West Coast United Egg Producers

P.O. BOX 2077, FOLSOM, CA 95763-2077
PHONE (916) 985-2323 FAX (916) 358-2961

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FDA/Dockets Management Branch (HFA-305)
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Docket No. 00N-0504

The West Coast United Egg Producers wishes to submit comments in response to the Egg Safety Action Plan following the public meeting held on April 6, 2000 in Sacramento California.

Reducing food illness is an admirable goal. It is one the egg industry shares to assure consumers the safest food supply in the world. Egg producers employ many management practices on a daily basis which seek to minimize the introduction of bacteria into the food supply. The California Egg Quality Assurance Plan (CEQAP) identifies those practices and gives farmers credit for what they do on an everyday basis that lessens the chance of producing an Se contaminated egg. The proposed Egg Safety Action Plan incorporates many of the same components as the CEQAP, such as using Salmonella enteritidis (Se) free chicks, providing Se free feed, farm sanitation and biosecurity practices. We differ in testing for Se. Since it's impossible to test your way to safety, we believe one test at the end of the production cycle presents an overall view on the success of the quality assurance plan. If the environment is positive, we encourage producers to conduct an intensive cleaning and disinfection program and to review their records to verify how the organism could have entered the farm. Many of these points were made during the public meeting; therefore we will not attempt to duplicate them here.

We believe the far-reaching goal of eliminating Se from farm environments is flawed. It's far too easy for regulators to simply write a rule declaring that bacteria be regulated off a premises. It's another to actually carry out that goal on a daily basis. The government is now attempting to enforce an unreasonable goal on the backs of 700 egg producers when the science tells us otherwise. The political goal of assuring 100 percent risk free food does not correlate with the scientific knowledge and real life experiences. Meaning, government cannot regulate bacteria off the planet because someone in Washington deems it politically correct. Bacteria have been on this plant before mankind. It's unrealistic to think that instituting a rule now will somehow make it go away. In-shell pasteurization is a lofty goal and as of yet, an unproven and expensive proposition.

We are enclosing a published paper "The Dillon Beach Project, A Five Year Epidemiological Study of Naturally Occurring Salmonella Infection in Turkeys and Their

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Environment" to support the fact that Salmonella cannot be kept off a farm premises. This was a 5-year joint project between the federal/state government and industry. Although there was extensive testing, the farm was eventually invaded by Salmonella. If the FDA and FSIS now believe they have the means to keep bacteria off a farm we invite them to demonstrate that process first under real life conditions. To enforce a regulatory program and expect the impossible from egg producers is not only unfair and costly, it is a gross misuse of regulatory power.

The government is overlooking the fact that Se food illnesses have been decreasing in recent years in absence of a mandatory regulatory program. The industry has initiated voluntary quality assurance programs that have been successful. The government should encourage a wider adoption of these programs because they are more effective and versatile. Voluntary programs can be readily changed as new information is learned. Government mandated programs take long periods of time to change because of regulatory red tape. Why not empower egg producers to continue to do what they have been successful in accomplishing?

The government should play a greater role in regulating the food handler side of the farm to table continuum since this area represents the greatest risk in the perpetuation of food illnesses. Cross contamination, poor sanitation and inadequate cooking procedures are always cited as the cause in food illness cases. Therefore, government would better serve consumers if it addressed education and certification at the food handler level. Since there is little known on the ecology of the Se, we recommend that research be conducted before any regulation is put in place. The research should include the correlation between environmental tests and Se infection in eggs; are eggs infected inside the shell or are they found on the shell surface and introduced when they are cracked and prepared?

Finally, the risk of becoming ill from an Se infected egg pales when compared to risks from other daily life activities. In the CDC Morbidity and Mortality Weekly Report (MMWR), February 4, 2000 Vol. 49, No. 4, page 91, titled National Child Passenger Safety Week – February 13-19, 2000, the article states that in 1998 there were 1,772 deaths of children aged 15 years or younger and 274,000 injured while riding in a motor vehicle in the United States. This statistic far exceeds the number of people who died from eating Se contaminated meals, which is reported in the same MMWR report. If the Clinton Administration is so concerned about saving lives, it can accomplish so much more by banning automobiles!

The West Coast United Egg Producers is a regional Capper-Volstead farmer cooperative trade association. Our membership is comprised of bonafide egg producers in California, Utah, Arizona and New Mexico. We are a regional office of the national United Egg Producers organization. Our member farmers control or contract approximately 90 percent of the egg production in the Western region.

We look forwarded to working with your department in making the Egg Safety Action Plan a success. However, common sense needs to be incorporated if the plan is to meet its goal.

Sincerely,

David Goldenberg

David J. Goldenberg
Manager

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The Dillon Beach Project — A Five-Year Epidemiological Study of Naturally Occurring Salmonella Infection in Turkeys and Their Environment

B. C. Zecha,[A] R. H. McCapes,[B] Wm. M. Dungan,[C]
R. J. Holte,[D] W. W. Worcester,[E] and J. E. Williams[F]

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SUMMARY

A 5-year epidemiological study of naturally occurring salmonella infection in 2 basic strains of turkeys and their environment was completed. Multiple generations of these turkeys, free from salmonella infection, were raised on a new commercial breeding ranch (the Dillon Beach Ranch) in California during a 3½-year period. Eight salmonella serotypes eventually gained access to the ranch. Egg transmission and prior contamination of the premises were ruled out as the source of these infections. The most likely means of their introduction was physical transport into the ranch by contaminated feed, personnel, and/or equipment. Several serotypes of salmonella were isolated from pelleted feed delivered to the ranch, demonstrating its role in introducing infection.

The low isolation rate from feed samples and environmental samples taken from many buildings indicates the need for intensive sampling as a reliable indicator of infection. The most severe and consistently detected infection occurred in young birds grown in forced-air-ventilated buildings.

