 cm and it had no blind pouch at the posterior end. Digital palpation per rectum revealed absence of a cervix, uterus, oviduct and gonads (ovaries). Instead rudimentary tubes were detected from the anterior end of the short vagina and the firm consistency of the cervix was absent. A tentative diagnosis of Freemartin syndrome was made from these observations. Subsequent per rectum scanning using a 7.5 MHz real-time linear transducer revealed a small, short echoic tubular structure thought to be the vagina but the distinctive hyperechoic cervix was not evident. The characteristic echo-texture of the uterus and oviducts were not discernible, instead small tubular-like structures with anechoic centres were observed. The ovaries could not be detected by ultrasonography. Further investigation by chromosomal analysis on cultured blood showed a varying degree of XX:XY chimerism. The Freemartin syndrome was confirmed in this heifer through external genital examination, rectal palpation, ultrasonography and karyotyping.

ORTHOPAEDIC CONDITIONS OF THE PELVIC GIRDLE IN COMPANION ANIMALS

Omamegbe, J. O., Chiti, L., Muleya, J., Hashimoto H. and Hankanga, C.

Samora Machel School of Veterinary Medicine, University of Zambia, P. O. Box 32379, Lusaka, Zambia.

Orthopaedic conditions of the pelvic girdle (excluding hip dysplasia/dislocations) have been said to account for up to 20% of all orthopaedic conditions seen in companion animals. These conditions include fractures of the bones of the pelvic girdle, ilio-sacral joint dislocations and, to some extent, fractures of the sacrum. This paper is based on orthopaedic problems of the pelvis of 55 dogs and 7 cats seen at the Small Animal Clinic of the School of Veterinary Medicine at the University of Zambia between 1989 and 1996. The cases are presented in two groups, 39 cases seen between 1989 and 1994 which were studied retrospectively and are presented with respect to age, sex, breed aetiology and type of pelvis pathology encountered. Twenty three cases seen and treated between 1995 and 1996 are presented as first hand cases with regard to radiographic presentation, management pattern and case outcome at the time of hospital discharge. The commonest cause of orthopaedic conditions of the pelvic girdle in both groups was motor vehicle-related accidents or trauma. The most frequent clinical signs were hind limb dysfunction shown as inability to stand in very severe cases, and weight bearing dysfunction in one or both limbs in relatively mild cases. A tentative diagnosis of fractures of the pelvic bones and ilio-sacral luxations was based on presenting history and findings at clinical examination. Confirmation of the diagnosis by radiographic case study was straight forward. Management of these cases was by either of two main protocols. They were managed either by strict cage rest or by open reduction and fixation with bone screws for ilio-sacral luxations and bone plating for pelvic bone fractures with varying degrees of cage restriction. At the stage of discharge from our clinic, 19 out of the 28 (67.9%) cases treated by open reduction and fixation method had gained sufficient ability to bear weight on the hind limbs and ambulation was considered acceptable.

SURGICAL APPROACHES AND REPAIR OF ILIAC FRACTURE AND SACROILIAC SEPARATION IN DOGS

[1]Hashimoto, H., [1]Nalumbamba, K., [1]Chiti, L., [1]Thomicroft, T. and [2]Koie, H

[1]Samora Machel School of Veterinary Medicine, University of Zambia, P. O. Box 32379, Lusaka, Zambia;
[2]Nihon University Animal Medical Centre, 1866 Kameino Fujisawa Kanagawa, Japan.

Iliac fractures in dogs are caused by major trauma such as following road traffic accidents. Surgical repair of iliac fractures is required frequently because of problems related to the patient's locomotion and reproduction. This report outlines a logical approach to the management of iliac fractures and sacroiliac separations in dogs. A lateral approach is the best method of exposure for all areas of the ilium. The skin incision starts from a little cranial to the iliac crest and ends at the greater trochanter. The subcutaneous tissue and fat should be separated. Thereafter the fascia between the middle gluteal muscle and the tensor fascia lata can be seen. After the fascia has been incised completely, the middle gluteal muscle becomes visible. The middle gluteal muscle and the tensor fascia lata are then separated completely. This leads to exposure of the lower part of the ilium as well as the lower part of the deep gluteal muscle. The middle gluteal muscle may now be separated from the cranial ilium with a curette and scissors. While the middle gluteal muscle is being separated the deep gluteal may be separated also. The separation of the muscles of the ilium from the bone should be done from the cranial ilium to the greater trochanter. An extensive exposure is required for the repair of iliac shaft fractures and sacroiliac separations. In the case of iliac shaft fractures, bone plating will be possible at this time. For the reduction of sacroiliac separations, extensive exposure between the lower ilium and the muscle which is attached to the ilium is required. The use of bone screws is recommended for the fixation. Many methods of fixation have been used for iliac fractures and sacroiliac separations. In this report, the use of bone plates and screws are presented. In every case, these methods were successful and good stability was obtained

SURGICAL APPROUCHES AND REPAIR OF ILLIAC FRACTURE AND SACROILIAC SEPARATION IN DOGS

Hashimoto,H. Nalumbamba,K. Chiti,L. Thornicroft,T. and Koie,H.
P.O.Box 32379,Lusaka,Zambia
Nihon University Animal Medical Center,1866 Kameino Fujisawa
Kanagawa,Japan

INTRODUCTION

Fractures of the pelvis are very common in dogs and they are mainly caused by major traumas including traffic accidents. One of studies records that 20 to 30 % of all fractures in dogs are pulvic fractures.

