An Outbreak of Conjunctivitis Due to Newcastle Disease Virus (NDV) Occurring in Poultry Workers

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NEWCASTLE disease, a disease of world-wide distribution in fowls, was first recognized as a human infection in 1943, when F. M. Burnet[1] reported a case of conjunctivitis in a laboratory worker, who became infected by the accidental introduction of infectious material into the conjunctival sac. The diagnosis was confirmed by virus isolation. Since that time, 8 cases of human infection in which the virus was isolated have been reported.[2-6] Each of these cases was manifested as a conjunctivitis. Two cases were reported by Anderson in Australia in 1946, and the remaining 6 cases, all from the United States, were reported in 1949 or later. The first case known in this country occurred in April, 1947, when a veterinarian developed conjunctivitis following the accidental splashing of Newcastle disease virus into his eyes. The virus was isolated from eye washings.[14]

Only 2 of these 8 cases occurred naturally. These 2 cases were reported by Ingalls,[4] one in a broiler plant operator, and the other in a veterinary student. In the case reported by Hunter,[5] the virus was also isolated from the serum of the patient taken on the second day, approximately 7 hours after a shaking chill.

Reports of outbreaks of multiple cases are not numerous. Yatom[7] reported 17 cases of conjunctivitis among kitchen workers handling poultry in the Agricultural School at Mikveh, Israel, where an outbreak of Newcastle disease had recently occurred in fowls.

Howitt[8] reported on the presence of neutralizing antibodies in sera from human beings in contact with fowl and suggested the possibility that Newcastle disease virus was responsible for atypical central nervous system infection in man. No virus was isolated from these cases. A restudy of the sera indicated that the Newcastle virus neutralizing factor was destroyed by heat.[9]

Bawell[10] reported the apparent isolation of a strain of Newcastle disease virus from excised lung tissue of a case of virus pneumonia followed by chronic atelectasis. However, the virus was lost on the second series of eggs inoculated.

The purpose of this report is to pre sent the findings obtained on an outbreak of 40 cases of conjunctivitis occurring in workers in a produce plant

where poultry is eviscerated, processed, and canned.

The outbreak first came to the attention of the Minnesota Department of Health and the Division of Veterinary Medicine of the University of Minnesota on March 7, 1951, and an investigation was instituted immediately. The plant involved is located in a rural Minnesota community with a population of about 1,500 persons. Approximately 300 workers, all adults, are employed at the plant, about 90 of whom work on an eviscerating line. Among this group there were 40 known cases of conjunctivitis. Only one male worker developed symptoms, but the exposed personnel was mostly female. The cases were limited to persons employed on evisceration. Since there was considerable turnover of employees in this group, the total number of exposed persons could not be determined. There were no secondary cases known to occur in family contacts, nor were there any other known cases of conjunctivitis occurring in the community.

Onset in the first case occurred February 7, and in the last case, April 5. There were no cases with onset between March 15 and March 28. Evisceration of chickens began on February 5 and continued until April 6, except for a period between March 12 and March 26, when turkeys were handled. As can be seen by Table 1, cases were scattered throughout the period, with a concentration of cases in the week of March 28 to April 3, when 15 cases occurred.

Symptoms consisted of irritation of the involved eye, lacrimation, redness and some swelling of the conjunctiva, but no involvement of the cornea; edema of the lids was present in varying degree. Photophobia or visual impairment was not common. Preauricular adenitis on the affected side was present in about half the cases. No constitutional symptoms were noted and headache was uncommon. Usually only one eye was involved. Of 19 cases on which detailed history was obtained, the left eye was involved in 13, the right in 4, and both eyes in only 2. Nine of these 19 cases gave a definite history of having something splashed into the eye previous to onset of conjunctivitis. The infection did not cause any time loss from work for the employees. Symptoms as a rule lasted only 3 to 4 days; however, in one instance they persisted for 3 weeks.

TABLE 1

Cases of Conjunctivitis Occurring in Poultry Workers as Distributed by Weeks During 1951

Period	*No. of Cases*
February 7–13	5
February 14–20	4
February 21–27	4
February 28–March 6	3
March 7–13	8
March 14–20	0
March 20–27	0
March 28–April 3	15
April 4–10	1
Total	40

A review of the company records for 1950 showed that 10 cases of conjunctivitis were reported during that year. These also were limited to workers on the eviscerating line. Two of the 1950 cases had recurrent attacks and one of them also had conjunctivitis in 1951. Intervals between attacks were 3 months, 5 months, and 8 months respectively.

