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Understanding the Molecular Methods for Typing Bacteria What does PFGE, RFLP, or BRENDA mean?

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University of Georgia

Traditionally, clinical microbiology has relied on phenotypic tests to type bacterial strains. These methods have often examined susceptibilities of a bacterial population to a battery of bacteriophages or antibiotics. Other methods have relied on immunological methods or serotyping to further differentiate a species. Collectively, these typing methods have been useful tools to understand the epidemiology of infectious disease. Often these methods have identified a specific phage type or serotype associated with an illness or geographic location. For example, there are at least 3 major *E. coli* O serotypes, O1, O2, and O78 associated with colibacillosis in birds. The serological and bacteriological methods for typing microbes are often limited to a few select reference laboratories (see Table 1).

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Broiler Performance Data (Region)
Live Production Cost

	SW	Midwest	Southeast	Mid-Atlantic	S-Central
Feed cost/ton w/o color ($)	121.17	111.61	130.25	127.07	125.05
Feed cost/lb meat (¢)	11.56	10.87	12.86	12.31	12.00
Days to 4.6 lbs	44	44	45	44	44
Med. cost/ton (¢)	2.47	1.61	3.19	3.00	2.82
Chick cost/lb (¢)	3.96	3.74	3.58	3.96	3.78
Vac-Med cost/lb (¢)	0.04	0.02	0.08	0.12	0.10
WB & 1/2 parts condemn. cost/lb	0.23	0.21	0.25	0.34	0.38
% mortality	4.12	4.25	4.09	5.06	5.32
Sq. Ft. @ placement	0.75	0.75	0.74	0.76	0.81
Lbs./Sq. Ft.	6.28	7.32	7.28	6.95	6.75
Down time (days)	16	16	16	17	18

Data for week ending 1/22/00

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Table 1. Comparisons of Traditional Methods for Typing Bacteria

Method	Advantages	Limitations	Time
Serotype	Simple.	Availability of reagents. Variation in antigen.	min.-Hrs.
Biotype	Simple. Availability of reagents.	Variability in carbohydrate metabolism.	1-2 Days
Phage Type	Simple.	Availability of reagents.	1 Day
Antibiotic profile	Simple. Availability of reagents.	Multi-drug resistance.	1 Day

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Broiler Performance Data (Company)
Live Production Cost

	Average Co.	Top 25%	Top 5 Cos.
Feed cost/ton w/o color ($)	125.54	119.10	118.98
Feed cost/lb meat (¢)	12.11	11.22	11.29
Days to 4.6 lbs	44	44	44
Med. cost/ton (¢)	2.80	2.07	2.34
Chick cost/lb (¢)	3.90	3.71	3.37
Vac-Med cost/lb (¢)	0.08	0.04	0.07
WB & 1/2 parts condemn. cost/lb	0.29	0.18	0.17
% mortality	4.67	3.73	4.70
Sq. Ft. @ placement	0.76	0.73	0.75
Lbs./Sq. Ft.	6.75	6.77	7.17
Down time (days)	16	16	16

Data for week ending 1/22/00

Broiler Whole Bird Condemnation (Region)

	SW	Mid-West	S. East	Mid-Atlantic	S. Central
% Septox	0.347	0.334	0.391	0.258	0.369
% Airsac	0.103	0.066	0.162	0.374	0.537
% I.P.	0.060	0.084	0.106	0.279	0.217
% Leukosis	0.006	0.003	0.017	0.003	0.003
% Bruise	0.009	0.006	0.011	0.018	0.015
% Other	0.019	0.005	0.013	0.025	0.015
% Total	0.545	0.498	0.700	0.965	1.156
% 1/2 parts condemnations	0.391	0.353	0.291	0.412	0.377

Data for week ending 1/22/00

Continued from page 2

These typing procedures are not sensitive enough to further differentiate bacterial isolates. Bacterial isolates, such as *E. coli*, can be differentiated further using procedures that identify differences in the genetic composition of a microbial population. These techniques involve multilocus enzyme electrophoresis, bacterial restriction endonuclease digest analysis (BRENDA), pulsed-field gel electrophoresis (PFGE), random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) analysis by Southern blot or PCR or DNA sequencing.

Bacterial Restriction Endonuclease Digest Analysis (BRENDA).