Veterinarians are frequently required to repair these fractures because of the problems related to the patient's locomotion and reproduction and paticularly the repairs of iliac fractures and sacroiliac separation which have a big role in bearing the patient's weight. Some authors have described methods for the repair of these fractures but every method has advantages and disadvantages. However in my opinion these methods are not enough to explain a logical approuch to the management of iliac fractures and sacroiliac separation.

The objective of this report is to explain a logical approach to fixation of the above mentioned fractures and separations

THE APPROACHES TO ILIAC SHAFT AND SACROILIAC JOINT

After aseptic preparations,the patient is laid down in lateral recumbency. Two points have to be marked out before starting a skin incision. These are : the iliac crest and the greater trochanter. During the operation bleeding must be controled adequately. The subcutaneous tissues and fats should be separated for next procedure.

After the subcutaneous tissues have been completely separated,the fascia between the middle gluteal muscle and the tensor fascia latae muscle can be seen. This fascia is an important mark for the approach to the iliac shaft because the middle gluteal muscle and the tensor fascia latae muscle attach to the ilium.

The fascia is then incised completely with scissors. After the incision,the border between the middle gluteal muscle and the tensor fascia latae muscle becomes visible and can then be completely separated. This leads to the exposure of the lower part of the ilium as well as the lower part of the deep gluteal muscle.

When the middle and deep glutial muscles have been separated from the iliac shaft completely with a bone curret,the separated gluteal muscles have to be lifted with forceps. The iliac shaft can then be seen.

The separation of the muscles from the iliac shaft should be done from the cranial ilium to the greater trochanter to repair the iliac fractures. For good repair,enough exposure is needed. It means that the middle and deep gluteal muscles have to be completely separated.

In the case of reducing sacroiliac separations,an extensive exposure between the cranial and lower ilium and the muscle which is attached to the ilium is required.These separations let the ilium free. When it can be done,the sacroiliac joint can be touched with the surgeon's fingers to reduce it.

CAES STUDY

The fist caes is that of a two years old mongrel female. The body weight is 6 kg. There are fractures of the iliac shaft and the pubis on the left side of the dog. The sacroiliac joint and the acetabulum are intact but the left iliac shaft and the pubis are broken. The iliac fracture was reduced and fixed with a bone plate and screws. The repair of the iliam shaft fracture only gives good reduction to the whole pelvis. In this case since bone plating also gives it very good stability,the fixation of the pubic fracture was not necessary.

The secound case is that of a two years old mongrel male.The body weight is twenty 2 kg. There are fractures of the iliac shaft and the pubis on the right side of the dog. Almost the same as the case 1, the sacroiliac joint is intact and acetabulum is also intact. The iliac shaft fracture was reduced and fixed with a bone plate and screws. Since this fixation gives good stability to the pubis, the fixation was not required.

The last case is that of a seven month old , jack russel, male dog. The body weight is 2 kg. There are the left sacroiliac joint dislocation and the acetabulum fracture. The right acetabulum is also broken. The sacroiliac joint was reduced and fixed with two bone screws. In my opinion, the use of bone screws is the best way of fixing this type of separation.

CONCLUSION

On the above mentioned cases,the use of bone plate and screws gives good stability to the iliac shaft fractures and the sacloiliac dislocation. In my opinion, the procedure of operation is easier than other methods.

Plevic fractures are ofen left without operation because of the difficulties in operation.However,most sacroiliac joint dislocation, iliac fractures and acetabulam fractures require reduction and fixation because these parts

allow the transfer of body weight from the spine and from the hind legs.

Therefore surgeons should not hesitate operating on pelvic fractures if by doing so,a patient will greatly be helped. A logical approach makes reduction and fixation of pelvic fractures very easy and makesthe patient very comfatable.

Finally I'd like to appreciate all staff who have assisted me,during operating.

SURGICAL REPAIR OF FRACTURES USING BONE PLATES AND SCREWS IN DOGS

Hashimoto.H,Nalumbamba.K,Chiti.L,Thornicroft.T,Hankangar.C,H.Phiri,B.Kubi,C and Omamegbe.J.O.

Samora Machel School of Veterinary Medicine,University of Zambia.P.O.Box 32379,Lusaka,Zambia.

The repair of bone fractures occupies a lot of surgical fields. The owner of the patient whose bone has broken requires our help.

Fractures happen in various places, starting from the head to the finger. There are several methods of surgical repair but in my opinion, bone plating and screwing are the best methods to repair various type of fractures.

In this presentation, five cases will be ilustrated. Case 1 is a mandibula fracture which was fixed with a bone plate and screws. The progress after the operation was very good . Case 2 is a humeral fracture which was fixed with a bone plate and screws. Since the patient was very young (three months old), the cortex of the humerus was very thin and the fracture was very near to the growth plate. Since it was a distal fracture , there was very narrow space for plating and pinning. Case 3 is a radial dislocation and ulna fracture in a small dog. The radial dislocation was reduced and fixed with a screw. The ulna was reduced and fixed with a bone plate and screws. Case 4 is iliac and pubis fractures. Repair of the iliac fracture also gives good stability to the pubis. Case 5 is a tibia communited fracture. Firstlly, this fracture was repared with a bone plate and screws but two days later the plate was bent and it was operated again. During the operation a bone plate and a pin were used.