A total of 17,858 bacteriological cultures representing about 102,756 environmental, feed, and bird samples, and 93,930 serological tests on 46,977 serum samples, were run during the study.

[A]Veterinary Services, USDA, APHIS, Veterinary Services, Room 237, 2490 West 26th Ave., Denver, Colorado 80211.
[B]Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616.
[C]Veterinary Department, Nicholas Turkey Breeding Farms, P.O. Box Y, Sonoma, California.
[D]Bureau of Animal Health, California Department of Agriculture, 1220 N Street, Sacramento, California.
[E]Veterinary Laboratory Services, California Department of Agriculture, 1220 N Street, Sacramento, California.
[F]Southeast Poultry Research Laboratory, USDA, ARS, 934 College Station Drive, Athens, Georgia 30601.

FROM : RICHARD H. MCCAPES PHONE NO. : 916 756 4284 Apr. 19 2000 12:08PM P1

INTRODUCTION

Salmonella infection and contamination of domestic animals and their products is important for economic and public-health reasons. Present and past efforts to control salmonella infection in turkeys has been directed largely toward preventing clinical illness in poults during the initial weeks of life. Control measures used effectively to prevent such illness include: 1) hatching-egg sanitation and therapy; 2) elimination of parent stock infected with specific serotypes; 3) premises sanitation; and 4) poult therapy. Salmonella infection, however, remains prevalent in turkey populations in spite of much reduction in clinical illness.

The public-health significance of salmonella infection in our domestic meat animals is of major societal concern. Means of preventing human salmonellosis from meat and poultry sources are being actively sought (1,3). A major step toward that end would be elimination of salmonella from populations of the major food animals.

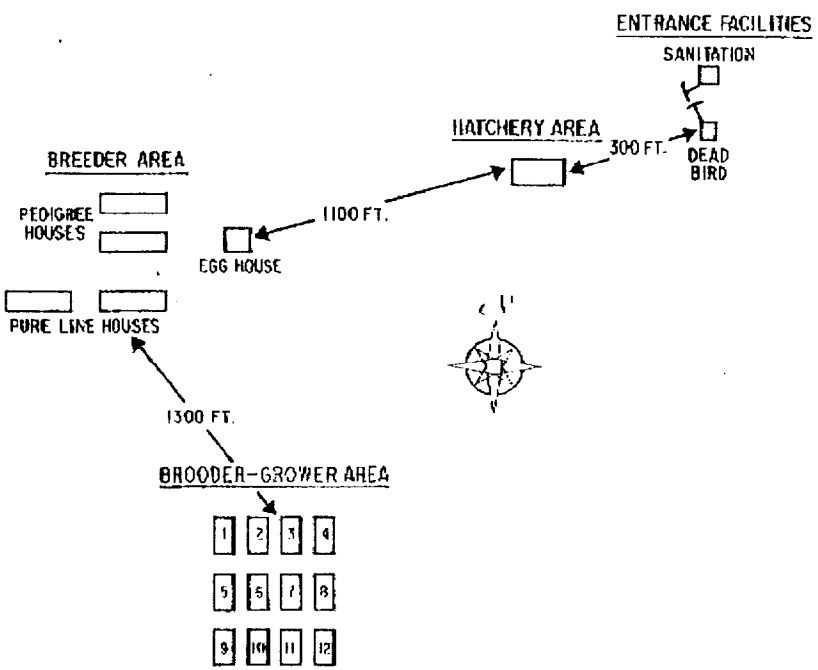

Fig. 1. Diagram of Dillon's Beach ranch by production area.

There are significant obstacles to the eradication of salmonella from livestock and poultry populations. Studies have shown that at least 3 abilities of the salmonella organism present formidable obstacles to their eradication from poultry populations: 1) their ability to remain viable in the environment for long periods; 2) their wide host range; and 3) their ability to be egg-transmitted by both ovarian and shell contamination (4,5,6,7,9,10,11,13,14,15).

Eradication of salmonella infection from domestic turkeys in the United States would require the elimination of infected flocks and all means of transmission of the organism to turkeys. The ability to produce hatching eggs from salmonella-free primary and commercial breeding flocks would be a valuable step toward eradication. This 5-year study was undertaken to determine whether 2 closed basic strains of turkeys introduced and maintained on a new primary breeding facility in California could be maintained free from salmonella infection under the commercial management system utilized. Intensive periodic surveillance of 6 successive generations of turkeys and their environment by bacteriologic and serologic testing procedures was utilized to establish a profile of salmonella activity.

MATERIALS AND METHODS

Duration of study. The study began in April 1969 and terminated in March 1974.

Ranch production facilities. The ranch selected for the study is at Dillon Beach, California, about 60 miles north of San Francisco on the Pacific Coast. Turkeys had not been raised on the premises before the study. The land had been used for a small bovine dairy. The facilities were divided into 3 major production areas as follows (See Fig. 1):

1) Brooder grower area. Twelve houses were utilized for brooding and growing 1600 hens and 1200 males each, and holding selected breeders until time of lighting. Four of these houses (nos. 9, 10, 11, 12) can be used for light-control purposes and had forced-air ventilation. Houses 9 and 10 have a combination forced-air exhaust and intake system in addition to natural ventilation. Houses 11 and 12 are closed and have baffled exhaust fans opposite baffled air-intake openings. The others had natural side-curtain ventilation. Construction was of wood, with screened walls, plastic curtains, sheet-metal roof, and dirt floors.