One of the earliest molecular methods for differentiating bacterial isolates, BRENDA, employed analysis of genomic DNA digested with a restriction enzyme and the separation of the high and low molecular weight (MW) DNA by gel electrophoresis. Differences were often observed in the DNA banding patterns for members of a species. This method has worked especially well for microorganisms with small genomes like *Haemophilus* or *Mycoplasma*, where discrete DNA bands and their pattern are easily discernible upon visual inspection. The disadvantage of BRENDA at the time of its inception was the unavailability of any mathematical analyses that would offer the unbiased analysis of the complex DNA patterns generated from restriction enzyme digests.

Pulsed-Field Gel Electrophoresis (PFGE).

a. Description. The advent of PFGE allowed the resolution of large molecular weight (MW) DNA fragments between 20 kb to 1,000 kb in size and permitted investigators the opportunity to fingerprint organisms with large genomes of 4 Megabase (Mb) or greater. Standard gel electrophoresis fails to separate DNA fragments greater than 20 kb. During PFGE, an electric field is periodically changed in terms of its orientation and duration, thus allowing large MW DNA to migrate through the gel. This method involves the imbedding of bacteria into an agarose plug and subsequent lysis. The DNA is left immobilized and intact. A restriction enzyme is then chosen which lyse the DNA into fragments (8-20 fragments) with a broad size range. **Typing by PFGE is useful because it can detect subtle changes in the bacterial genome resulting from the insertion or deletion of gene sequences or mutations that alter the restriction enzyme sites.** These genetic changes are identified by PFGE as changes in a single DNA fragment among the DNA fragments observed on PFGE gel. Isolates with identical PFGE DNA pattern are considered indistinguishable while bacteria with 2 to 3 different DNA bands are closely related. Bacteria are unrelated when there are 7 or more different bands. Molecular typing by PFGE is currently used in epidemiology to fingerprint many bacterial isolates, such as *E. coli* O157:H7.

b. Molecular epidemiology of food-borne pathogens by PFGE.

i. *Escherichia coli* O157:H7. The Center for Disease Control (CDC) in Atlanta currently uses PFGE to study the molecular epidemiology of *Escherichia coli* O157:H7. The method is reproducible with several different laboratories generating comparable results. More medically important, microbes have been examined by PFGE than any other molecular typing method. Pulsed-field gel electrophoresis is the closest thing to a universal method for molecular typing.

ii. Salmonella and Campylobacter. The molecular epidemiology of food-borne outbreaks associated with *Salmonella spp*. have been investigated using PFGE. Baquat et al. compared *S. brandenburg* isolates from sporadic cases to a 1992 outbreak. They were able to discriminate the outbreak isolates from sporadic isolates of *S. brandenburg* isolated in other countries with PFGE. Pulsed-field gel electrophoresis had greater discriminatory power in terms of typing isolates than plasmid profiling or ribotyping. Other investigators have examined PFGE to genetically type *S. enteriditis* isolates. Using two different restriction enzymes to generate restriction enzyme digest profiles (REDP) with PFGE, Suzuki et al. were able to identify outbreak isolates and distinguish them for sporadic case isolates. The resolving power of PFGE to identify genetic differences among bacteria is dependent on whether single or multiple restriction enzymes are selected to generate REDP and the choice of restriction enzyme(s). If PFGE is to be a useful tool for investigating *S. enteriditis*, it will be important to implement an accepted, standardized protocol. Laboratories are now starting to examine various molecular techniques for typing *Campylobacter spp*. Pulsed-field gel electrophoresis was compared to RFLP-PCR of rRNA and flagellin genes and found to have the greatest discriminatory power to genetically type campylobacter.