Bone plates and screws give good stability to the above mentioned fractures. Bone plates and screws scarcely damage the bone marrow and the growth plate. They are adaptable to distal fractures and bone fractures which pinning hardly work on, such as mandibula and iliac fractures. Also they are adaptable to joint dislocations. Bone plating and screwing in combination with pinning are adaptable to multiple and comminuted fractures.

REPAIR OF MULTIPLE PELVIC FRACTURES IN A DOG

[1]Kubi C., [2]Hashimoto H. and [1]Nalubamba K. S.

[1]Departments of Clinical Studies and [2]Disease control, Samora Machel School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka.

ABSTRACT

A four year old male Dachshund involved in a traffic accident was presented to the Small Animal Clinic of the School of Veterinary Medicine with a complaint of lameness and falling over on attempts to stand on its hind quarters. Physical examination revealed non-weight bearing lameness of the hind limbs. Radiology revealed bilateral ilial fractures, fracture of the pubic contribution of the acetabulum and sacroiliac dislocation. Repair of the fractures was achieved in two stages, beginning with the plating of the right ilial fracture, followed three days later, by the plating of the left ilial fracture and excision arthroplasty of the left femoral head. Good fracture reduction was observed on post-surgical radiographs. The dog was able to ambulate on both limbs without difficulty following two weeks of confinement.

A Case Report : Wound management using Chitipack[R].

[1] Hankanga C., [2]Hashimoto H., [1]Kubi C. and [1]Phiri B
[1] Department of Clinical Studies and [2] department of Disease Control, Samora Machel School of Veterinary Medicine, University of Zambia, Box 32379 Lusaka.

Abstract

A 6 month old female Ridgeback was presented to the University Small Animal Clinic following a road traffic accident. The dog had suffered multiple open fractures involving the metacarpals and severe soft-tissue trauma of the left forelimb. Radiographs revealed transverse fractures of the first digits of all the metacarpal bones. The fractures were repaired by intra-medullary pinning and the severe soft-tissue using daily dry bandaging and Chitipack[R] for three days using a three day cycle. Chitipack[R] is a crab-shell derivative which promotes wound granulation. Chlorhexidine (2%) was used as an antiseptic for wet bandaging. Healthy granulation tissue was noted by day 4 and by day 10 most of the exposed bones had been covered by healthy granulation tissue. Serial photographs were taken to show the different stages of wound healing.

CONTACT BETWEEN DOMESTIC ANIMALS AND WILDLIFE-USING COMPUTER IMAGING ANALYSIS IN LOCHINVAR NATIONAL PARK OF ZAMBIA

A.K. SUZUKI
NATIONAL INSTITUTE FOR ENVIRONMENTAL STUDIES
JAPAN

ABSTRACT

To clarify the contact or mixing between domestic animal and wildlife, computer imaging analysis method was used. I chose 255 points in the 32 photographs that were suitable for the analysis. I got all of the photographs exposed in the Lochinvar National Park during 1991 and 1992. The exposures were arranged by the Cannon Camera (IOS10 with 300 mm AF lens). I could get a minute characteristics of the lens and the detail advice from Cannon Camera Co. I memorised the photographs in the computer's extendel memory (16 mega bytes) through image reader (NEC-PC, Japan) using a computer's image analysis program (SAYCO in Win, Tech., Japan). On the display of the computer, I got a co-ordinate (x,y) in a unit of pixel or dot. I could calculate easily the distance between two points from the co-ordinate of the two points.

In the dry season, wildlife gathered each species separately. I could find the mixing groups of cattle and Kafue Lechwe in a picture. I could calculate the distance from the most closed cattle and the Kafue Lechwe of 16 groups. I found that the nearest distance was within 50 metres (3 of 8 groups), and within 100 meters (4 of 8 groups). Some groups of cattle broke straight into the scattering wildlife around small water streams.

In the rainy season, I could not recognise the same contact or mixing. These results suggest that the condition is not very good, because it has a high potential to affect and spread the common diseases mutually.

2.10. BRUCELLOSIS IN A KAFUE LECHWE AND EAST COST FEVER IN AN ELAND - PATHOLOGICAL ASPECT

K. MATSUKAWA
SCHOOL OF VETERINARY MEDICINE, UNIVERSITY OF ZAMBIA
P. O. BOX 32379, Lusaka, Zambia

ABSTRACT

Fourteen testes of seven Kafue lechwes were collected during August 1994. Six animals had almost same-sized testis averaged 6.8x4.4x4.0 cm, but one case had regular-sized right testicle and left enlarged to 8x7x5 cm.

Histologically, the testes of the former six animals did not show active spermatogenesis and inflammation. In the latter, seminiferous tubules were atrophied and lined with edematous Sertoli cells and aspermatogenetis. Many tubules contained thick pus and irregular lesions Intertubular interstitium was granulomatously widened, hyperemic, infiltrated with monocyte and fibrous proliferation. In the epididymis also, the chronic progressive granulomatous inflammation was observed. This testis was diagnosed as brucellosis histopathologically. The opposite side did not show any inflammation.

A four month-old male eland kept at a game farm in Kabwe city died after two days of sickness and autopsied 4 hours after death on 14th February 1995.