2) Breeder area. Two pure-line breeder houses were used to

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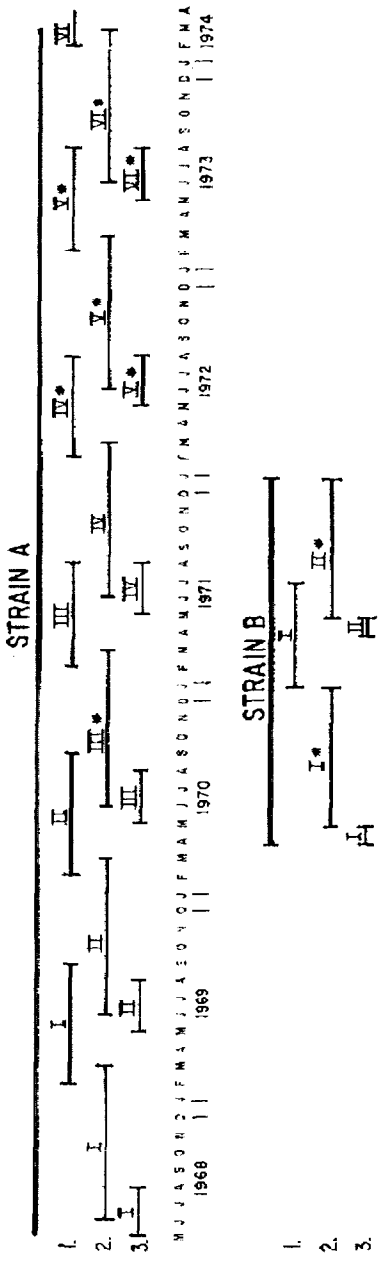

Fig. 2. History of Dillon's Beach ranch production periods by turkey strain, generation (Roman numerals), calendar (month and year), and salmonella status.

house 1800 hens each for multiplication and export of the basic turkey strains under development on the ranch. Each house had 8 breeder pens, 4 on each side, separated by a center aisle and 4 broody pens. Nests were located along the aisle. Two pedigree breeder buildings housed about 500 pedigree hens and 225 toms each. Each had 36 pedigreed-hen pens, 18 on a side separated by an 8-ft center aisle. Each pen, housing up to 15 hens, had 5 trap nests. Broody cages were suspended from the walls. Tom pens in each building were separated from the hen area by a maintenance and storage area in the center of the building. Each building had 36 pedigreed-tom pens holding 2 toms each, and 5 pure-line tom pens holding up to 30 birds each. The tom area can be darkened for light control. The aisles and floor area under the broody pens and the center maintenance floor area were concrete. All pure-line and pedigree breeder houses were bird-proof, with wooden wall, sheet-metal roof, and dirt floors in the pens.

An egg house was used to grade, clean, and sanitize hatching eggs from all breeder units. It consisted of a cleaning-grading room, a packing room, and a storage room. A pass-through fumigation cabinet was located in the wall between the grading and packing rooms. Construction was wood walls, with sheet-metal roof and concrete floors.

3) Hatchery area. The hatchery building consisted of an egg holding and packing room, an incubation room, and a hatching room. An outside concrete platform was used for equipment sanitation. Construction was of sheet-metal roof, concrete walls, and floors. Two Robbins incubators (H-32 converted to I-26) and two Robbins H-10 hatchers were used.

Ranch-entrance sanitary facilities. A wood building with concrete floor at the ranch entrance was used to store clean overalls and rubber boots for personnel entering the premises. Facilities were present for boot disinfection. A garden-type sprayer and attached hose was used for sanitation of vehicle tires. An enclosed wooden platform was located at the entrance for storage of all dead turkeys for daily pickup.

Turkeys. Two primary strains (A and B) of large Broad-Breasted White turkeys populated the ranch during this study. Both strains had been bred for many generations on premises in other states, were imported into California in the form of hatching eggs, and were placed at the Dillon Beach ranch as day-old poults.

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Strain A eggs were imported twice from a ranch in Oregon. The 1st eggs were incubated and hatched at the Nicholas hatchery in Sonoma, California, and were placed at Dillon Beach at 1 day of age in June, 1968. The 2nd egg importation in 1969 and all Strain A eggs thereafter were incubated and hatched at Dillon Beach. The Oregon flocks producing the eggs were negative to *S. typhimurium* and *S. pullorum* serum tube agglutination tests. Prior generations of breeders on those premises had the following history of salmonella infection: 1966, *S. worthington;* 1967, *S. san diego;* 1968, *S. infantis*. Except for the 1st Dillon Beach generation, about 20,000 poults of this strain were hatched each year and grown on the ranch. About 5,000 of the females and 500 of the males were retained as pure-line and pedigree breeders each year. The remainder were marketed as meat. About 34,000 eggs were set each season to produce the next year's hatch. First-year numbers were about half as many. During the 1-year interval between placement of the 1st poults on the ranch and the start of the study, salmonella surveillance on the ranch was carried out by Nicholas Turkey Breeding Farms, and no evidence of infection was noted. Test samplings were made of environmental swabs, hatchery debris, dead embryos and cull poults, birds dying early in life, and serologic testing of all selected breeders for *S. pullorum* and *S. typhimurium*.

Strain B eggs were imported once from 1 ranch in Wisconsin, and were hatched at Dillon Beach in May, 1970. Litter samples obtained in the spring of 1970 from the Wisconsin environment of the breeder flock producing these eggs were negative for salmonella, as were *S. pullorum* and *S. typhimurium* serology tests of all these breeders prior to egg production. Previous generations of breeders on these premises had a history of *S. heidleberg, S. st. paul,* and *S. chester*. About 2500 poults were hatched from this strain in each of the 2 generations on the Dillon Beach ranch. About 300 females and 40 males were retained as pure-line breeders in 1971, and 575 females in 1972. Strain B was removed from the ranch in early 1972 following isolation of *S. typhimurium* var. *copenhagen*.