In this plant, poultry is canned for commercial sale. Frozen "New York dressed" chickens, purchased in carlots and shipped in from various parts of the country, are kept frozen until the time of canning, when they are thawed in large tanks by running water at a temperature of about 50° F. until easily handled. However, ice is often still present in the viscera at the time of handling. After thawing, the fowls are then passed along the eviscerating line, hanging from and propelled by an "endless" conveyor chain. Each worker has specific duties in cleaning and eviscerating the birds, so that in about 30 minutes a bird reaches the end of the

line completely eviscerated, washed, and weighed. During the process there are opportunities for the splashing of contaminated material. The hands and arms of the workers become soiled with material from the carcasses and the viscera of the chickens. The only facility readily available for handwashing for this group was a single wash basin with hand-operated faucets, and it was observed that proper washing of hands with soap and water was not carried out when employees left the eviscerating line. Since there were no first-aid facilities for treatment of individuals who accidentally splashed material into the eye, they did not report for treatment until there were actual symptoms of conjunctivitis. Thus, in addition to introduction of the virus into the eye by splashing or spraying of infected material, there was also the possibility of introduction of such material by contaminated hands. Each worker works approximately an 8 hour shift.

Specimens of material for bacteriological studies from the eyes of 7 acute cases were collected by rubbing a cotton swab over the conjunctiva and placing specimens in blood agar slants. From these cultures the bacteria isolated were bacteria not generally considered as being causative agents of conjunctivitis. Specimens obtained in a similar manner from 10 cases were placed in 10 per cent horse serum in broth and were agitated and frozen for virus studies.

With the coöperation of the management and employees, blood specimens were obtained from 26 employees on or about April 5 and July 5, 1951. Sixteen of these were paired specimens. These were divided into three groups according to the presence or absence of conjunctivitis and to the date of onset of symptoms. Group I is an acute group, consisting of 7 employees whose onset of conjunctivitis was within the preceding week. Group II consists of 10 employees whose conjunctivitis developed between 2 weeks and 2 months prior to the date of collection of the first blood specimen. This is considered a chronic group. Group III consists of 9 employees from the eviseration line who had no history of conjunctivitis. Group IV is a control group of 6 individuals who have had no known contact with NDV. The sera in Group IV were random samples taken from blood specimens submitted to the Section of Medical Laboratories of the Minnesota Department of Health for routine serological studies from Minneapolis residents.

PROCEDURES AND MATERIALS

Hemagglutination (HA) titration—The chicken embryo allantoic fluids tested for hemagglutination (HA) capacity were diluted twofold in buffered saline, starting with a dilution in the first tube of 1:8 to 1:8,192 dilution in the last tube. To 0.5 ml. of each dilution, 0.5.ml. of the 0.25 per cent suspension of chicken red blood (CRB) cells was added. The tubes were shaken and allowed to stand at room temperature 45–60 min., or until the control tubes containing only saline and CRB cells had settled to form a button at the bottom of the tube. The HA titer was read as that of the last tube showing complete and even agglutination on the bottom surface of the tube.

Hemagglutination - inhibition (HI) tests—Serial twofold dilutions of serum, starting with a 1:8 dilution in the first tube, to 1:256 in the last tube, were made in saline containing 8 HA units of test virus per tube, leaving a final volume of 0.5 ml. After 10 min., 0.5 ml. of the 0.25 per cent chicken red blood cell suspension was added to each tube. Tests were read between 45 and 60 minutes, time of reading being determined by the saline and red blood cell control. After the red blood cells had

settled in the saline control and would flow freely in a teardrop formation when the rack was tilted, the tests were read using the same method.* The results were interpreted as follows:

All tubes negative	=	negative
Tube 1 positive	=	suspicious (+8)
Tube 2 positive	=	suspicious (+16)
Tubes 3–6 positive	=	positive (+32 to +256)

Heat stability test—Heat stability tests were run on pooled allantoic fluids from eggs dying within 2–3 days after inoculation and exhibiting an HA titer. A precision water bath was used, and the temperature was held constant at 56° C. Samples from the same pools were removed at intervals of 0, 15, 30, 45, 60, 90, and 120 min. All were chilled at 40° C. and a hemagglutination titration was run after all tubes had been removed and chilled.

Serum neutralization (SN) test—Tests were run as recommended by the Neurotropic Virus Disease Commission in 1942.[11] The Newcastle disease virus (NDV) strain KD-NJ–1945 was used throughout the series of serum neutralization (SN) tests. The virus was stored at —45° C. and lots from the same pool were used for all the tests. Human negative sera were obtained from individuals having no known contact with the fowls or the virus, and these served as the negative controls (Group IV, Table 5). All sera were stored at —45° C. until ready to be used. They were then heat-inactivated at 56° C. for 60 min. before any tests were run. For NDV in poultry, a serum neutralization index (SN index) of 0–50 may be considered as negative, 50–100 suspicious, and over 100 significant.

