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EXPECTED OUTCOMES

The proposed studies will provide much needed basic and applied information that should directly impact our ability to diagnose and control infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILTV), avian mycoplasmas, and the immunosuppressive viruses (IBDV and CAV). In addition, this proposal calls for avian influenza virus (AIV) surveillance studies to be performed to monitor AIV in wild bird populations in the Eastern and Midwestern regions of the U.S.

Diagnostic applications will focus on developing the most rapid, sensitive and accurate tests using polymerase chain reaction (PCR) assays and novel expression systems for producing highly specific microbial antigens for serological diagnosis.

Control of the diseases under study will also use contemporary molecular and conventional approaches. The identification of immunogenic genes or their products (proteins) will likely result in their use as whole virus, subunit, DNA, or recombinant (vector) vaccines. A significant effort in this regard will be directed at the identification of genes or proteins that stimulate cross-protection against heterologous strains, particularly in the case of IBV. Furthermore, antisense antiviral technology will also be evaluated as an alternative method of control.

Importantly, the cooperating participants in the proposed Northeast Regional Research project maintain active research/service relationships with the poultry industry and thus will be able to extend the new project findings to commercial production practices.

ORGANIZATION

Representatives from cooperating Agricultural Experiment Stations, Veterinary Medical Experiment Stations, and USDA ARS will make up the regional technical committee for the project. A technical committee representative will be appointed by the director of each participating experiment station. This individual will be responsible for receiving and providing necessary information such as the scheduling of the annual technical committee meeting, the compilation and distribution of the annual technical committee report, etc. In addition, an administrative advisor appointed by the Association of Regional Experiment Station Directors and a representative of the Cooperative State Research, Education, and Extension Service (CSREES) will also serve on the committee. All representatives from the participating experiment stations and other research institutions (e.g. USDA ARS) will constitute the voting membership of the technical committee.

The regional technical committee will nominate and elect a chairperson to oversee and coordinate the project objectives for the full, five-year period. The chair, if not a member of a cooperating institution, will be a full voting member of the committee. A secretary and vice-chairperson will be elected for one-year terms by the voting membership. The vice-chair will be responsible for preparation of the yearly and final project reports. The secretary will be responsible for other correspondence on behalf of the technical committee as required, and will be responsible for making arrangements (hotel, meeting room, etc.) for the annual meeting. After a one year term, the secretary will ascend to the vice-chair position. Subcommittees may be appointed by the chair as needed for specific assignments.

The annual technical committee meeting will continue to be a joint meeting held in conjunction with the annual North Central Regional Research Technical Committee. The joint meeting will be held in Chicago, Illinois in November, on the Friday and Saturday immediately preceding the Conference of Research Workers in Animal Diseases.

ATTACHMENT

TECHNICAL COMMITTEE MEMBERS AND OTHER PROJECT PARTICIPANTS WITH THEIR AREAS OF SPECIALIZATION

Institution	Name	Area of Specialization
Alabama	L. Lauerman[1]	Molecular Biology/ Diagnostics
	V. Panangala	Microbiology
	V. vanSanten	Molecular Biology
	J. Wright	Epidemiology
Connecticut-Storrs	M. Khan[1]	Pathology/Molecular Biology
Delaware	S. Cloud	Immunology
	J. Dohms	Microbiology
	M. Emara	Molecular Immunology
	J. Gelb[1]	Virology
	C. Keeler	Molecular Virology
	R. Morgan	Molecular Virology
	C. Pope	Pathology
	J. Rosenberger	Microbiology/Virology
Georgia	M. Jackwood[1]	Molecular Microbiology
	S. Kleven	Microbiology
Illinois	D. Tripathy[1]	Molecular Virology
Maryland	V. Vakharia[1]	Molecular Virology
New York - Cornell	S. Naqi[1]	Virology/Immunology
North Carolina	D. Ley[1]	Microbiology
Ohio	D. Jackwood	Molecular Virology
	M. Saif[1]	Microbiology
	R. Slemons	Virology
Texas	E. Collisson[1]	Molecular Virology
Tennessee	R. Webster[2]	Virology
USDA - ARS	D. Swayne[1]	Pathology
	M. Perdue	Molecular Biology
	D. Suarez	Molecular Virology

[1] Technical Committee voting member

[2] St. Jude Children's Research Hospital, Dept. Of Virology and Molecular Biology - See Attachment

INSTITUTIONAL PARTICIPATION ON THE PROPOSAL OBJECTIVES.

