Ovine Fetal Infection due to *Salmonella arizonae*

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INTRODUCTION

The bacterium now called *Salmonella arizonae*, was first recovered from reptiles (3). It has variously been referred to as *Salmonella* sp., Arizona group, paracolon bacteria, *Arizona arizonae* and *Arizona hinshawii* (2, 3). *S. arizonae* is presently classified under subgenus III of the salmonelleae (2, 5). Specific serotypes of this organism are recognized pathogens of poultry, especially of young turkey poults. Numerous other serotypes are widely distributed in nature (6, 7, 8, 9). *S. arizonea* was first identified from newborn lamb carcasses in 1952. This isolate was identified as Arizona paracolon and was serotyped O26:H29-30 (10). More recently this serotype has been recovered from various tissues of sick or dead adult sheep and from the tissues of aborted ovine fetuses (1, 4, 9). Under present classification, Arizona 26:H29-30 is O61:K:1,5,7 (2, 3).

This paper reports the isolation of *S. arizonae* from ten ovine fetuses aborted or stillborn, from six ewes on four different farms in Nova Scotia and Prince Edward Island during the winter and spring of 1977.

Laboratory Specimens

Aborted fetuses, placentas or stillborn lambs were submitted from ewes as indicated in Table 1.

Gross Pathology

Four fetuses were in good condition for examination. Lungs were dark red, firm and did not float. Four fetuses were undergoing autolysis but lungs appeared similar. In addition, body cavities contained serosanguineous fluid and subcutaneous edema was present. Two fetuses were undergoing mummification when aborted along with their more normal appearing twin. Tissues for bacteriology or histology were not taken from the two mummified fetuses (cases O16, O47).

Bacteriology

Lung, spleen, abomasal contents and placenta, when the latter was available (Table I), were the standard tissues submitted for bacteriology. Liver and kidney were also cultured from O16 and O52. Tissues were seared with a hot spatula, opened aseptically and wire loop scrapings were streaked onto MacConkey agar and blood agar plates containing five percent ovine blood. All plates were incubated overnight at 37° C. Representative nonlactose fermenting colonies from MacConkey plates were inoculated onto the following media in the Minitek System:[1] dextrose, arabinose, inositol, rhamnose, phenylalanine, urea, H_2S, indol, citrate, ONPG, lysine and ornithine decarboxylase and malonate. In addition TSI, OF and SIM media were also inoculated. All media were incubated at 37° C for 24 hours and biochemical reactions were recorded.

All *S. arizonae* isolates gave a positive reaction on dextrose, ONPG, citrate, H_2S, lysine and ornithine decarboxylase, arabinose, rhamnose and malonate in Minitek media. All isolates showed motility on SIM medium fermentation in OF and on TSI gave an alkaline slant and acid butt with a narrow ring of H_2S at the aerobic junction. These organisms gave a negative reaction on all other inoculated media. An isolate from each of four cases (O36, O37, O47 O72) was subjected to antimicrobial susceptibility testing using .the Bauer-Kirby disc method. One isolate from each case, was sent for confirmation of identification and serotyping to the National Enteric Reference Centre, Bureau of Bacteriology, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario.

Histopathology

Representative tissues including lung, kidney, liver, spleen and, placenta when available, were selected from eight carcasses and fixed in 10% buffered formalin. These tissues were embedded in paraffin, sectioned at 6 μ, and stained by the hematoxylin and eosin (H & E) method.

Specimens from Ewes

Vaginal swabs[2] were collected from two ewes (cases O36 and O37) 30, 50 and 90 days following

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[1]BBL, Division of Becton, Dickinson & Co., Mississauga, Ontario.

[2]Securline, culture collection transport system. Precision Dynamics Corporation, 3031 Thornton Ave., Burbank, California.

TABLE I

OVINE FETAL SPECIMENS FROM WHICH *S. ARIZONAE* WAS ISOLATED AND THEIR FLOCKS OF ORIGIN

Case No.	Specimens Submitted	Breed	Ewes		
			Age (Years)	Approximate stage of Gestation (Days)	No. in Flock
O16[a]	2 fetuses	Suffolk	5	100	50
O152[a]	2 fetuses, placenta	Cheviot	4	120	50
O36[b]	Carcass, placenta	"	6	Term	80
O37[b]	2 carcasses, placenta	"	6	Term	80
O47	2 fetuses, placenta	Scottish Blackface	4	140	250
O72	Fetus	Cheviot	8	140	220

[a,b]Cases with the same superscript letter are from the same flock.

the delivery of stillborn lambs. All swabs were immediately streaked onto MacConkey and blood agar plates and incubated overnight at 37°C. Following the final swabbing, both ewes were euthanized and their reproductive tracts were removed for laboratory examination. Swabs were collected from the uterus, cervix and vagina of each tract and cultured on MacConkey and blood agar plates. All cultures were processed as outlined for fetal tissues.