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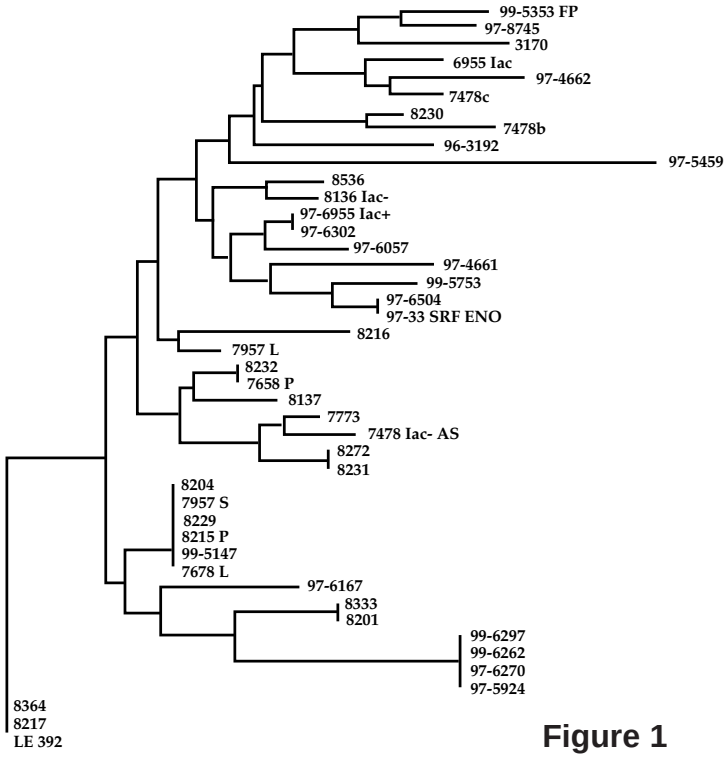

Figure 1

c. Limitations of PFGE. Pulsed-field gel electrophoresis requires the use of special, single-purpose gel electrophoresis equipment. The cost of the PFGE equipment ranges from $4,000 for a simple Field Inversion Gel Electrophoresis (FIGE) system to $10,000 for the Clamped Homogenous Electric Field (CHEF) gel electrophoresis system routinely used in human genome analysis. Results are obtained in 2 days for eighteen samples. As with many molecular typing methods, procedures for PFGE have to be empirically derived for each microbe. For example, bacteria like *Staphylococcus aureus* are recalcitrant to lysis with lysozyme and therefore require another cell wall hydrolase (lysostaphin) to release the chromosomal DNA. Choice of restriction enzymes for typing must be empirically determined for each microorganism. Fortunately, since its inception as a molecular typing tool, PFGE procedures have been developed for typing many medically important pathogens. As is the case for BRENDA, PFGE requires that bacterial DNA remain intact prior to restriction enzyme digestion. Problems with DNA degradation has precluded PFGE from use in typing certain species. See Tables 2 and 3 for advantages and limitations of the various techniques used in molecular epidemiology.

Finally, analysis of large data sets will require the use of a special computer program. Software has been designed to perform distance-based phylogenetic analysis of RFLP. The advantage of using this software is its: 1) application to any technique that identifies RFLP s; 2) comparison of large sample sizes or results from past experiments; and 3) identifies problems with reproducibility. The software also manipulates the images and erases distortions in the gel that might interfere with the phylogenetic analysis. In the end, the program generates a phylogenetic tree or dendrogram (Figure 1). Isolates that cluster together in this dendrogram are considered to be genetically related.

Table 2. Comparison of Molecular Typing Methods for Bacteria

Method	Advantages	Limitations	Time
BRENDA	Only requires gel electrophoresis equipment	Complex pattern Pure culture. "Cutable" DNA.	2-3 Days
PFGE	Simple pattern for comparisons. Reproducible. Availability of reagents.	Equipment: single purpose & cost. Pure culture. "Cutable" DNA.	2 Days
RFLP-Southern	Simple pattern for comparisons.	Pure culture. "Cutable" DNA. Many steps.	5 Day

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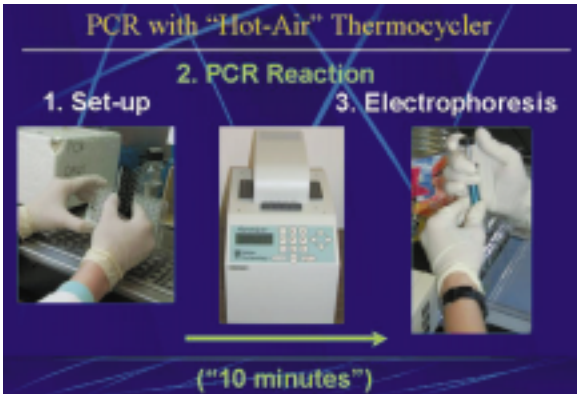
Table 3. Comparison of PCR Methods for Typing Bacteria