Institution	Objective Number			
	(1) Diagnosis	(2) Immunosuppression	(3) Epizootiology	(4) Control
Auburn	X		X	
Connecticut	X			
Delaware	X	X	X	X
Georgia	X		X	X
Illinois				X
Maryland				X
New York			X	
North Carolina	X		X	
Ohio	X	X	X	X
Texas			X	
USDA-ARS			X	

ATTACHMENT

Resources

Station	SY	PY	TY
SAES			
Alabama	0.50	1.00	0.40
Connecticut-Storrs	0.20	0.25	0.25
Delaware	3.30	10.00	4.40
Maryland	0.10	0.00	0.00
New York-Cornell	0.10	0.25	0.25
Ohio	0.20	0.30	0.50
Texas	0.10	0.00	0.80
VMES			
Georgia	0.15	0.00	0.00
Illinois	0.10	0.00	0.00
North Carolina	0.10	0.20	0.20
USDA			
ARS	0.15	0.00	0.00
TOTAL	5.00	12.00	6.80

ATTACHMENT

Critical Review

The previous regional research project NE-138, Interactions between IBDV, IBV, and *E. coli* in a respiratory disease complex in chickens, was initiated on October 1, 1991 and will end September 30, 1996. The following critical review summarizes the original NE-138 project.

1. Objectives of previous project

1. Study the interrelationships of infectious bursal disease virus (IBDV), chicken anemia virus (CAV), infectious bronchitis (IBV), and *E. coli* in the development of the respiratory disease complex (RDC); to include:

a. The events of early IBDV, IBV, and *E. coli* pathogenesis in the development of the RDC.

b. The role of IBDV and IBDV/CAV-induced immunosuppression on the pathogenesis of IBV and *E. coli* infections.

c. Local and systemic immune responses in IBV and *E. coli* infections in immunocompetent and immunosuppressed chickens.

2. Develop strategies for improving immunization of chickens to control RDC.

3. Develop methods by which variant serotypes or pathotypes of IBDV, CAV, IBV, and *E. coli* may be readily diagnosed (identified) and characterized.

2. Work accomplished under previous project

Research performed under the research objectives resulted in substantive findings on these important respiratory diseases affecting the poultry industry. The highlights are as follows:

Major IBV-associated field losses were documented in 1992 in the Delmarva and northeast Georgia poultry regions. Initially, there was considerable speculation that the epornitics in Delmarva and Georgia were caused by the same serotype of IBV. Scientists in the NE-138 project quickly determined that this was not the case, rather two different serotypes were involved. Over the next several years, project participants isolated and identified the new virus serotype responsible for the Delmarva outbreak (Delaware variant 92-072), characterized its pathogenicity, discovered its origin, developed an effective vaccine and improved surveillance using molecular approaches developed under Objective 3. In addition, the Georgia variant was also isolated and characterized by members of the NE-138 group. Sequence analysis and serological and cross-challenge studies indicated the Georgia variant was similar to the Arkansas serotype, the predominant serotype linked to the Georgia outbreak in 1992. The IBV diagnostic tests, detailed in the next paragraph, were used to identify the many field isolates recovered from commercial flocks in both outbreaks. More recently, members of the project have been documented the spread of the Delaware variant 92-072 serotype to other chicken producing areas in the U.S.