Tissues from the oviduct, uterus, cervix and vagina were collected from each of the two tracts and fixed in 10% buffered formalin. Sections were prepared for examination as indicated for fetal tissues.

Single serum samples were collected from three ewes (036, 037 and 047) approximately 90 days following the delivery of infected fetuses. These sera were submitted to the National Enteric Reference Centre for *S. arizonae* titres.

RESULTS

S. arizonae was recovered in moderate to large numbers and essentially in pure culture from most fetal tissues submitted for bacteriology. From case 047 a light growth of this organism was recovered from placenta only. Coliforms were recovered along with *S. arizonae* from cases 016 and 072. The four isolates examined were sensitive to ampicillin, neomycin, kanamycin, streptomycin, tetracycline, chloramphenicol, polymyxin B, Gentamycin, furoxone, triple sulfa and bactrim. All six isolates were confirmed as *S. arizonae* and serotyped as O61:K:1,5,7 SG III (Ar. 26:H29-30).

Autolysis interfered with examination of four fetuses but results were similar in the remaining four. In lung (Figure 1) scattered foci of neutrophils were present in bronchioles and alveolar ducts. Within and between cotyledons there was bacterial colonization and neutrophil infiltration (Figure 2). Significant lesions were not seen in liver and kidney.

S. arizonae was recovered in low numbers from vaginal swabs of both ewes at 30 and 50 days but only from O36 at 90 days postpartum. The organism was recovered from the vagina of O36 only at necropsy.

Significant lesions were not present on gross or histological examination of the reproductive tract of either ewe.

Using *S. arizonae* isolate O72 as antigen, serum from the ewes of case O36 and 037 gave a 3+ and 2+ tube agglutination respectively at a dilution of 1/320. Serum from the ewe of case O47 gave a 3+ tube agglutination at a 1/10 dilution only.

DISCUSSION

Serotype O61:K:1,5,7 (26:H29-30) is recovered from man and is the common serotype in monkeys and sheep (9). These workers consider this serotype to be host adapted to sheep and it is the one consistently isolated from ovine specimens (1, 4, 10). It was isolated from abattoir drain swabs where sheep and cattle were slaughtered (5). In our experience this serotype is not uncommonly recovered from the small and/or the large intestine of sheep in the absence of intestinal lesions. The above observations suggest that *S. arizonae* may frequently reside in the intestinal tract of normal sheep and presumably may become invasive when animals are stressed or debilitated. Available history did not indicate illness in any of the ewes (Table I) before or at the time of abortion, although those tested mounted a serological response to *S. arizonae*.

The two ewes studied (cases O36, O37) continued to discharge the organism from the vagina at 50 days postpartum and O36 had *S. arizona* present after 90 days. One would assume contaminated vaginal discharge to be a significant method of spread for this bacterium. However, since only one or two ewes were infected in each of the flocks studied it would suggest that *S. arizonae* is not highly communicable and/or highly virulent.

Histological findings were not included in previous reports of ovine abortion due to *S. arizonae* (4, 10). In our cases the placental and

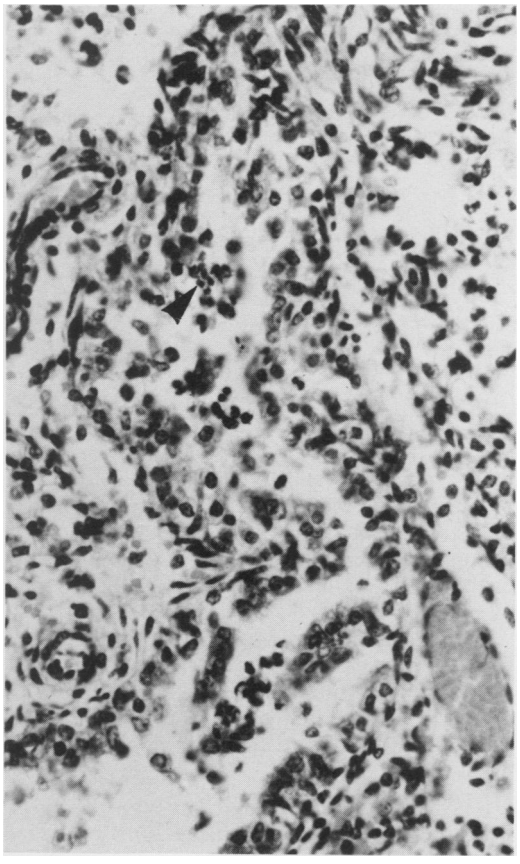

FIGURE 1. Lung showing neutrophils (*arrow*) present in bronchiole. H & E. X500.