Dillon Beach ranch utilization and production schedule (see Fig. 2). Hens and toms selected from the Strain A ranch population and placed in the pedigree pens represented the outstanding individuals of this primary strain. The pedigree hens produced eggs from February to early September. Many of these eggs were

used to repopulate the ranch in the following year. They were incubated in the ranch hatchery in 6 or 7 hatches at 2-week intervals from May to early August.

Each hatch was started and grown in 1 or 2 brooder-grower buildings from June to the following March. The outstanding birds of each hatch were selected for placement in the pedigree breeder pens for the following year's repopulation. Additional hens of exceptional quality were selected for egg production in the pure-line houses. Eggs from these hens, produced during the same breeder period, were shipped away from the ranch and used to multiply the basic strains being developed. Birds not selected for breeders were marketed as meat. All breeders were marketed at the end of each breeder season.

Strain B breeders were not placed in the pedigree houses the first generation. The Strain B breeder period, hatchery period, and brooder-grower periods were 1 month in advance of Strain A. All of Strain B was removed from the ranch following the official blood test in early 1972.

Husbandry and sanitary procedures. These were considered representative of commercial practices at the time. Personnel entering the ranch were instructed to put on clean overalls and rubber boots at the entrance. Boots of personnel and tires of vehicles entering the ranch were disinfected with a chlorinated phenolic or a formalin solution. Each farm building had boot-disinfectant pans at the entrance. Traffic between the major production areas was limited as much as possible.

Following a season's use, each building was cleaned and disinfected. Walls, ceilings, floors, nests, and equipment were washed and sanitized by a quaternary ammonium solution. Disinfection following sanitizing in 1968–70 was with 1 gallon of 46.7% cresylic acid per 400 gallons of diesel fuel, in 1971 with a chlorinated phenolic solution; in 1972–1973 with 1 gallon of a 2.7% formalin solution per 15–20 square feet of floor space (12). Clean pine shavings were replaced each season.

Eggs were fumigated with formaldehyde gas shortly after being laid, at the time of incubation, and at transfer time. Eggs in 1973 were treated prior to incubation by the temperature-differential method of egg dipping in a solution of 3000 ppm tylan tartrate, 1000 ppm gentamicin sulfate, and 500 ppm quaternary ammonium compound.

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All day-old poults were injected subcutaneously with 10 mg of streptomycin and 2000 IU of penicillin. Tylan tartrate was added to the drinking water for the 1st 10 days in the 1973 and 1974 hatches. A coccidiostat was fed during the brooder-grower period.

Feed during this study was provided by three different commercial mills. Mill no. 1 produced the feed from May 1968 through September 1972; Mill no. 2 from October 1972 through May 1973; and Mill no. 3 from June 1973 on. Animal by-products were utilized as a protein source in Mill no. 1 and 2, whereas only plant-origin protein was utilized in the manufacture of turkey feed in Mill no. 3. All feed was pelleted. The pelleting process the mills used was chosen to provide the desired physical properties. Bacteriological quality was not considered separately. Starting rations were crumbled following pelleting.

The Dillon Beach ranch was several miles from the closest poultry operation. Wild birds, rabbits, small rodents, deer, and reptiles frequented the property. Sporadic attempts were made to control the rodents and birds.

Sampling specimens and procedures. *1) Litter sampling.* A total of 24,812 samples placed in 5496 composite samples were obtained for bacteriologic examination. A large sterilized mixing spoon was used to place litter samples in sterile Whirl-Pac or other types of plastic bags. After mixing in the laboratory, 10 g from each composite sample were cultured.

Pedigree hen and tom pens and nest units were sampled at the 4th and 16th weeks of egg production and just after the breeders had been removed. Five spoonfuls made up each composite hen-pen and pure-line tom-pen sample, and 2 spoonfuls made up each pedigree-tom-pen composite samples. One spoonful was taken from each nest.

Pure-line hen pens were samples at the 4th week of production and shortly after the breeders were removed. Litter sampling was by the same procedure used in the brooder-grower houses.

Brooder-grower houses were sampled in 1970, 1971, and 1972 at the 4th, 12th, and 18–20th weeks of life. The floor area in each house was divided into 8 × 10-ft grids, and a composite sample of 5 spoonfuls per grid was obtained. In 1973 and 1974, samples were obtained at the 2nd, 10th, and 18th weeks of life. A composite sample of 5 spoonfuls of litter across the width of the house

was obtained every 15 feet for the length of the house. Environmental swabs only were taken in 1969.

2) Environmental swabs. About 4,325 samples placed in 866 composite samples were obtained for culture. Samples were taken with cotton-tipped applicators moistened with Selenite Brilliant Green (SBG) broth. Five or fewer were pooled in one composite sample in sterile culture tubes. SBG broth was added at the laboratory. Areas swabbed included ledges, ventilation fans, window-screens, droppings, hatching equipment, walls, and floors.

3) Nonviable incubated eggs. Samples from 8,025 nonviable eggs incubated for 10 to 21 days were placed in 535 composite samples for culture. Fifteen or more eggs were pooled per composite sample. After shell disinfection, a hole was drilled through the shell and 2 ml of fluid was removed for culture.

4) Dead embryos and cull poults. A total of 1,890 dead embryos and 2,776 cull poults were examined. A swab of yolk and a portion of the liver were obtained from each and placed in 933 composite samples.

5) Hatcher debris. A total of 153 composite samples of fluff, and egg shells were taken with tongue depressors from trays, around the fan, and from the hatcher floor. Samples were placed in Whirl-Pac bags. After mixing, 10 grams from each sample was cultured in 100 ml of SBG broth.

6) Sexing debris. A total of 28 composite samples were obtained.

7) Birds experiencing early deaths. Cultured in the same manner as cull poults were 971 dead poults.

8) Adult birds. A total of 883 gastrointestinal and reproductive tracts were obtained at time of processing, placed in individual plastic bags, cooled, and returned to the laboratory. Areas cultured by sterile swab were the cloaca, rectum, ileocecal junction, magnum, isthmus, uterus, and vagina.