Method	Advantages	Limitations	Time
RAPD	No prior knowledge of gene sequences required.	Pure culture. Equipment cost. Reproducibility.	4-8 Hours
RFLP-PCR	Pure culture may not be necessary. "Specificity"	Knowledge of gene sequence. Equipment:cost.	4-8 Hours
PCR + DNA	Sequencing. Pure culture may not be necessary. Identify "subtle" differences. "Specificity".	Knowledge of gene sequence. Expensive software for analysis. Access to automated DNA sequencer.	2 Days

Restriction Fragment Length Polymorphism (RFLP).
Methods involving RFLP analysis are used to examine repetitive elements and differences present in restriction enzyme sites that exist within or flanking a repetitive element. The earliest RFLP procedures referred to as ribotyping identified genetic differences within a species by Southern Blot analysis of genomic DNA probed with labeled rRNA genes. Ribotyping has been used to fingerprint food-borne pathogens including *Salmonella sp.* and *Campylobacter sp.* Differences in gene sequences translate into presence or absence of specific restriction enzyme recognition sites. This inherent variability in a gene sequence has been exploited by combining PCR with restriction enzyme digests to type bacteria isolates. Methods based on PCR technologies have decreased the time required to type bacterial strains from days to hours. The quality of the template is also critical for Southern analysis but less so for RFLP-PCR.

Random Amplification of Polymorphic DNA (RAPD) or Arbitrary Primer PCR.
It was recognized early in the development of PCR that arbitrary primers can randomly amplify DNA sequences. Using M13 sequencing primers, Welsh and McClelland reported that with high Mg 2+ concentration and low annealing temperatures, PCR produced distinctive DNA banding patterns in bacteria and plants. Arbitrary primer PCR or RAPD PCR has been useful in fingerprinting microbial isolates including Salmonella, *E. coli, Histoplasma capsulatum, Campylobacter*, and *Helicobacter pylori*.



DNA Sequencing.
Although primarily a tool for typing viruses. DNA sequencing has been recently applied in the molecular epidemiology of bacterial diseases. Polymerase chain reaction is used to amplify the gene for sequencing. The targets selected for sequencing range from surface proteins and enzymes to rRNA. The gene chosen for typing may have to be empirically determined for each microorganism. In most cases, DNA sequencing is used in conjunction with other typing methods like PFGE to examine the horizontal transfer of genes within a species. This method, although expensive, may have more practical applications for the identification and typing of microorganisms which are difficult to culture.

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Understanding the Molecular Methods for Typing Bacteria

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The ability of microbiologists, veterinarians, or physicians to epidemiologically trace bacteria has been greatly limited by our ability to type or serotype these organisms. These molecular techniques give us the ability to sift through the normal background bacteria and find the true source of the pathogenic or foodborne bacteria. Our ability to make full use of these new tools is still growing and I hope that I have been able to help clarify some of the terminology and methods that are available for your use. [1]

[1] The References used in Dr. Maurer's review are available upon request.

Broiler Whole Bird Condemnation (Company)

	Average Co.	Top 25%	Top 5 Co.'s
% Septox	0.354	0.271	0.227
% Airsac	0.259	0.073	0.061
% I.P.	0.141	0.049	0.089
% Leukosis	0.009	0.006	0.011
% Bruise	0.013	0.010	0.018
% Other	0.020	0.007	0.003
% Total	0.797	0.417	0.409
% 1/2 parts condemnations	0.351	0.285	0.263

Data for week ending 1/22/00

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Excerpts from the latest USDA National Agricultural Statistics Service (NASS) "Broiler Hatchery," "Chicken and Eggs" and "Turkey Hatchery" Reports and Economic Research Service (ERS) "Livestock, Dairy and Poultry Situation and Outlook" Reports

According to the most recent USDA Economic Research Service (ERS) reports, U.S. livestock producers are benefiting from inventory declines that are reducing production and a booming economy that is fueling demand for meat products. Livestock prices rallied in the last half of 1999 and feed costs remain relatively low, boosting producers' returns. Although poultry producers are benefiting from the low feed costs, rising production is pressuring prices. Broiler production is expected to moderate in 2000. Declining red meat production and moderating poultry production are expected to push total meat production below a year earlier for the first time since 1982.