VIRUS ISOLATION

Specimens of material obtained from the conjunctiva of the 10 acute cases were held at —45° C. until they were inoculated into eggs. Penicillin and streptomycin were used as bacterial inhibitors and the inoculum was incubated at 37° C. for 1 hr. Five eggs were inoculated from each washing with 0.1 ml. each into the allantoic cavity of 10-day-old embryonated chicken eggs. A total of 4 NDV isolations was made, 3 upon first passage and 1 upon second passage into eggs.

The presence of the virus was verified by the early death pattern of eggs inoculated with the suspected fluids, by the HA titers of allantoic fluids recovered from these eggs, and by passage of these positive allantoic fluids into chickens and their subsequent rise of HI titer or death.

TABLE 2

Results of NDV Isolation in Specimens from the Conjunctiva of 10 Persons Collected on Day of Onset to 7 Days Later

Identification	*Days After Onset*	*NDV Isolation*
1. LF	Same day	Negative
2. MT	2	NDV
3. SL	2	NDV
4. MS	7	NDV
5. BM	8	Nevative
6. ML	8	Nevative
7. JO	Same day	Negative
8. MP	5	NDV
9. RN	7	Negative
10. LN	4	Negative

Pathogenicity of Newcastle disease virus for chickens—Allantoic fluids (0.5 ml.) from eggs dying on the second and third day after inoculation of material from No. 2 (MT) and No. 3 (SL) listed in Table 2 were injected intramuscularly into two adult Newcastle-negative white Leghorn chickens. These birds displayed no typical Newcastle infection symptoms but their HI titers

* Detailed information for HI testing procedures will be found on the directions enclosed with Lederle's Newcastle disease antigen (diagnostic).

TABLE 3

Mortality Rate in 6-Week-Old Chickens When Inoculated with 4 NDV Solutions

Identification	*No. of Chickens*	*Route of Inoculation*	*Percentage Mortality Rate*
MT:	10	Intranasal	70
	10	Intramuscular	90
SL:	10	Intranasal	90
	10	Intramuscular	100
MS:	10	Intranasal	90
	10	Intramuscular	100
MP:	10	Intranasal	30
	10	Intramuscular	80

TABLE 4

Results of Heat Stability Studies of the 4 NDV Isolations

	Hemagglutination Titer						
	Time in Min. at 56° C.						
Identification	*0*	*15*	*30*	*45*	*60*	*90*	*120*
MT	1,024	1,024	1,024	1,024	1,024	1,024	1,024
SL	1,024	512	512	512	256	256	256
MS	1,024	1,024	512	512	256	128	64
MP	512	512	256	256	256	256	32

rose from negative to positive-256 within 8 days. The LD_{50} titer on 12-day-old chicken embryos of No. 3 (SL) was 10^{-8}. Two control birds were injected at the same time with allantoic fluids from eggs dying on the second and third days after inoculation from No. 1 (LF). These fluids exhibited no hemagglutination titer and the chickens were negative to HI tests made respectively 8 days and 16 days after inoculation.

Allantoic fluids (0.1 ml.) from the fourth egg passage of the 4 NDV isolations were injected into HI-negative 6-week-old white Leghorn chickens. Results are given in Table 3.

The mortality rate was highest 4 days after the virus had been injected, with only a few deaths occurring after 12 days. Of the 15 survivors, 8 showed nervous symptoms and paralysis and are not included in mortality rates. Of the 10 contact controls used, 3 died within the 3 week period that the chickens were held, 4 were paralyzed or showed severe nervous symptoms, and 3 were normal. All survivors had uniformly high HI titers of 256 or greater.

Heat stability of virus—Heat stability studies of the HA activity of the virus isolations were run on samples taken from the same pools as the preceding experiment. All 4 isolations resisted 56° C. for 2 hr. to exhibit some titer at the end of that period. Hanson, *et al.*,[12] found great variations in the stability of the HA ability of individual strains of Newcastle disease virus. (See Table 4.)

Results of serological tests of four groups studied—A week after the NDV was isolated, four groups of people were selected as indicated above. Sera from these individuals were tested by SN and HI tests. Three months later blood samples were again drawn from as many of the people as could be located and were tested in the same manner. The results are given in Table 5.