9) Feed samples. A total of 2252 samples were cultured. One sample of feed per ton of a delivery lot was collected in 18-ounce sterile Whirl-Pac bags. Ten grams from each sample was added to 100 ml of SBG broth for culture. In 1969, 1970, 1971, and 1972 the samples were obtained as the feed was augered into the ranch feed bins. In 1973 and 1974, samples were obtained at the mill as the feed was augered into the delivery truck.

10) Cloacal swabs. A total of 54,380 swabs were pooled in 5,438 composite samples for culture. Cotton-tip applicators were

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Table 1. Salmonella serotypes detected during a sixty-one-month epidemiological survey of Dillon Beach Ranch by turkey strain, generation, calendar year, production period, and specimen type.

Production period and specimen type	Turkey strain, generation (in roman numerals), and calendar years involved: Strain A I 1968–69	Strain A II 1969–70	Strain A III 1970–71	Strain A IV 1971–72	Strain A V 1972–73	Strain A VI 1973–74	Strain B I 1970–71	Strain B II 1971–72
1) Hatchery period								
a) Embryos & culls		–	–	–	+G	–	–	–
b) Hatchery debris		–	–	–	+D,G	+D,G	–	–
c) Candle-out		–	–	–	–	–	–	–
2) Brooder-grower period								
a) Feed		–	+A	–	–	–	+A	–
b) Early mortality		–	–	–	–	+A	–	–
c) Litter		–	–	–	+D	+A	–	–
d) Cloacal swab		–	–	–	–	+A	–	–
e) Environmental swab		–	–	–	–	+A	–	–
f) Market tissue		–	–	–	+D,F,G	+A,I		
g) Serologic test								
3) Breeder period								
a) Serologic test	–	–	–	–	–	–	–	+B
b) Feed	–	–	–	+G	+A	+I,J,K	–	–
c) Pedigree								
Litter	–	–	–	–	–	–		
Environmental swab	–	–	–	+C	–			
Market tissue	–	–	–	+D,G	–			
d) Pure-line litter	–	–	–	–	+A	–		
Environmental swab	–	–	–	–	–			
Market tissue	–	–	–	+D,F	+M			
4) Feed-mill area	–	–	–	+B	+P	–	–	+B

AS. *tennessee* — NS. *heidelberg* — NS. *reading*
NS. *typhimurium* var. *copenhagen* — NS. *bareilly* — NS. *anatum*
OS. *infantis* — NS. *arizonae* — NS. *kentucky*
OS. *san diego* — NS. *worthington*

utilized and placed in sterile tubes for transport to the laboratory where 30 ml of SBC was added.

11) Serum samples. A total of 46,977 serum samples were obtained, and 93,930 serologic tests performed as described below.

Testing procedures. *1) Bacteriological examination.* Difco selenite brilliant-green (SBG) was used as the selective enrichment medium for all specimens cultured for isolation of salmonella. Enrichment broth cultures were incubated at 37 C for 18–24 hours. A minimum of 3 suspect colonies were transferred to Difco triple sugar-iron-agar (TSIA) slants and tryptonic broth. Slants showing typical salmonella reactions were tested biochemically by inoculating 0.5 ml of the 24-hour tryptone broth culture to dulcitol, salicin, malonate, and urease and incubated at 37 C for 48 hours. Salmonella serotypes were identified by the Spicer-Edwards technique. Representative isolates were sent for confirmation to USDA's National Animal Disease Center, Ames, Iowa.

A total of 17,858 samples were cultured.

2) Serological testing. The standard tube agglutination test as described in the National Poultry Improvement Plan (2) was utilized for *S. pullorum* and *S. typhimurium* employing separate antigens. Each selected breeder serum was tested for these infections each season. A total of 51,282 tests were performed at the California State Livestock and Poultry Pathology Laboratory in Petaluma, California.

Microagglutination and microantiglobulin tests for salmonella utilizing group B, C, and D antigens were also performed at the USDA Southeast Poultry Research Laboratory, Athens, Georgia, and the Petaluma Laboratory according to techniques described by Williams and Whittemore (2,16). Sera from all 1972 Strain B breeders and 1973 Strain A pedigree breeders were tested by these methods.

A total of 38,874 microantiglobulin and microagglutination tests were performed on sera from 1973 Strain A brooder-grower birds at 5, 12, and 18 weeks of age. Twenty-four additional birds were tested at 24 weeks.

RESULTS

Table 1 summarizes salmonella isolations and serotypes identified during the study according to turkey strain and generation, calendar year, production period, and specimen type. Table 2 summarizes the rate of recovery of salmonellae by serotype in each

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Table 2. Rate of recovery of salmonella by serotype in each specimen type from which isolations were made during a calendar period of any generation of strain A or B.

Production age period and specimen type	Calendar period 1970-71 No. cult.	1970-71 No. isol.	1970-71 Serotype[A]	1971-72 No. cult.	1971-72 No. isol.	1971-72 Serotype[A]	1972-73 No. cult.	1972-73 No. isol.	1972-73 Serotype[A]	1973-74 No. cult.	1973-74 No. isol.	1973-74 Serotype[A]
1) Hatchery age												
a) Embryo and culls[B]							86	1	3			
b) Hatchery debris[B]							49	5	4	92	2	4
								15	3		9	3
2) Brooder grower age												
a) Feed												
b) Early mortality	8	1	1									
c) Litter[B]										358	50	1
d) Cloacal swab[B]							281	6	4	367	85	1
e) Environmental swab[B]										4308	902	1
f) Market tissue										118	8	1
GI tract							400	2	4			
Spleen							7	1	7			
								1	6			
g) Serologic test										24	3	1
Reactor bird											1	9
3) Breeder age												
a) Serologic test				30	3	2						
Reactor bird												
b) Feed				570	1	3	266	1	1	38	1	9
											1	10
c) Pedigree											1	11
Environmental swab[B]				38	1	3						
Market tissue—GI tract				78	22	4						
					1	2						
d) Pure-line												
Litter[B]							127	2	1			
Market tissue—GI tract				147	14	4	4	2	8			
4) Feed-mill area				43	1	5	23	1	6			