Autopsy for diagnosis included: 1. anemia, 2. medium splenomegaly, 3. lymph nodes hyperplasia, in cervical area, 4. cloudy swelling of liver and kidney, 5. adrenal cortex hyperplasia, 6. subendocardial paintbrushed hemorrhage, 7. foamy exudation in air duct, 8. slight jaundice, 9. tick infestation.

In the blood smear, there were several comma-like or ovoid intracellular bodies (protozoa) in most erythrocytes, and schizonts in some leukocytes.

Histologically, schizont-laden gigantic cells were found in hepatic sinusoid and lymph node sinus.

As a result, the case was diagnosed as East Coast Fever (Theileriosis).

Journal of Wildlife Diseases, 30(4), 1994, pp. 586–588

Dermatophilosis (Cutaneous Streptothricosis) in Kafue Lechwe (*Kobus leche kafuensis*)

G. S. Pandey,[1] A. Mweene,[1] A. K. Suzuki,[2] A. Nambota,[1] and T. Kaji,[1] [1] School of Veterinary Medicine, Department of Disease Control, University of Zambia, P.O. Box 32379, Lusaka, Zambia; [2] National Institute for Environmental Studies, 16 2 Onogawa, Tsukuba Science City, Ibaraki 305, Japan

ABSTRACT: Extensive dermatitis caused by *Dermatophilus congolensis* was identified in two kafue lechwe (*Kobus leche kafuensis*) in Lochinvar National Park of Zambia. The lesions were characterized by thickening of the skin, crusts, and nodule formation. Almost all parts of the body were affected. Histologically there was an exudative dermatitis with acanthosis, parakeratosis, hyperkeratosis, and an exudate rich in neutrophils. This is the first known report of dermatophilosis in lechwe.

Key words: *Dermatophilus congolensis*; Kafue lechwe; *Kobus leche kafuensis*; Zambia.

Dermatophilosis is an exudative dermatitis caused by *Dermatophilus congolensis*, which affects a wide variety of animals, both wild and domesticated and occasionally humans. The disease commonly affects cattle, sheep, goats and domesticated equines in many parts of the world, often creating severe economic problems (Stewart, 1972; Yager and Scott, 1992). The disease also has been reported in the pig, cat, dog, donkey, and buffalo (*Syncerus caffer*) (Losos, 1986). The only non-mammalian host so far encountered are the Australian bearded lizards (*Amphibolurus barbatus*) (Montali et al., 1975) and marble lizard (*Calotes mystaceus*) (Anver et al., 1976). Human cases also have been reported (Dean et al., 1961). Our objective was to describe cutaneous streptothricosis in two free-living Kafue lechwe.

The Kafue lechwe (*Kobus leche kafuensis*), a medium size antelope, is found only in Kafue flats of Zambia. Lechwe are semi-aquatic in nature and prefer swamps and wetland habitat feeding on grass and water plants (Howard et al., 1984). Lochinvar National Park (16°03'S, 27°17'E) has an area of 480 km² and is a flood plain on the southern side of the Kafue river, a major habitat for Kafue lechwe. The other major wildlife in the park are zebra (*Equus burchelli*), buffalo (*Syncerus caffer*), wildebeest (*Connochaetes gnu*), kudu (*Tragelaphus strepsiceros*) and orbi (*Ourebia ourebi*). Domesticated animals, especially cattle, also enter the park for about 4 to 5 mo every year to share grazing and drinking of water during the dry season.

During September and October 1989, 92 adult lechwe were shot in a World Wide Fund for Nature sponsored study into the use of lechwe meat by local communities. The lechwe were examined for external and internal abnormalities to certify meat for public consumption. Two of the 92 lechwe had extensive skin lesions. The skin of the two lechwe showing cutaneous thickening, crusts and nodule formation was examined thoroughly. The surface of the skin was covered by exudative debris. The skin and hair felt greasy and had a dirty whitish-yellow coloration. There was little loss of hair and lesions were not visible until the carcass was examined closely. Almost all parts of the body were affected and characterized by exudative dermatitis. The paint brush lesion consisting of focal matting of hair by exudate was observed. Lesions ranged from small nodule-like formations to large areas with extensive accumulation of crusts. The lesions around the mouth, ear, hindlimb, forelimb, and tail resembled papillomas. Free nodular crusts also were entangled between hairs. Upon palpation the skin felt rough and when scabs were removed they took some hairs with them. Both carcasses were very emaciated. External lymph nodes were enlarged. Fibrosis of liver, enlarged thickened bile ducts full of trematodes resembling *Fasciola gigantica*, and the presence of mature amphistomes in the rumen were noted internally in both animals. Pieces of the skin and crusts from the two lechwe,

which were suspected of having mange, were evaluated further.

Skin scrapings containing crusts and purulent exudate were soaked in distilled water, smeared on glass slides and stained with Giemsa and Gram's stain (Carter and Cole, 1990). Two forms of organisms were noticed. One had hyphae 1 μm in width with transverse bands and the other had large structures measuring 3 to 5 μm in width. Degenerating neutrophils and epithelial cells were seen. No mange mites were seen in a skin scraping dissolved in 5% potassium hydroxide and examined microscopically.