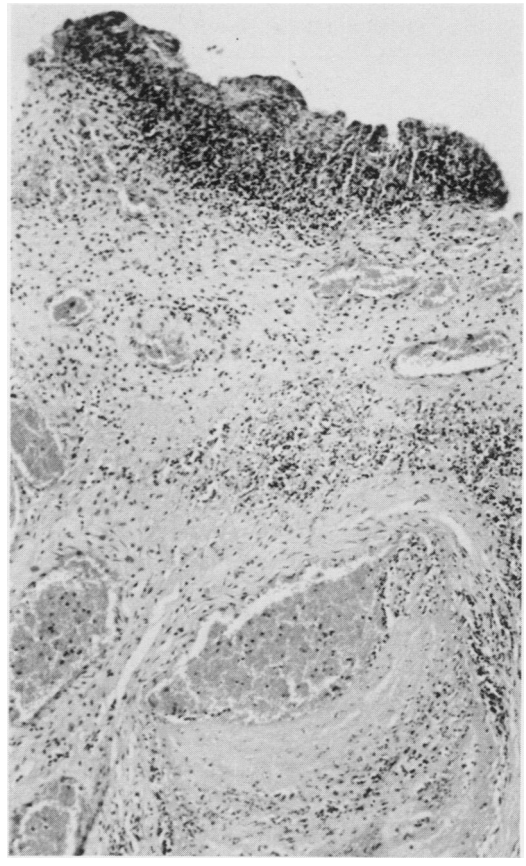

FIGURE 2. Placenta with neutrophil infiltration. H & E. X130.

pulmonary lesions (Figures 1 and 2) while variable in degree, are typical of bacterial placentitis and subsequent bronchopneumonia.

These findings indicate *S. arizonae* may be a more common cause of ovine abortion than present literature indicates. The organism is not difficult to culture or identify, thus slow lactose fermenting colonies, recovered on selective medias, should be examined biochemically.

SUMMARY

S. arizona serotype O61:K:1,5,7 was recovered from the tissues of ten ovine fetuses and/or placentas. Histological lesions were placentitis and bronchopneumonia. Two ewes examined continued to excrete the organism from the vagina at 50 days postpartum and one was still shedding at 90 days.

RÉSUMÉ

Les auteurs ont isolé *Salmonella arizonae* (sérotype O61:K:1,5,7) des tissus de dix foetus ovins et/ou de leur placenta. Les lésions histologiques se caractérisaient par une placentite et une broncho-pneumonie. Le vagin de deux brebis recelait encore cette salmonelle, 50 jours après l'agnelage; le même phénomène s'observait chez une autre brebis, 90 jours après l'agnelage.

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BOOK REVIEW

Proceedings of the Twentieth Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians. Obtainable from Dr. M.W. Vorhies, Secretary-Treasurer, AAVLD, 6101 Mineral Point Road, Madison, Wisconsin 53705. 390 pages. Price $10.00.

These proceedings are a report of the most recent annual meeting of the AAVLD which was held in Minneapolis, Minnesota on October 16, 17 and 18, 1977. The editors are to be congratulated on the speed of publication and the quality of this presentation.

The majority of the thirty-seven papers in this publication describe the etiology, pathology and the laboratory diagnoses of a wide variety of infectious disease conditions in cattle, swine, goats, sheep, cats, dogs, chickens, psittacine birds and fish.

As a reflection of some of the current concerns in animal production in the U.S.A., there are five papers dealing with pseudorabies, three with bluetongue, and three with bovine abortion. A few examples of other disease conditions discussed include channel catfish virus disease, canine histoplasmosis, *Cytauxzoon felis* infection in a cat, porcine parvovirus-induced reproductive failure, acute bovine pulmonary emphysema and malignant catarrhal fever.

Noninfectious diseases have received appropriate attention in papers discussing such topics as the toxicological and residual aspects of pentachlorophenol, the diagnostic problems of anticoagulant rodenticide toxicoses and the toxicity of plants containing perilla ketone.

Those persons interested in diseases of wildlife will find the article discussing the problems in diagnoses and control of wildlife disease of particular interest.

Diagnostic methods and techniques are constantly being improved. The application of some of these advancements in pseudorabies diagnoses are described in considerable detail in papers outlining (a) a serological test for this disease using a cell-bound antigen and a peroxidase enzyme labelled assay method and (b) an agar-gel immunodiffusion test. A third paper prepared by the Pseudorabies Diagnostic Standardization Committee of the AAVLD discusses the recommended minimum standards for tests employed in diagnosing this disease.

The constitution and by-laws of the AAVLD have been included in these proceedings. Space limitations do not permit further description of its contents.

Membership in this association is open to any laboratory worker engaged in the field of disease diagnoses in animals and applications for membership should be forwarded to the Secretary-Treasurer.

Readers of the Canadian Veterinary Journal will be pleased to know that Dr. Julius Frank, Director, Animal Pathology Division, Agriculture Canada, is the 1977-78 President of the AAVLD.

It has been stated that receipt of the Proceedings each year is, by itself, well worth the membership fee for the AAVLD. This reviewer agrees with that statement. *C.C. Stewart.*