[A]Salmonella serotypes: 1, *S. tennessee*; 2, *S. typhimurium* var. *copenhagen*; 3, *S. infantis*; 4, *S. heidelberg*; 5, *S. bornum*; 6, *S. arizonae*; 7, *S. san diego*; 8, *S. reading*; 9, *S. anatum*; 10, *S. lexington*; 11, *S. worthington*.
[B]Composite samples.

specimen type from which isolations were made during the calendar period of any generation.

The first ranch isolation was *S. tennessee*, from brooder-grower feed in December, 1970, 31 months after the ranch was populated. This isolation was not associated with prior or subsequent bird infection (Table 1). No bacteriologic or serologic evidence of bird-associated salmonella infection was obtained during the 3½ years that the Dillon Beach ranch was in operation. This included 3 successive generations and the brooder-grower period of the 4th generation of Strain A turkeys and the 1st generation and the brooder-grower period of the 2nd generation of Strain B taken (Table 1).

The first salmonella isolation from turkeys on the Dillon Beach ranch was in 2nd-generation Strain B breeders in January 1972 (Table 1). Five birds reacted to the *S. typhimurium* standard tube agglutination test, 8 to the *S. pullorum* tube agglutination test, and 1 to both. The microagglutination test on these sera was negative at the 1:20 dilution. The microantiglobulin test revealed 2 birds positive to Group B antigen, 16 to Group C, and 1 to Group D at titers of 1:20 to 1:160. *S. typhimurium* var. *copenhagen* was isolated from 1 Group B and 1 Group C microantiglobulin test reactors, and from the one which had reacted to both standard tube tests for *S. typhimurium* and *S. pullorum*. Known infection with *S. heidelberg*, a group B Salmonella, in Strain A turkey generations 5 and 6 was not detected by testing all selected breeders with the standard tube agglutination test for *S. typhimurium*.

The initial isolations from Strain A turkeys or their immediate environment were *S. infantis* and/or *S. heidelberg* from hatchery debris of five of the six 1972 hatches of the fifth generation (Table 1). *S. infantis* was also recovered from culled day-old poults of the same hatching period. Shortly after these hatchery isolations, the same serotypes were isolated from the 4th-generation pedigreed and pure-line breeders which were still on the ranch and had produced the eggs for these hatches. *S. infantis* was isolated from a feed bin supplying the 4th-generation Strain A pure-line breeders and from an environmental swab taken from the hen section of 1 pedigree house. *S. heidelberg* and/or *S. infantis* were found in the gastrointestinal tracts of these 4th-generation pedigreed and pure-line breeders at time of their marketing after egg production (Table 1).

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Table 2. Rate of recovery of salmonella by serotype in each specimen type from which isolations were made during a calendar period of any generation of strain A or B.

Production age period and specimen type	Calendar period 1970–71 No. cult.	1970–71 No. isol.	1970–71 Serotype[A]	1971–72 No. cult.	1971–72 No. isol.	1971–72 Serotype[A]	1972–73 No. cult.	1972–73 No. isol.	1972–73 Serotype[A]	1973–74 No. cult.	1973–74 No. isol.	1973–74 Serotype[A]
1) Hatchery age												
a) Embryo and culls[B]							86	1	3			
b) Hatchery debris[B]							49	5	4	92	2	4
								15	3		9	3
2) Brooder grower age												
a) Feed	8	1	1									
b) Early mortality										358	50	1
c) Litter[B]							281	6	4	367	95	1
d) Cloacal swab[B]										4208	902	1
e) Environmental swab[B]										113	8	1
f) Market tissue												
GI tract							400	2	4			
Spleen							7	1	7			
								1	6			
g) Serologic test										24	3	1
Reactor bird											1	9
3) Breeder age												
a) Serologic test				30	8	2						
Reactor bird												
b) Feed				570	1	3	266	1	1	86	1	9
											1	10
c) Pedigree											1	11
Environmental swab[B]				38	1	3						
Market tissue—GI tract				73	22	4						
					1	2						
d) Pure-line												
Litter[B]							137	2	1			
Market tissue—GI tract				147	14	4	4	2	8			
4) Feed-mill area				43	1	5	23	1	6			

[A]Salmonella serotypes; 1, *S. tennessee*; 2, *S. typhimurium* var. *copenhagen*; 3, *S. infantis*; 4, *S. heidelberg*; 5, *S. bornum*; 6, *S. arizonae*; 7, *S. sandiego*; 8, *S. reading*; 9, *S. anatum*; 10, *S. lexington*; 11, *S. worthington*.
[B]Composite samples.

specimen type from which isolations were made during t calendar period of any generation.

The first ranch isolation was *S. tennessee*, from brood grower feed in December, 1970, 31 months after the ranch w populated. This isolation was not associated with prior or su sequent bird infection (Table 1). No bacteriologic or serologic e dence of bird-associated salmonella infection was obtained duri the 3½ years that the Dillon Beach ranch was in operation. Th included 3 successive generations and the brooder-grower peri of the 4th generation of Strain A turkeys and the 1st generati and the brooder-grower period of the 2nd generation of Strain taken (Table 1).