Specifically, the assays developed for IBV identification and diagnosis were the monoclonal antibody (Mab) based ELISA (NY-AES), restriction fragment length polymorphism's (RFLP) assay (GA VMES), and serotype-specific PCR (DE AES). These stations shared reagents, confirmed each others work, and co-authored publications. Most recently, in a survey of IBV field

types in production areas in Virginia, Pennsylvania, and Delaware/Maryland, both RFLP, PCR and Mab ELISA data were in agreement.

In the area of immunosuppression, important work was conducted on characterization of chicken anemia virus (CAV). In contrast to IBDV that damages bursal derived lymphocytes, CAV attacks T-lymphocytes. Because of the widespread distribution and potential economic impact of this virus in U.S. broiler and layer flocks, it is important to detect CAV, antibodies to CAV, and study its role in conjunction with IBDV in causing immunosuppression. PCR-based, conventional virus isolation, and serological detection of CAV have improved steadily over the project period due to work conducted in Objective 3. The role of CAV in immunosuppressive disease complexes was delineated under Objective 1. CAV was shown to causes dramatic effects on T-lymphocyte sub-populations using both *in vitro* and *in vivo* determinations. CAV infection in the young chicken was determined to cause immunosuppression in pathogenesis studies. Combined IBDV and CAV infection produce more severe immunosuppression than caused by either agent individually. However, under field conditions it was found that the timing of natural infections in chickens varied due to the presence of maternal antibodies to either IBDV and CAV in progeny of infected breeders.

IBDV control via vaccination continues to be a central element of chicken health programs. Protection of broilers relies on the consistent transfer of maternal antibody from breeders receiving live and inactivated vaccines at specified times in the pullet/breeder lay cycle. Significant advances were also made in detecting and recognizing the antigenic variability of IBDV using highly specific Mabs. This is important for following the evolution of IBDV strains in the field as well as for the future design of IBDV vaccines. Progress has been made on recombinant vaccines designed to provide the broad spectrum of protection needed to prevent emergence of "break through" strains. Work on this area will continue in the proposed project.

The repository of reagents and bacterial and viral strains proposed in the project were inventoried. These agents became available for exchange and were exchanged in many cases. This type of activity has been superseded by the American Association of Avian Pathologist (AAAP) committee that has compiled a listing of available reagents.

3. Degree to which objectives have been accomplished

The objectives of this project were narrow and focused, concentrating on RDC caused by a limited number of disease agents. Nevertheless the objectives were ambitious given the number of participants (total SY = 2.65). In addition, there was the loss of .55 SY's during the project when Pennsylvania SAE participation ended. Nevertheless, substantial progress was made in understanding the relationship of underlying immunosuppression on interactions with respiratory agents in Objective 1. In Objective 2 and 3, major contributions were made and implemented in the diagnosis and control of IBV. Work accomplished on *E. coli* was more limited as only .05 SY were dedicated to *E. coli* research (NC VMES).

4. Incomplete work or areas needing further investigation

The poultry industry continues to suffer major losses caused by respiratory disease agents. The virus, ILTV and mycoplasmas, MG and MS, have re-emerged in recent years. IBV and AIV continue to evolve through mutation and/or their potential introduction into commercial poultry populations (AIV). Reservoirs such as backyard poultry (ILTV, IBV, AIV and mycoplasma) and wild birds (AIV, mycoplasma) harbor these agents and represent an ongoing threat to the nation's multibillion dollar poultry industry. Although much has been learned about these agents, we need to develop more rapid, sensitive and specific diagnostic tests and control measures.