Broiler Returns and Prices Lower

Broiler returns (before interest and overhead), at the whole-bird level, are not expected to remain in double digits in 2000 as they did in 1999. Slightly higher soybean meal prices and slightly lower corn prices are expected to keep 2000 feed prices nearly unchanged from 1999 while broiler prices are expected to be lower. As a result, production is expected to continue increasing but at a slower rate, near 5 percent. Prices for whole birds are expected to remain weaker than a year ago as production increases remain relatively strong. Prices for most broiler parts in January are 10-20 percent below a year ago, while strength in the export market is keeping leg quarter prices about 5 percent above last year. Competing supplies of red meat will be an important factor in overall meat prices in 2000. Projected declines in beef and pork production should prevent broiler prices from dropping even lower.

In response to attractive 1998 net returns, pullet hatch for potential placement in the hatchery supply flock was above a year ago in 1999. Cumulative potential placements indicate that the hatchery supply flock could be 3-8 percent larger than a year ago through the first half of this year. The broiler-type hatching egg production flock was 1 percent larger than a year ago on December 1, 1999.

Turkey Net Returns Stay Positive

A return to profitability in July 1998, and continued profitability since then, brought higher turkey production in the second half of 1999 and are expected to encourage a 2-percent rise for 2000, the strongest increase since 1996. The number of turkeys raised is expected to increase 1 percent based on producer intentions reported in USDA's recent Turkeys report. Additional meat production increase will come from heavier average bird weights. Lower stocks of turkey, 20 percent below a year ago, have kept whole bird and parts prices above January 1999. The most strength has been in drumsticks and wings, with prices nearly 50 percent higher than last year.

Egg Production Increases Expected

With continued positive average net returns in 1999, egg production is expected to continue increasing 2-3 percent in 2000. Table egg production is expected to increase about 2 percent with a 4 to 5-percent increase in hatching production raising the total.

Egg production increased nearly 4-percent in 1999, lowering wholesale large egg prices about 10 cents per dozen. Increasing production is expected to continue putting pressure on egg prices, with the 2000 forecast for wholesale prices about 5-6 cents lower. Net returns are expected to be slightly negative in 2000. Negative returns during October probably encouraged a decline of 7 percent in eggs in incubators for December 1, 1999, but the 6-percent increase in chicks hatched during 1999 will carry over into a larger production flock for 2000. The table egg production flock was 3 percent larger than a year ago on December 1.

Poultry Trade Slows

U.S. shipments of broiler products through the first 10 months of 1999 totaled 3.8 billion pounds, down only 3 percent from the same period the previous year. The estimate for fourth quarter shipments is 1.225 billion pounds. If this holds, the yearly total will be 4.631 billion pounds, down less than 1 percent from 1998.

While shipments to Russia through November are down 64 percent, the decline in the Russian market has not been that severe. The drop in direct exports to Russia has been partially offset by much higher exports to Estonia and Latvia. Shipments through November to these two Baltic countries has been close to 1 billion pounds. Most of the broiler products entering these countries are transshipped to Russian markets. The other major factor in the broiler export market has been the strong shipments to the China/Hong Kong and Korean markets. Over the first 11 months of 1999, shipments to Hong Kong and direct shipments to China have totaled 1.2 billion pounds, up 28 percent. As the market for imported poultry has opened in Korea, shipments have expanded very rapidly, rising over 400 percent through November.

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Excerpts from the latest USDA National Agricultural Statistics Service (NASS) ...

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The strengthening export markets for broilers have not yet generated higher prices. Through October, broiler exports were down 3 percent from the previous year on a quantity basis, but down 24 percent on a value basis. The relatively low cost of broiler meat has been one of the chief factors in its export growth and overall unit values are very low. The export value of broiler legs to Russia through November averaged only 21 cents a pound in 1999, down from 31 cents the previous year. With domestic production expected to expand again in 2000, little additional strength in broiler export prices is expected.

In 2000, broiler exports are expected to expand, with greater shipments going to a number of Asian markets and to a slowly recovering Russian market. The overall estimate for U.S. exports has been increased 75 million pounds since the December forecast, and the revised figure for 2000 is 4.750 billion pounds, up 2.6 percent from 1999, or about the same as 1998. This expansion is expected to continue driven by the poultry industry's ability to efficiently convert feed to meat, lowering its cost relative to both beef and pork. Additionally, demand in developing countries is expected to expanded driven by rising populations and a growing preference for a western type diet.

Mature chicken exports in October were 34.9 million pounds, down 10 percent from the previous year. Exports over the first 10 months of 1999 totaled 336 million pounds, down 6.5 percent from a year earlier. Although shipments to Asia have grown steadily in 1999, lower exports to Canada, Russia, and Poland have pushed the totals lower. The present estimate for fourth-quarter 1999 is 105 million pounds, which would bring the yearly total to 406 million pounds, down 20 million from the 1998.