The first salmonella isolation from turkeys on the Dill Beach ranch was in 2nd-generation Strain B breeders in Janua 1972 (Table 1). Five birds reacted to the *S. typhimurium* standa tube agglutination test, 8 to the *S. pullorum* tube agglutination te and 1 to both. The microagglutination test on these sera was neg tive at the 1:20 dilution. The microantiglobulin test revealed birds positive to Group B antigen, 16 to Group C, and 1 to Group at titers of 1:20 to 1:160. *S. typhimurium* var. *copenhagen* was is lated from 1 Group B and 1 Group C microantiglobulin test r actors, and from the one which had reacted to both standard tu tests for *S. typhimurium* and *S. pullorum*. Known infection wi *S. heidelberg*, a group B Salmonella, in Strain A turkey generatio 5 and 6 was not detected by testing all selected breeders with t standard tube agglutination test for *S. typhimurium*.

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Table 3. Bacteriologic and serologic testing for *Salmonella tennessee* infection in 1973 Dillon Beach Strain A brooder-grower birds, according to sample type, age, hatch, and house number.

Sample type and age	Percent of samples positive to *Salmonella tennessee* by culture or microantiglobulin test															
	Hatch 1		Hatch 2		Hatch 3		Hatch 4		Hatch 5		Hatch 6		Hatch 7			
	Hse 1	Hse 2	Hse 3	Hse 4	Hse 5	Hse 9	Hse 6	Hse 10	Hse 7	Hse 11	Hse 8	Hse 12				
1) Early Mortality[A]	-	-	-	-	-	23	42	29	-	27	11	15				
2) Litter[B]																
2 weeks	-	-	-	-	-	100	-	100	-	83	-	100				
10 weeks	-	-	-	-	-	42	-	94	-	68	-	92				
18-20 weeks	-	42	-	17	-	17	-	25	17	87	88	25				
3) Cloacal swab[C,D]																
5 weeks	-	-	-	-	-	73	-	79	-	69	-	42				
12 weeks	-	-	-	-	-	35	-	93	-	91	-	80				
18 weeks	-	-	-	-	6	15	14	23	-	48	20	22				
4) Serum[C,E]																
Group C Antigen																
5 weeks	-	-	-	-	-	30	-	26	-	51	-	33				
12 weeks	-	-	-	-	-	19	-	6	-	37	4	22				
18 weeks	3	-	-	-	1	1	12	5	1	14	27	12				
Group D antigen																
18 weeks	-	-	-	-	-	-	-	-	-	-	-	-				
Group D antigen																
19 weeks	-	-	-	-	-	-	-	-	-	-	-	-				
5) Environmental swab[F]	-	-	13	-	-	13	-	17	-	21	-	-				

[A]14 to 90 poults per house during first two weeks.
[B]6 composite samples per house at 2 weeks, 12 composite samples per house thereafter. Samples at 18-20 weeks obtained shortly after birds removed from house.
[C]Cloacal swabs and serum samples obtained from same birds. Approximate no. of birds swabbed: 5 weeks 17,000; 12 weeks 17,000; 18 weeks 8,000. No. of sera tested: 5 weeks 3,600; 12 weeks 3,600; 18 weeks 8,000.
[D]10 swabs pooled in each composite sample.
[E]Positive reactions to the microantiglobulin test at dilutions of 1:20 or higher.
[F]5 to 16 composite samples per house.

The *S. bornum* isolated from fish meal in Mill no. 2 in 1972 was not associated with prior or subsequent bird infection (Table 1). *S. heidelberg* was isolated from the litter of 1 brooder-grower house of the 1st hatch of 5th Strain A turkey generation at 3 and 7 weeks of life. Litter culture was discontinued after the 4th hatch of this generation (Table 1). The *S. san diego* and *S. arizonae* isolated from tissues of marketed culls of the 5th Strain A generation were not associated with prior or subsequent bird infection on the ranch (Table 1). *S. tennessee* was isolated from the litter of the 5th Strain A generation pure-line breeders and from the feed of these breeders in June 1973 (Table 1).

S. reading was isolated from 2 of 6 fifth-generation pure-line hens retained on the premises for egg production for meals of ranch personnel. *S. reading* had also been isolated from birds of another of the company's farms in the area. The *S. arizonae* isolated from Mill no. 3 during the 5th-generation breeder season was not associated with prior or subsequent turkey infection on the ranch (Table 1). *S. infantis* and/or *S. heidelberg* were again isolated from hatchery debris of seven of eight 1973 hatches of the 6th Strain A generation (Table 1). Before incubation of eggs in these hatches they had been treated with Tylan, gentamicin sulfate, and quaternary ammonium compound. *S. tennessee* was isolated from all groups, several specimen types, and several age groups of the 6th-generation Strain A turkey brooder-grower birds (Tables 1, 3). *S. anatum* was isolated from one 6th-generation Strain A blood-test reactor (Table 1).

Table 3 summarized the bacteriologic and serologic test results for *S. tennessee* infection in the 6th-generation Strain A brooder-grower birds. Isolations were made from poults dying in the first 2 weeks of life in houses 6, 8, 9, 10, 11, 12, including four hatches. Subsequent testing in these 6 houses revealed positive cultures and serologic tests to be most consistent, persistent, and widepread in houses 9, 10, 11, and 12, with all age groups tested being infected. There was good correlation in ability to detect infection between all specimen types and testing procedures used in these 4 houses. The rates of isolations from cloacal swabs and litter samples and of serologic reactions to the microantiglobulin test in these houses decreased as the infected population became older. Culture at 24 weeks of age of 24 reactors to the microantiglobulin test revealed *S. tennessee* in 3 and *S. anatum* in 1.

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Infection in houses 6 and 8, first detected in dead poults during the 1st 2 weeks of life, was not detected again until 18 weeks of life, whereas infection was widely detected throughout the growing period in hatchmates in houses 10 and 12 as mentioned above. No isolations were made in houses 1, 2, 3, 4, 5, and 7 (involving 4 hatches) until late in the brooding-growing period, with inconsistent specimen types being involved in positive tests. The rate of serologic reactions to the microtiter test in house 8 increased between 12 and 18 weeks (Table 3).