However, there is a also a need to establish regional and national surveillance and response networks, linking government and university laboratories and agencies with the industry. This proposal will address surveillance of AIV, MG, MS and other mycoplasmas. AIV surveillance will involve coordinated wild bird sampling and virus isolation protocols. Serotypes H5 and H7 isolates will be further evaluated for pathogenic potential at Southeast Regional Research Laboratory, USDA-ARS, and the new BL3 containment facility at Delaware AES. This facility is currently under construction using joint funding from USDA, the State of Delaware, the Delmarva Poultry Industry, and the University of Delaware. Additional molecular characterizations will then be accomplished. Similarly, mycoplasma surveillance will conducted in wild and domestic/commercial avian species. It is the goal to have molecular analysis of respective mycoplasma isolates and strains become available on the Internet. Poultry diagnostic laboratories at all participating institutions are positioned to determine increased incidence (isolations) of AIV, ILTV, IBV and mycoplasmas. This new project will have as its goal, the capability to cooperatively react to emerging and re-emerging threats by joining the diagnostic and research infrastructure of each institution into a single network.

Finally, the project will continue to address the need to develop safer, more efficacious vaccines and other control measures for the respiratory agents as well as IBDV and CAV to protect commercial flocks at a reasonable cost.

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ATTACHMENT

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November 27, 1995

Dr. John E. Dohms
and Dr. Jack Gelb, Jr.
University of Delaware
College of Agricultural Sciences
Dept. of Animal and Food Sciences
040 Townsend Hall
Newark, DE 19717-1303

Dear Drs. Dohms and Gelb:

Thank you for your letter and information concerning the project entitled "Epidemiology and Control of Emerging Strains of Poultry Respiratory Disease Agents" that has just arrived. As I mentioned in our telephone conversation of 11-22-95, I am very interested in this subject and am prepared to assist you in whatever way is possible, but it is not possible in the short time before December 7, 1995 to send you a grant application covering each of the items that you listed on the sheet headed "New North East Regional Project", for my calendar is completely full through that date.

I will be pleased to provide expertise on molecular characterization of influenza virus and the development of strategies for the control of emerging pathogenic strain. It is vital at this time that we are prepared for the reappearance of H5N2 viruses in the United States, for they continue to circulate in Mexico.

If I can be of assistance to you, please do not hesitate to contact me.

Sincerely,

Robert G. Webster, Ph.D.
Rose Marie Thomas Chair,
Department of Virology
and Molecular Biology

RGW/db

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AGRICULTURE

Playing Chicken With an Epidemic

As if Mexico's current economic and political troubles weren't enough, now its chickens have the flu. The virus, avian influenza A, poses no threat to human health, but it can be lethal to poultry, and some 26 million chickens in three Mexican states are already infected with a deadly strain, says Eduardo Rivera, coordinator of Mexico's National Avian Influenza Campaign. Although Rivera and other authorities are determined to contain the epidemic, some veterinarians say their efforts may be too little, too late to prevent Mexico's flocks from being devastated.

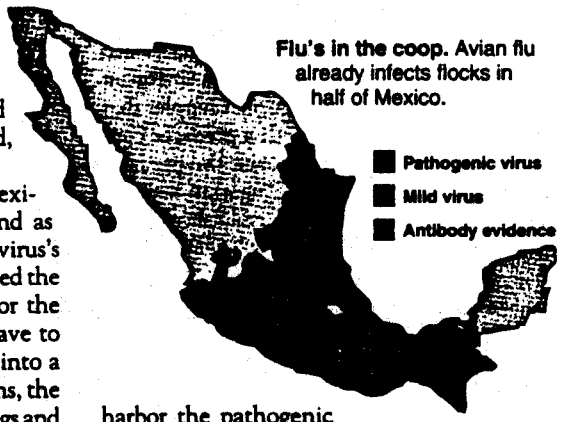
By deciding not to act when a mild form of the virus surfaced last spring, "Mexico made the same mistake we made in 1983," says Charles Beard, a veterinary virologist with the Southeastern Poultry and Egg Association in Tucker, Georgia. In that year, poultry producers allowed a similar mild strain to spread freely; after 6 months it mutated into a virulent form that claimed 17 million birds and cost $63 million to eradicate. Now Mexico has experienced the same grim progression. With the mutation-prone mild strain present in at least half the country, the epidemic is likely to spread beyond the three states that have already been hit hard by the pathogenic mutant, perhaps even reaching the United States. Says Robert Webster, a virologist at the St. Jude Children's Research Hospital in Memphis, Tennessee, "The virus scares the hell out of the whole poultry industry in the U.S."