Exports are expected to rise slightly in 2000 to 415 million pounds due to an overall increase in shipments to the major Asian markets of Hong Kong and Japan. Increases in Asia will also be helped by a small rebound in shipments to Russia and Poland, markets that almost completely disappeared in 1999.

Turkey Export Market

Exports of whole turkeys and turkey parts have fallen 16 percent during the first 11 months of 1999 compared to the previous year. Average prices have also declined, driving down the value of exports by 21 percent. The estimate for 1999 turkey exports is 361 million pounds, down 85 million pounds, or 20 percent from 1998. Turkey exports have been weak to most of the traditional markets. The only large importing countries where shipments have increased are Korea, Taiwan, and Canada. Exports to Korea have expanded sharply, up 151 percent. Shipments to Taiwan, which fell strongly in 1998, have increased over 1200 percent and are 86 percent above their total for all of 1997. Shipments to Canada, almost entirely turkey parts, have shown strong growth, expanding 40 percent.

The current forecast for U.S. turkey exports in 2000 calls for an increase of 8 percent to 390 million pounds. The increase is based on some growth in the Hong Kong market, which has been stronger over the second half of 1999. Continued strength is also expected in the Korean and Taiwan markets, although growth there is expected to be much smaller than the large jumps of 1999. There is also reason to believe that the Russian market has bottomed out after falling somewhat in 1998 and very heavily in 1999. In all cases, turkey parts will face strong competition from broiler and other chicken products, especially in mixed products such as sausages.

Table Egg Export Market

Overall exports of eggs and egg products (on an egg equivalent basis) are estimated at 159 million dozen in 1999, down 60 million dozen or 27 percent from the previous year. At this pace egg exports in 1999 would be the lowest since 1993. In October, exports of eggs and egg products totaled 13.9 million dozen, down more than 5 million dozen from the previous year. While exports have been lower to a number of markets, much of the decrease has come from a 70-percent decline in shipments to Mexico. The drop was concentrated in the egg product market. Higher Mexican egg production increased the number of eggs available to the egg product sector and lower the need for imports. Shipments also declined strongly to Hong Kong, where U.S. producers face strong competition from Chinese egg producers.

Egg exports in 2000 are forecast at 160 million dozen, about even with 1999. After falling sharply in 1999, exports to most of the major markets are expected to level out.

Turkey Eggs in Incubators on January 1 Up 1 Percent

According to the latest USDA National Agricultural Statistics Service (NASS) report, turkey eggs in incubators on January 1, 2000, in the United States totaled 31.8 million, up 1 percent from January 1 a year ago. Eggs in incubators were up 2 percent from the December 1 total of 31.0 million. Regional changes from the previous year were: East North Central, down 4 percent; West North Central, up 2 percent; North and South Atlantic, up 2 percent; South Central, up 2 percent; and West, down 2 percent.

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Excerpts from the latest USDA National Agricultural Statistics Service (NASS) ...

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Poults Placed During December Up 1 Percent

The 25.3 million poults placed during December 1999 in the United States were up 1 percent from the number placed during the same month a year ago. Placements were up 8 percent from the November total of 23.5 million. Regional changes from the previous year were: East North Central, down 9 percent; West North Central, down 1 percent; North and South Atlantic, up 12 percent; South Central, unchanged; and West, down 8 percent.

December Egg Production Up 3 Percent

U.S. egg production totaled 7.27 billion during December 1999, up 3 percent from the 7.05 billion produced in 1998. Production included 6.19 billion table eggs and 1.08 billion hatching eggs, of which 1.02 billion were broiler-type and 64.0 million were egg-type. The total number of layers during December 1999 averaged 329 million, up 2 percent from the total average number of layers during December 1998. December egg production per 100 layers was 2,213 eggs, up 1 percent from 2,190 eggs in December 1998.

All layers in the U.S. on January 1, 2000 totaled 328 million, up 2 percent from a year ago. The 328 million layers consisted of 270 million layers producing table or commercial type eggs, 55.6 million layers producing broiler-type hatching eggs, and 2.73 million layers producing egg-type hatching eggs. Rate of lay per day on January 1, 1999, averaged 70.7 eggs per 100 layers, up 1 percent from the 70.3 a year ago.