S. anatum, *S. lexington*, and *S. worthington* were isolated from the feed of the 6th-generation Strain A breeders (Table 1).

Miscellaneous wild-animal specimens cultured during the survey were negative. These included 1 rat, 4 mice, 1 rabbit, 1 snake, 1 swallow, 12 composites of fly pupae, and rat and mouse feces.

DISCUSSION

There was no evidence that salmonella was introduced into the Dillon Beach ranch by egg-transmitted infection. None of the salmonella serotype infections *S. worthington*, *S. san diego*, and *S. infantis* experienced by Strain A turkey source breeders in Oregon were detected in three full generations raised at Dillon Beach following importation of eggs. Likewise, no turkey infections were experienced in the first full generation of Strain B turkey breeders following egg importation. These observations indicate that no ovarian transmission of salmonella occurred at the time the hatching eggs were produced in Oregon or Wisconsin for use at Dillon Beach. It also suggests that salmonella either did not contaminate the shells of these imported eggs or was completely eliminated by the farm and/or hatchery shell sanitation procedures.

The elimination of imported hatching eggs as a source of the eventual infection in turkeys and the contaminations of production areas indicates that the salmonellae involved were either present on the premises at the time the ranch was populated or were introduced later. The absence of any detectable infection in turkeys or contamination of production areas (other than in feed) during the first 3½ years was considered sufficient evidence that prior contamination was not the source. It is therefore felt that all salmonella serotypes associated with bird or environment infection were physically carried in.

A total of 11 salmonella serotypes were identified during this study. Three (*S. arizonae*, *S. bornum*, and *S. san diego*) were not associated with infections on the ranch and hence were felt not to have established themselves on the Dillon Beach ranch premises. These isolations were from feed-mill and processing-plant premises where the specimens were obtained.

Eight serotypes gained access to the ranch, and all were believed to have been introduced in feed or by personnel or supplies. These were *S. tennessee*, *S. anatum*, *S. lexington*, *S. worthington*, *S. heidelberg*, *S. infantis*, *S. reading*, and *S. typhimurium* var. *copenhagen*. Four of these serotypes (*S. tennessee*, *S. anatum*, *S. lexington*, and *S. worthington*) were isolated directly from pelleted feeds. This demonstrated that feed was one vehicle conveying salmonella organisms to the ranch during the survey period. A 5th serotype (*S. infantis*) was also associated with feed. The remaining serotypes were associated with turkey infections only and were not isolated from feeds or premises. Their routes of access are thus obscure.

However, outside personnel and equipment routinely entered the Dillon Beach Ranch premises. These included insemination crews and their vehicles, often closely associated with other Nicholas turkey populations in the surrounding area. The routine entrance-area sanitary procedures, including personal hygiene practices and vehicle sanitation, could not have assured the prevention of entrance of salmonellae by this route. The *S. reading* isolation, in particular, strongly suggests the entrance of infection by personnel traffic. The possible roles of wild birds, rodents, and reptiles in the introduction of salmonella infections to the premises were not determined, although such possibilities are documented in the literature (9,10,13).

The importance of this study from the standpoint of the possibility of maintaining salmonella-free turkey populations was the demonstration that progeny from breeder turkeys with a history of salmonella infection can be hatched free of infection and that such progeny, and at least several generations thereafter, can be raised in present production facilities free of salmonella providing the premises they are grown on have no endemic infection or prior contamination and viable organisms can be prevented from being physically introduced. Present-day sanitary practices in commercial feed manufacture and the access to premises of outside

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personnel and equipment are not adequately designed to prevent the entrance of salmonellae.

In terms of this study, the most serious obstacle to maintaining primary turkey breeders indefinitely free of salmonellae is felt to be the inability to consistently produce and deliver salmonella-free finished feed. Also considered sizable problems are other sources of infection, such as premises contamination by personnel, equipment, and wild animals.

Following their introduction into the ranch, the salmonella serotypes isolated were quite erratic in persistence in birds or the environment as observed by the testing systems utilized. An interesting observation was that environmental contamination with salmonellae was transient, when occurring, and mostly absent in the open brooder-grower houses (nos. 1–8) and the breeder houses. The age of the birds in the breeder houses (i.e., adults) may have been an additional factor for low recovery in those areas.

Equally interesting was the high incidence and long duration of infection in young birds and environmental contamination found in the forced-air ventilated brooder-grower houses (nos. 9–12).

Building sanitation between seasons appeared to be effective in eliminating environmental contamination. That egg transmission may have played a role in the persistence of *S. heidelberg* and *S. infantis* in turkey Strain A generations 5 and 6 is suggested by recovery of these organisms from hatchery debris. If that was the case, it would suggest that the fumigation practices and the antibiotic dipping were not effective in preventing all transmission. Assuming that the dipping and fumigation were done as effectively as possible and eliminated shell contamination, this would suggest the possibility of ovarian transmission in the seven hatches involved. There was, however, no breeder infection indicated in repeated sampling of breeder-house litter during production. However, these serotypes were isolated from 4th-generation breeders going to market. Consequently, some mode of environmental contamination at the hatchery level must also be considered a possibility.

It is also of interest that none of the selected breeders from generations 5 and 6, which revealed *S. heidelberg* infection at hatch time, reacted serologically to the *S. typhimurium* tube agglutination test.

The low rate of salmonella isolations from feed samples may indicate a low level of contamination. This low recovery rate suggests that feed sampling procedures should be improved and that large numbers of samples may be needed to assure detection of low levels of contamination. The varying isolation rate from the environment suggests the necessity for extremely intensive sampling to detect transient contamination.

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