Last June, U.S. and Mexican veterinarians and agriculture officials held one of several meetings in Mexico City to discuss the situation. At that point, infected chickens were suffering a mild infection confined to the lungs and gut, and Mexican officials were reluctant to take the steps needed to forestall an outbreak of more serious disease: quarantining infected farms and increasing hygiene measures elsewhere. "I don't think they were convinced that the mild virus could turn into a real problem," says Beard, who spoke at the meeting.

Like the United States before it, Mexico allowed the virus to circulate—and as before, a few critical mutations in the virus's hemagglutinin (HA) protein transformed the mild virus into a serious pathogen. For the virus to infect a bird, host enzymes have to cleave the HA protein, converting it into a form that can infect cells. In mild strains, the protein is cleaved by enzymes in the lungs and gut. But in a process unraveled after the 1983 outbreak, a series of mutations can turn the protein into a more readily activated form, converting a relatively benign virus into a killer. "Once the HA protein acquires a series of basic amino acids, it can be cleaved by an enzyme that occurs in every tissue in the body, including the brain," says Webster. Chickens infected with the mutated form suffer a mortality of 20% to 100% from a devastating systemic infection with internal hemorrhaging and central nervous system collapse.

By the start of this year, this lethal form had surfaced 150 miles southeast of Mexico City in Puebla, on a farm of 1.25 million chickens. Since then it has turned up in another 35 flocks. By this point in the 1983 U.S. epidemic, agriculture officials and farmers had taken aggressive steps. Soon after detecting the virulent virus, the U.S. Department of Agriculture (USDA) killed all chickens infected with either the mild or the virulent strains. The federal government established a quarantine zone, compensated owners, and disinfected farms—and the outbreak was stopped dead.

Mexico, unable to afford the cost of destroying tens of millions of birds, is instead trying to contain the epidemic by vaccinating flocks and enforcing quarantines. Because Mexico has a limited supply of the vaccine, it will be available at first only in states that harbor the pathogenic virus, reports Beard. Birds will be vaccinated based on a pecking order, with grandparent genetic stock getting the first doses, followed by broiler breeders, egg layers, and broilers. By setting up checkpoints and requiring permits for moving poultry into and out of infected areas, Mexico hopes to maintain disease-free havens, especially in the remote states of Yucatán and Sonora. "The government wants disease-free areas for the breeders so there will be a source of chicks to repopulate the industry," says Beard.



Flu's in the coop. Avian flu already infects flocks in half of Mexico.

ILLUSTRATION: C. FABER SMITH SOURCE: CPA

In this country, meanwhile, the USDA and the poultry industry are urging producers to watch for sick birds, send samples to diagnostic laboratories, and step up hygiene practices, such as disinfecting vehicles, equipment, and clothes. For producers with affiliates in Mexico, the authorities recommend limiting travel to the Mexican facilities.

The USDA is also considering a plan to monitor the wild waterfowl that will soon begin their migration northward from South and Central America. Shore birds such as the ruddy turnstone and the red knot are known reservoirs of the mild avian flu, says Webster. And that is raising fears that their arrival could herald not only the coming of spring but also the reappearance of a deadly visitor.

–Bernice Wuethrich

Bernice Wuethrich is a writer in Washington, D.C.

SIGNATURES

TITLE: EPIDEMIOLOGY AND CONTROL OF EMERGING STRAINS OF POULTRY DISEASE RESPIRATORY AGENTS

William W. Hagler — July 31, 1996

Administrative Advisor — Date

Chairperson, Regional Research Committee — Date

Chairperson, Regional Association of Directors — Date

Administrator, Cooperative State Research, Education And Extension Service — Date