Laying flocks in the 30 major egg producing States produced 6.84 billion eggs during December, up 3 percent from December 1998. The average number of layers during December, at 309 million, was up 2 percent from a year earlier.

Egg-Type Chicks Hatched Down 8 Percent

Egg-type chicks hatched during December totaled 32.7 million, down 8 percent from December 1998. Eggs in incubators totaled 30.5 million on January 1, 2000, down 7 percent from a year ago. Domestic placements of egg-type pullet chicks for future hatchery supply flocks by leading breeders totaled 175,000 during December 1999, down 4 percent from December 1998.

Broiler Hatch Up 1 Percent

The December 1999 hatch of broiler-type chicks, at 748 million, was up 1 percent from December of the previous year. There were 624 million eggs in incubators on January 1, 2000, up 1 percent from a year earlier.

Leading breeders placed 7 million broiler-type pullet chicks for future domestic hatchery supply flocks during December 1999, up 1 percent from December 1998.

Broiler Eggs Set In 15 Selected States Up 3 Percent

Commercial hatcheries in the 15-State weekly program set in incubators 182 million eggs during the week ending January 22, 2000. This was up 3 percent from the eggs set the corresponding week a year earlier. Average hatchability for chicks hatched during the week was 82 percent. Average hatchability is calculated by dividing chicks hatched during the week by eggs set three weeks earlier.

Broiler Chicks Placed Up 2 Percent

Broiler growers in the 15-State weekly program placed 145 million chicks for meat production during the week ending January 22, 2000. Placements were up 2 percent from the comparable week in 1999. Cumulative placements from January 2, 2000, through January 22, 2000, were 434 million, up 1 percent from the same period a year earlier.

Meetings, Seminars and Conventions

2000 February

Feb. 14-18: ***Pepa Annual Conv.,*** Paradise Point Resort, San Diego, Calif. Contact: Pacific Egg & Poultry Association, 1521 I St. Sacramento. Calif. 95814. Phone: 916-441-0801.
Feb. 16-17: ***NGFA Conv. & Industry Show,*** Holiday Inn Kearney, Neb. Contact: Nebraska Grain & Feed Assn., 1233 Lincoln Mall, Suite 200, Lincoln, Neb. 68508-3911. Phone: 402-476-6174.
Feb. 22-23: ***Antimicrobial Resistance Wkshp.,*** DoubleTree Hotel, Rockville, Md. Contact: U.S. Food & Drug Administation, Center for Veterinary Medicine, Office of Management and Communications, HFV-12, 7500 Standish Place, Rockville, Md. 20855. Phone: 301-594-1755.
Feb. 24-25: ***VSFA Annual Mtng.,*** The Homestead, Hot Springs, Va. Contact; Virginia State Feed Assn., 3437 Grandview Drive, Richmond, Va. 23225-1201. Phone: 804-272-5333.

2000 March

March 4-7: ***49th Western Poultry Disease Conference.*** Capitol Plaza Holiday Inn, Sacramento, CA. Contact: Lina Layiktez at 530-757-3331; e-mail: events@ucdavis.edu. Web site: conferences.ucdavis.edu
March 9-11: ***49th - Afia* Argo De Las Americans.*** Expo Guadalajuara, Jalisco, Mexico. Contact: Denuse C. Selesnick, Agro Food International Associates c/o International Trade Information Inc., 23241 Ventura Blvd. Suite 308, Woodland Hills, Calif. 91364-1003. Phone: 818-591-2255.
March 14-15: ***Feed Mill Management Seminar,*** Nashville, Tenn. Contact: U.S. Poultry & Egg Association, 1530 Cooledge Road, Tucker, Ga. 30094. Phone: 770-493-9401.
March 15-16: ***29th Annual Midwest Poultry Federation Convention,*** RiverCentre, St. Paul, MN. Contact: Laura Ginsburg at 651-646-4553; Fax: 651-646-4554.
March 22-23: ***New England Poultry Health Conference,*** Sheraton Hotel, Portsmouth, NY; William Bell, William Bell Assoc., Inc., P.O. Box 725, Augusta, Maine 04330
March 22-23: ***WPSA (UK Branch), Poultry Science Spring Meeting,*** Scarborough, UK. Contact: Dr. Paul Rose, Harper Adams University College, Newport, Shropshire TF10 8NB, UK. Fax: +4 (0) 1952 815217
March 29-30: ***Nebraska Poultry Industries Annual Convention,*** New World Inn, Highway 30 and 81 South, Columbus, NE. Contact: Nebraska Poultry Industries, Inc., A103 Animal Sciences, University of Nebraska, P.O. Box 830908, Lincoln, NE 63583-0908. Phone: 403-472-2051.