Crusts were washed in sterile distilled water to remove superficial contaminating microorganism inoculated onto 5% sheep blood agar plates (Difco Company, Detroit, Michigan, USA), thioglycollate medium (Nissui Pharmaceutical Company, Tokyo, Japan) and Sabouraud's Agar (Difco Company). The cultures were incubated aerobically and anaerobically at 37 C. Bacterial identification was based on Carter and Cole (1990). A bacterial sensitivity test was conducted using Oxoid antimicrobial susceptibility test discs (Unipath Limited, Basingstoke, Hampshire, England) containing 50 μg chloramphenicol, 300 μg sulfonamide, 25 μg ampicillin, 10 μg penicillin, and 50 μg tetracyclin.

Dermatophilus congolensis was isolated from scrapings of both skins of lechwe. Colonies on blood agar were whitish, punctate, and hard; beta hemolysis was evident in 48 hr. Organisms did not grow on Sabouraud's Agar. Branching filaments and coccoid forms of the organisms were Gram-positive and non-acid fast. The isolates were sensitive to tetracycline, penicillin, chloramphenicol, and ampicillin, but resistant to sulfonamide. Samples of affected skin were fixed in 10% formal saline, embedded in paraffin, sectioned at 5 μm thickness and stained with hematoxylin and eosin. The dermatitis was characterized by acanthosis, parakeratosis, hyperkeratosis and exudate rich in neutrophils. A thick scabrous layer of necrotic and cornified epithelial cells, degenerate neutrophils and coagulated proteinaceous fluid was attached to the surface of the epidermis. Focal accumulations of neutrophils were present in many places between the epidermis and the overlying scab. The long parallel chains and individual coccoid cells characteristic of *Dermatophilus congolensis* were evident in huge numbers, primarily in the stratum corneum, with foci of organisms frequently associated with hair follicles. The hair root usually was intact. Hyphae were predominantly found in the upper epidermis, especially in the stratum corneum and sometimes in the papillary layer of the dermis. There was no histopathological evidence of mange. The definitive diagnosis was based on gross lesions, histopathology, and cultural and morphological characteristic of the organism.

The gross and histopathological lesions were similar to those described in cattle, sheep, goats, deer and other herbivorous animals (Roberts, 1965; Stewart, 1972; Yager and Scott, 1992). In herbivorous animals this organism grows within the epidermis as filaments from which zoospores are formed; subsequent hyphal growth and formation of a new generation of zoospores occurs only in epidermis. Zoospores resist drying and heating but do not survive long in wet environments outside the lesion (Smith and Cordes, 1972).

The lechwe in the present study by virtue of their habit stay either in water, wetlands or around water and get soaked easily (G. S. Pandey, unpubl.). This wetness may have aggravated skin lesions facilitating spread of infection and softening of the skin. In dry crusts the zoospores can survive for a long time whether on the body or on the ground and when crusts become wet the zoospores are released which are infective (Oppong, 1976). Zoospores can be spread between animals by ticks, biting flies, insects, and contaminated pastures (Macadam, 1970; Yager and Scott, 1992).

Dermatophilosis in cattle is widely prev-

ıl in Zambia including the area around
hinvar National Park (Samui and
gh-Jones, 1990). There is close contact
veen cattle and lechwe, particularly
ng the dry season when the availability
ater and grazing is restricted. The area
nd Lochinvar is densely populated
ı cattle which enter the park, graze and
k water together with lechwe and re-
ı in close contact with them from June
ctober. During the end of October and
ember, when the rain starts, the cattle
aken back to their villages.
icks and flies are abundant on cattle
are present on lechwe but less nu-
os. Infected cattle may have contam-
d the pasture on the Kafue flat. In-
on in lechwe is presumed to have been
mitted from cattle sharing the pas-
and. Although lechwe have been
d in Lochinvar for a number of years,
· is no past mention of any type of
natitis. Meat inspection is done rarely;
hen only after skinning the carcass so
the inspector has no chance to check
kin for dermatitis. We are not aware
y other published reports of this dis-
in lechwe. Preventing contact be-
o cattle and wildlife may be necessary
luce the incidence of dermatophilosis
chwe and other species.

: are grateful to Prof. C. E. A. Love-
Dean of the School, for her keen in-
and for providing the facilities, to
Director of the Department of Na-
l Parks and Wildlife for allowing us
e samples, to R. C. V. Jeffery, WWF,
o the Japan International Co-opera-
Agency.

LITERATURE CITED

, M. R., J. S. Park, and H. W. Bush. 1976. rmatophilosis in the marble lizard (*Calotes staceus*). Laboratory Animal Science 26: 817–3.

Carter, G. R., and G. R. Cole. 1990. Diagnostic procedures in veterinary bacteriology and mycology. Academic Press, San Diego, California, pp. 280–283.

Dean, D. J., M. A. Gordon, C. W. Severinghaus, E. T. Kroll, and J. P. Reilly. 1961. Streptothricosis: A new zoonotic disease. New York State Journal of Medicine 61: 1283–1287.

Howard, C. W., R. C. V. Jeffery, and J. J. R. Grimsdell. 1984. Census and population trends of lechwe in Zambia. African Journal of Ecology 22: 175–179.

Losos, G. J. 1986. Infectious tropical diseases of domestic animals. Churchill Livingstone Inc., New York, New York, pp. 693–708.

Macadam, I. 1970. Some observations on bovine cutaneous streptothricosis in northern Nigeria. Tropical Animal Health and Production 2: 131–136.