Since Kilham[13] reported the presence of neutralizing and antihemagglutinating

TABLE 5

Results of Serological Tests on Sera From 32 Individuals for NDV, Mumps, Heterophile Antibodies, and Influenza A, A1, B

Group	Case	Onset of Conjunctivitis	Newcastle Disease Virus: Serum Neutralization (SN) Index April 5	Newcastle Disease Virus: Serum Neutralization (SN) Index July 6	Newcastle Disease Virus: Hemagglutination Inhibition (HI) April 5	Newcastle Disease Virus: Hemagglutination Inhibition (HI) July 6	Mumps Complement-Fixation July 6	Heterophile Antibodies July 6	Influenza Hemagglutination (HI) A July 6	Influenza Hemagglutination (HI) A1 July 6	Influenza Hemagglutination (HI) B July 6	Comments
I	ML	3–29–51	Neg.	Neg.	Qns †	Neg.	Neg.	Neg.	256	512	256	Mumps as a child
	BM	3–29–51	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	128	128	256	Mumps as a child
	MS*	3–30–51	Neg.	32	Neg.	Neg.	+1:8	Qns	128	64	128	No history of mumps
	MP*	4– 1–51	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	64	128	128	Mumps as a child
	AT	4– 2–51	Neg.	Qns †	Neg.	+8	—	—	—	—	—	—
	BP	4– 2–51	>10,000	>10,000	+8	Neg.	Neg.	Neg.	128	256	256	No history of mumps
	LM	4– 5–51	Neg.	—	Neg.	—	—	—	—	—	—	—
II	RP	2– 8–51	27	—	+8	—	—	—	—	—	—	—
	GH	2–26–51	27	Neg.	Neg.	Neg.	Neg.	Neg.	64	128	256	Mumps as a child
	CM	2–27–51	32	—	+8	—	—	—	—	—	—	—
	BT	3– 2–51	Neg.	Qns	Neg.	Neg.	+1:32	Qns	64	128	1,024	Mumps as a child
	MO	3– 2–51	Neg.	—	Neg.	—	—	—	—	—	—	—
	MJ	3– 9–51	32	—	Neg.	—	—	—	—	—	—	—
	ST	3– 9–51	>10,000	—	+8	—	—	—	—	—	—	—
	MT*	3–10–51	—	>10,000	—	+32	Neg.	Neg.	64	64	128	No history of mumps
	SL*	3–10–51	—	Neg.	—	Neg.	Neg.	Neg.	64	128	256	No history of mumps
	IK	3–23–51	550	550	Neg.	Neg.	±1:16	Neg.	64	128	256	Mumps as a child
III	CN	None	>10,000	>10,000	+8	Neg.	Neg.	Neg.	256	512	512	Mumps as a child
	DS	None	>10,000	>10,000	+16	+16	Neg.	Neg.	256	128	512	Mumps in 1942
	MS	None	6,900	3,200	Neg.	Neg.	+1:32	Neg.	128	256	512	Mumps in 1949
	JS	None	Neg.	Neg.	Neg.	Neg.	+1:16	Neg.	256	128	256	Mumps as a child
	SH	None	Neg.	Neg.	Neg.	Neg.	Neg.	±1:40	512	256	512	No history of mumps
	NS	None	79	246	+8	Neg.	Neg.	Neg.	512	256	256	Mumps as a child
	MB	None	2,200	6,900	+32	+32	±1:8	Neg.	128	256	512	Mumps as a child
	DM	None	>10,000	—	Neg.	—	—	—	—	—	—	—
	MT	None	170	Neg.	Neg.	Neg.	+1:32	+1:40	128	64	128	No history of mumps but family exposure in 1938
IV	70544	Unknown	Neg.		+8							
	70545	Unknown	Neg.		Neg.							
	70546	Unknown	32		Neg.							
	70554	Unknown	Neg.		Neg.							
	70555	Unknown	Neg.		Neg.							
	70556	Unknown	Neg.		Neg.							

* NDV isolated.
† Quantity not sufficient.

factors against NDV in sera of convalescent mumps patients, mumps complement-fixation tests were run on the specimens obtained July 6, 1951. Tests for heterophile antibody titer and influenzal hemagglutination inhibition were also run on these single specimens. These results are also listed in Table 5.

SUMMARY

Although the number of specimens and the number of isolations of Newcastle disease virus in this study were relatively small, the isolation of the virus from 4 out of 10 cases seems to establish that Newcastle disease virus was the etiological agent in the outbreak of 40 cases of conjunctivitis among the 90 workers employed in eviscerating poultry at the Minnesota plant.

There were no significant serological changes in the acute or convalescent sera of the paired specimens. However, it is of interest to note that the SN index was significantly high in 64 per cent of the individuals in Group III who had no conjunctivitis, while it was high in only 25 per cent of the individuals in Groups I and II. This suggests the possibility that a high SN index may be associated with resistance to conjunctivitis. In Group IV (the control group), the SN index was negative in each instance. This study indicates that contact with poultry on the evisceration line may result in circulating antibodies for the NDV. The possibility of a nonspecific antibody response occurring in man not associated with exposure or with any of the antigens studied must also be considered.

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