2000 April

April 5-7: ***Food Irradiation 2000,*** "A food safety process for the new millennium", will be held at the Sheraton National Hotel in Arlington, Virginia (Washington DC). Contact: Deborah Crommett, Conference Co-ordinator, Intertech Conferences, 411 US Route One, Portland, Maine 04105 USA, Fax + (207) 781-2150.
April 12-13: ***Poultry Industry Exhibition,*** Western Fairgrounds, London, ON. Sponsored by the Ontario Poultry Industry Council. Contact Phone: (519) 837-0284; Fax: (519) 837-3584; e-mail: pic@easynet.ca
April 18-21: ***VIV China,*** International Trade Fair for Intensive Animal Production and Processing. Contact: Mr. Richard de Boer, Product Manager VIV Royal Dutch Jaarbeurs, PO Box 8500, 3503 RM Utecht, The Netherlands, Fax: +31 30 295 5709.

2000 May

May4-5: ***National Breeders Roundtable,*** St. Louis, Mo. Contact: U.S. Poultry & Egg Assoc., 1530 Cooledge Road, Tucker, GA 30094. Phone: 770-493-9401.
May10-12: ***Avicola 2000, The Second International PoultryExhibition and Conference***. Predio Ferial de Palemo, Buenos Aires, Argentina. Contact: Sr. Diego Forte, EJ Krause y Asociados, Av de Mayo 605, 40 piso (1084) Buenos Aires,Argentina. Fax: + 54-14343-4833.
May 20: ***GPF Night of Knights,*** Cobb Galleria Center, Atlanta, GA. Contact: Georgia Poultry Federation, P.O. Box 763, Gainsville, GA 30503. Phone: 770-532-0473.
May 31-June 4: ***GVMA Meeting,*** Destin, Florida. Contact: GVMA. Phone: 770-416-1633; Fax: 770-416-9095.

2000 June

June 1-3: ***AP&EA Convention,*** Birmingham, AL. Contact: Alabama Poultry & Egg Association, P.O. Box 240, Montgomery, AL 36101-0240. Phone: 334-265-2732
June 9-10: ***Ark. Poultry Festival,*** Arlington Hotel, Hot Springs, Ark. Contact: Judy Kimbrell, Poultry Federation, P.O. Box 1446, Little Rock, Ark. 72203. Phone: 501-375-8131

2000 July

July 2-6: ***Xth International Congress on Animal Hygiene,*** Maastricht, The Netherlands. Contact: Royal Netherlands Veterinary Association, P.O. Box 14031, NL-3508 SB Utrecht, The Netherlands. Fax: +31 30 251 1787
July 6-14: ***XXth Gala International Symposium/Workshop*** on Rapid Methods and Automation in Microbiology. Manhattan, Kansas, Contact: Daniel Y.O. Fung, Kansas State University, Manhattan, KS 66506-1600. Phone: 785-532-5654; Fax: 785-532-5681; dfung@oz.oznet.ksu.edu: http://www.dec.ksu.edu/dec/con/microciology
July 22-26: ***AVMA 137th Annual Convention,*** Salt Lake City, Utah. Contact: American Veterinary Association, 1931 North Meacham Road, Schaumburg, IL 60173. Phone: 847-925-8070, Fax: 847-925-1329.

2000 August

Aug. 20-25: ***XXI World's Poultry Congress,*** Convention Centre, Montreal, Quebec. Contact: Rosaleen Rinzler, WPC 2000 Congress Secretariat, Events International Meeting Planners Inc., 759 Victoria Square, Suite 300, Montreal, Quebec, Canada H2Y 2J7. Phone: 514-286-0855.

2000 November

Nov. 27: ***Coccidiosis 2000,*** Hannover, Germany. Contact: Elaine Robson, Conference Organizer, Positive Action Conferences, P.O. Box 4, Driffield, East Yorkshire Y025 9D1 England. Phone: +44 (0) 1377-256316; Fax: +44 (0) 1377-254663.