Montali, R. J., E. E. Smith, M. Davenport, and M. Bush. 1975. Dermatophilosis in Australian bearded lizards. Journal of the American Veterinary Medical Association 167: 553–555.

Oppong, E. N. W. 1976. Epizootiology of dermatophilus infection in cattle in the Accra plains of Ghana. *In* Dermatophilus infection in animals and man, D. H. Lloyd and K. C. Sellers (eds). Academic Press, New York, New York, pp. 17–32.

Roberts, D. S. 1965. The histopathology of epidermal infection with the actinomycete *Dermatophilus Congolensis*. Journal of Pathology and Bacteriology 90: 213–216.

Samui, K. L., and M. E. Hugh-Jones. 1990. The epidemiology of bovine dermatophilosis in Zambia. Veterinary Research Communications 14: 267–278.

Smith, C. F., and D. O. Cordes. 1972. Dermatitis caused by *Dermatophilus congolensis* infection in polar bears (*Thalactos maritimus*). British Veterinary Journal 128: 366–371.

Stewart, C. H. 1972. Dermatophilosis: A skin disease of animals and man. The Veterinary Record 91: 555–561.

Yager, J. A., and D. W. Scott. 1992. The skin and appendages. *In* The pathology of domestic animals, Vol. 1, 4th ed., K. V. F. Jubb, P. C. Kennedy, and N. Palmer (eds). Academic Press, Inc. San Diego, California pp. 531–738.

Received for publication 15 November 1993.

FC8.2.3

LUNG CANCER IN A CLOUDED LEOPARD (NEOFELIS NEBULOSA)

Yanai T*, Tomita T, Takeda H[1], Yamamoto Y[1], Mori H[1], and Masegi T.
Dept. of Veterinary Pathology, Gifu University, Yanagido 1-1, Gifu and Yokohama Municipal Nogeyama Zoo[1], Yokohama, Japan

Lung cancer is fairly common in humans but rare in animals. Only a few cases have been reported in wild felidae, and there are no known reports of such cancer in the clouded leopard.

We describe a necropsy case of lung cancer of a 12-year-old male clouded leopard that was referred on October 1992 because of depression, anorexia and respiratory rate increases. He became less active and died while in a coma on 29 November 1992.

At necropsy, the thoracic cavity contained 2000 ml of bloody fluid. There were several grayish-white nodules, from 5 to 10 mm in diameter, implanted on the pleural surface. A grayish-yellow solid mass with necrosis, hemorrhage and calcification, 85 X 55 X 80 mm in site, was found in the hilar region of left lung. Hilus lymph nodes were markedly enlarged. Several white nodules, 10 mm in size, were also observed in the spleen.

Microscopically, the pulmonary mass consisted of infiltrative growth of cancer. The cancer cells were round to cuboidal with high cell atypia and showed glandular patterns. Numerous mitotic figures were present. Considerable anthracosis admixed with cancer cell nests was prominent. Metastatic deposits of lung cancer were observed in the spleen and adrenal.

We classified this case as a bronchiolar-alveolar carcinoma derived from hilar region of the lung.

FC8.2.4

EMERGING DISEASES OF LIVESTOCK AND WILDLIFE THROUGH THEIR MIXING AND GAME RANCHING IN ZAMBIA

Pandey G.S[1]*, Sunkel A.K[2], Kaji T[1] and Takahashi S[1]
Dept. of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia[1] National Institute For Environmental Studies, 16-2 Onogawa, Tsukuba Science city, Ibaraki 305, Japan[2]

Game ranching is becoming an increasingly important industry and game meat (venison) has become an important by-product of game farming. Most game camps form part of mixed farming enterprises with domestic stock, mainly cattle. The development of the game farming industry in Zambia has not always been viewed with optimism especially with regard to the problems associated with diseases and their transmission from wildlife to domestic animals and vice-versa.

The present paper describes (i) the occurrence of heartwater in translocated Kafue lechwe (Kobus leche kafuensis) due to mixing with cattle (ii) risk of occurrence and adaptation of warble fly larvae (Stephanocyrus spp.) into cattle from warble infested lechwe as a result of translocation.

Diseases such as foot and mouth, corridor disease carried and transmitted by African buffaloes (Syncerus caffer), malignant catarrhal fever transmitted by wildebeest (Connochaetes taurinus) cause fatal disease in cattle. Bovine tuberculosis affects Kafue lechwe in Zambia. The authors have also observed dermatophilosis in Kafue lechwe and believe the occurrence is a result of mixing lechwe with cattle.

Farmers mix their cattle particularly in the rainy season with game animals to reduce the tick burden in game. During the dry season cattle mix with wildlife on the in Kafue flats to share grazing and drinking water. Therefore the presence of disease in wildlife is of considerable concern to livestock farmers including disease transmission from wildlife to domestic animals and vice versa. This should be an important consideration when translocating game into cattle farming area and mixing them with cattle. The need for accurate disease diagnosis and translocation of disease free game animals has been discussed. In view of wildlife as carriers of disease and potential disseminators of certain diseases of importance to the farming industry, statutory control over the movement of wildlife, establishment of game ranches, veterinary certification, provision of quarantine has been advocated in the present paper.

FC8.2.5

DISEASES FOUND IN NECROPSY CASES OF CRANES WINTERING IN IZUMI, KAGOSHIMA, JAPAN

Shimizu T[1]*, Yasuda N[1] and Koshimizu K[2]
Lab. of Veterinary Pathology[1] & Lab. of Veterinary Microbiology[2], Faculty of Agriculture, Kagoshima University, Korimoto 1-21-24, Kagoshima, 890 Japan

Almost ten thousands of cranes, consisting of about 90% hooded cranes (*Grus monacha*) and 10% white-naped cranes (*Grus vipio*), winter in Izumi, Kagoshima prefecture, southwestern Japan. Protection of such a large colony of cranes is a great concern of inhabitants of the district and the supervisory offices. As a branch of basic research project on the cranes the Association of Crane Protection in Kagoshima have asked pathological examination of dead cranes to the Laboratory of Veterinary Pathology, Faculty of Agriculture, Kagoshima University since 1983. In this presentation we report on diseases found in 68 necropsy cases comprising 59 *Grus monacha*, 8 *Grus vipio* and 1 *Grus nigricollis*; 32 males and 36 females in the last 5 years.

Fracture of the skeletal bone is the most frequent cause of death(19 cases) especially in windy Feburary. Hepatomegaly(26) and splenomegaly(16) mostly containing white spots were often encountered. A half of the cases was associated with infection of Hepatozoon-like organisms. Innumerable merozoites and/or meronts were seen mostly in enlarged mononuclear cells in the liver, spleen, bone marrow etc. but organisms that belong to the sexual phase were not recognized.

Aggregates of minute nodules in the mucous membrane of oropharynx and esophagus were seen in 14 cases. They consist of compactly gathered mononuclear cells each containing an intracytoplasmic inclusion and invade in the interstitium of the spleen, heart, lung and skeletal muscle. Various degrees of pseudomembranous enteritis were observed in 13 cases, 7 of which were ascertained association with *Escherichia coli* spp. by bacterial culture. There were 14 cases of *Tetrameres grusi* infection in the proventriculus and 11 cases of aspergillosis in air ways. Various flukes were found in the small intestine (6), bursa fabricii (2) and trachea (1). A Haemoproteus-like organism was found in a crane.

Hepatozoon-like organisms and oropharyngeal esophageal granulomata have much in common with disseminated visceral coccidiosis reported in *Grus canadensis* and *Grus americana*. We have not observed a typical disseminated visceral coccidiosis, although Eimerian oocysts having much similar morphological characteristics to *Eimeria gruis* and *Eimeria reichenowi* have been detected in the feces of some cranes.

FC8.2.6

INHIBIN SECRETION DURING THE ESTROUS CYCLE OF FEMALE ASIAN ELEPHANTS

Watanabe G[1]*, Funakoshi M[2], Arai K[1], Araki K[1], Otsuka H[2], Taya K[1], Sasamoto S[3], Tajima H[3], Saito K[3], Miyoshi Y[3], Shichiri S[3], Hose M[3], Sato J[3], Kanaya K[4], Hashitaki E[4], Tanabe K[4], Hiramatsu H[4], Yabe T[4], Tasaka K[4], Yamaguchi A[4], Narushima E[4]
Tokyo University of Agriculture and Technology[1], Ueno Zoological Gardens[2], Tama Zoological Park[3], Tokyo Japan

Female elephants are known to have a long estrous cycle, which is as long as four months. The estrous cycle consists with five weeks of follicular phase and ten weeks of luteal phase, estimated by profiles of plasma sex steroid hormones. However, regulatory mechanisms responsible for follicular development and luteal functions during such a long estrous cycle are poorly understood. We have demonstrated that inhibin plays an important role in the regulation of secretion of follicle-stimulating hormone (FSH) from the pituitary gland in various mammals. In the present experiment, we investigated the secretion of inhibin during the estrous cycle in Asian elephants.

Follicular fluid was collected from ovaries of a female Asian elephant died accidentally. Bioactivity as well as immunoreactivity of inhibin was detected in the follicular fluid. Granulosa cell layers were positively stained immunohistochemically by anti-inhibin serum. Inhibin immunoreactivity was also examined in the homogenate of corpus luteum collected from an African elephant, whereas bioactivity of inhibin was not detected.

The plasma concentrations of inhibin were measured by specific radioimmunoassay once a week in two female Asian elephants. The plasma inhibin profile represented clear cyclic changes along with the estrous cycle. Plasma levels of inhibin were high in luteal phase and correlated positively with plasma concentrations of progesterone. Plasma levels of testosterone also correlated positively with inhibin. Plasma concentrations of FSH were low during follicular and early luteal phases when plasma levels of inhibin were high, then increased concomitantly with the decrease of plasma inhibin levels.

These results suggest that the granulosa cells of ovarian follicles is one of the sources secreting inhibin in female elephants, and that the corpus luteum is an another source which produces immunoreactive inhibin during the luteal phase.

Emerging problems on diseases of livestock and wildlife due to their mixing in the developing country

A. Suzuki [a], G. S. Pandey [b], K. Matsukawa [b], S. Takahashi [a], A. Nambota [b] and T. Kaji [b]

[a] *National Institute for Environmental Studies, Japan Environment Agency 16-2, Onogawa, Tsukuba-Science City, Iboraki 305 Japan.*
[b] *School of Veterinary Medicine, University of Zambia, P.O.Box 32379 Lusaka, Zambia*

Introduction

In the developing country, domestic animal pasture has been enlarged in area to make a larger amount of milk and meat production. In these county the pasture has been closed to the national parks(Suzuki, 1995). As the control of domestic animals mainly cattle is not enough, domestic animals contact or mix with wildlife. The mixing may has a high risk potential to contagious common diseases.

Contacts or Mixing between domestic animal and wildlife, however, has been unclear. Therefore, using computer analysis method, we tried to clear the contact or mixing between domestic animals and wildlife in the Lochinvar National Park. Serological diagnoses of brucellosis were tested in Kafue Lechwe and cattle to confirm the transmission of common disease.

The authors describe the actual mixing and brucellosis of wildlife and domestic animal in the Republic of Zambia as an example of developing country.

Materials and Methods

Three hundred ninety two photographs has been taken in the Lochinvar National Park since 1990. Thirty two of them, that were suitable for computer analysis, were selected to examine the relationship between domestic animal and wildlife. Two hundred and fifty five points in 32 photographs were marked to calculate the correlation. Exposures for photographs were arranged and controlled by the microcomputer of Cannon Camera(IOS 10 series with 300 mm Auto Focus lens). Data of characteristics of lens were presented by Canon Co. Ltd.

Calculations of regression curve were performed to gain the most fitted formula or a function using computer soft ware programme (NEC, PC 9801 series, MS-Excel [R]). The relationship between distances from camera(lens) and length on a film shows the linear regression on the logarithmic scale. It is a sort of TRELOG [R] function, then a distance was clearly defined by a measurement of length on the film using of TRELOG function.

Recently, computer system on the image analysis has been developed to enlarge digitally the picture without interference such as lens' deviation. Digital enlargement is useful for these analysis, as the enlargement is proportional to the multiple. In this research, the photographs were enlarged digitally from 20 times to 30 times by image processing system (SAYCO, Digital Arts Co.). A figure was shown as 700 pixel(X-axis) and 500 pixel(Y-axis) at 20 times and as 1050 pixel(X) and 750 (Y) at 30 times, relatively. A point on a display of computer was recognise as a co-ordinate(X, Y),.

At first, a difference of two points on individual animal were calculate to gain a distance from camera to the object by using a function of TRELOG. Secondary, a distance between animals was gain by calculation using triangle analysis.

Serological diagnoses of Kafue Lechwe (*K. lechwe kafuensis*) and cattle were examined to

confirm the brucellosis (*brucella abortus, brucella canis*), after the authors got a special permission of catching and blood sampling. Blood samplings were done from immobilised Kafue Lechwe by injection of immobile drug (M99, D. M. Pharma.). The diagnosis was standard serum agglutination tests using test tube and complete agglutination at dilutions of 1 : 100 or more were considered positive.

Results and Discussion

In the dry season, mixing or contact between the domestic animal (mainly cattle) and wildlife was frequently occurred and the main mixing wildlife was Kafue Lechwe*(K. Lechwe kafuensis)* in the Lochinvar National Park. The Kafue Lechwe were scattered to take a grass around a ponds or streams. When some groups of cattle broke into the scattered Kafue Lechwe to drink water or take green grass, mixing and nearness were occurred. Sixteen groups of cattle and Kafue Lechwe were mixed in the narrow area, it's nearest distance was within 50 m(range 15-50m, 3 of 8 groups), and within 100 m (range 50lm - 100 m, 4 of 8 groups).

In the rainy season, contact and mixing as same as in the dry season was very scarcely, because domestic animals were almost in human control and were using as a agricultural machine and they can have enough green grass and water in a village.

Common infectious disease between domestic animal and wildlife were well known (Pandey et all, 1995). Main infectious mechanism are ticks, flies and others. Contact or mixing might has a high potential to affect and spread the common infectious disease special regard to contagious disease such as brucellosis.

Eight Kafue Lechwe were hit and injected immobile drug but three of them escaped from blood sampling. On the serological diagnoses under double blind tests, one of five heads showed positive in *brucella abortus*. Five heads, however, showed negative in *brucella canis*. Two of five sera of cattle feeding in a village near by the National Park showed positive in *brucella abortus*.

Matsukawa (1995) reported pathological evidence seemed to brucellosis in Kafue Lechwe. Our serological result was supported by the pathological aspects. Kafue Lechwe in the other place where the mixing was not found, brucellosis has not yet reported. The authors considered that brucellosis of the Kafue Lechwe in Kafue flat might be infected from the disease cattle by mixing.

Conclusion

The results indicates that mixing or contact of domestic animal with wildlife shall make emerging problem of common disease and the isolation of domestic animal from wildlife was very important for meat production and wildlife conservation, also human.

References

Matsukawa, K., 1995. Brucellosis in a kafue lechwe and east coast fever in an eland -- Pathological aspect--.Symposium on the effects of enlargement of domestic animal pasture on the wildlife in Zambia. : s1-p8

Pandey, G. S., A. K. Suzuki, T. Kaji, and S. Takahashi, 1995 . Emerging diseases of livestock and wildlife through their mixing and game ranching in Zambia. XXVth W. Vet. Ass. :114

Suzuki, A. K., 1995 Contacts between domestic animals and wildlife--using computer imaging analysis in Lochinvar National Park of Zambia, : s1-p1