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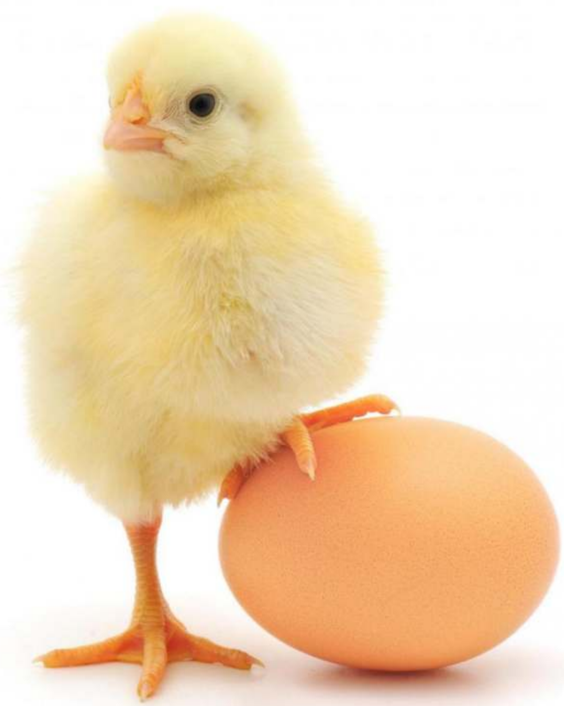

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Risk Factors Associated with Prevalence of Swollen Head Syndrome (SHS) in Broiler Chickens in Eastern Province - Saudi Arabia

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Abstract: A case-control study was conducted to identify risk factors associated with prevalence of swollen head syndrome (SHS) in broiler chickens in Eastern Province of Saudi Arabia (EPSA). A total of 200 birds - originated from 25 broiler flocks - confirmed cases and 200 controls were used in this study. Source of all data were the necropsy reports of the diagnostic investigations carried out at the Veterinary Teaching Hospital-King Faisal University, between July 1999 and June 2000 and the corresponding results of serological testing for turkey rhinotracheitis virus (TRTV) antibodies. Furthermore, a questionnaire was developed and used to collect data from 47 broiler farms on putative risk factors hypothesized to be associated with the prevalence of SHS in production farms. Multiple logistic regression was performed to identify factors associated with SHS. Analysis of the data revealed significant association between TRTV antibodies and clinical signs of SHS (Chi^2=292.6; P$\leq$0.001) was concluded. Meanwhile, farm's hygiene and stocking density found to be contributed in prevalence of SHS in open and closed housing system (R^2= 87.99 and 53.86%, respectively).

Key words: Swollen head syndrome (SHS), TRTV, risk factors, broiler chickens, Saudi Arabia

Introduction

Swollen head syndrome (SHS) is usually a multifactorial disease; it is believed that the initial lesions are caused by viruses. There is strong evidence of the association of turkey rhinotracheitis virus (TRTV) with SHS in chickens (O'Brien, 1985; Jones *et al*., 1991) and the virus isolated from chickens have been indistinguishable from those isolated from turkeys (Cook *et al*., 1992). However the etiology of SHS remains unclear. Clinical signs are a consequence of bacterial interventions and some other factors such as environmental factors. SHS occurs only in a small numbers of birds in affected flocks and the virus has been isolated from chicken flocks without signs of SHS (Jones *et al*., 1991). Chicken free of SHS signs may have antibodies to the pneumovirus (Cook *et al*., 1988; Hafez and Lohren, 1990).

Escherichia coli have been consistent feature of SHS, and it was isolated from internal organs, middle ear, meninges and brain in outbreaks of SHS in broilers and broiler breeders with pneumovirus antibodies (Pattison *et al*., 1989).

In the last few years, SHS have stroked many broiler flocks in Eastern Province of Saudi Arabia (EPSA) and caused economic losses to the poultry industry at the area. A literature search revealed that, apart from this study, no work has been done in this area to date. The objective of this study is to investigate the epidemiologic status of SHS in broiler chickens in EPSA. More specifically:

1. Document seroprevalence of TRTV in the broiler chickens.
2. Investigate associations between TRTV seropositive chickens and clinical and pathological signs of SHS.
3. Evaluate risk factors associated with prevalence of SHS in broiler farms in EPSA.

Materials and Methods

Experimental approach

Study population: The study was conducted in EPSA, an area with 136 poultry farms produce about 7 % of the total Saudi Arabia poultry production (Al-Hammad and Gamaa, 1996).

Identification of cases: A case was defined as any broiler chicken expressed clinical signs of SHS (oculo-facial edema and/or swollen of the head). It comprised a total of 200 broiler chickens, selected randomly from those, which were submitted for diagnostic purposes to the Avian Clinic at the Veterinary Teaching Hospital, College of Veterinary Medicine, Al-Ahsa, Saudi Arabia for a period of 12 months (from July 1998 to June 1999). They were originated from 28 broiler production farms in the EPSA. Sera of cases were used for detection of TRTV antibodies using the ELISA technique described by Chettle and Wyeth (1988).

Identification of controls: The control subjects were randomly selected from the broilers birds' population at large, and not from a limited group of Avian Clinic. Control was consisting of random samples of broiler chickens collected from poultry farms of EPSA region. The number of chickens sampled was calculated in such a way that valid inferences were made on the whole flock. The poultry samples-consist of sera of chickens enrolled in the study.

Sample size calculations: There are 47 broiler farms in the area produce more than 20,000,000 birds per year. The number of collected samples in these farms determined using the following assumptions: 1) the expected prevalence of SHS in poultry is 10%; 2) The level of error protection for the estimated prevalence is 95%; 3) The acceptable error in the prevalence estimates 10%. Using these assumptions and the sample size formula suggested by Levy and lemeshow (1980), a sample 175 birds was computed to enroll in the study. To allow for sampling error and sampling spoilage the sample size was increased to 200 birds. The total number of collected samples is 400 (200 cases and 200 controls).

Factors affecting the likelihood of SHS: A questionnaire was developed and used to collect data on risk factors hypothesized to associate with the likelihood of the occurrence and morbidity of SHS in the broiler chicken farms. Putative factors considered in this study were related to the potential sources of infection and operate at each of the two levels. The data include: management, and health practices. Data on management level include: farming management (all-in-all out or multi-age flocks), stocking density, type of bedding (sand or saw dust), number of labors per flock, and farm's hygiene (includes: cleaning and disinfecting of transport vehicles, limitation of entrance, and presence of foot bath at entrance of each house, cleaning and disinfecting between flocks as well as labor's hygiene include; the use of precautionary measures before and after entrance to each flock such as regular use of a labor's uniform, gloves, mask, boot, soap washing, disinfections). Data on health practices level include: history of disease problem of the farm, the use of veterinary services. Information was collected by four interviewers (who were trained to obtained as near a uniform response as possible) during personal interviewers with farm's owners or managers.

Data analysis: The data were analyzed in two stages; in the first stage, Chi squared test was used in order to assess the degree of association between TRTV seropositive birds and the SHS. In the second stage, data collected from the questionnaire were coded and analyzed statistically. For all analyses, the dependant variable, SHS epidemic status of the flock had two categories: uninfected, coded=1 or infected, coded=2. Other variables are set out in Table 1. Multiple logistic regressions were used to identify variables, which were associated with the risk of SHS. Backwards stepwise was then employed, the P-value to remove a variable in the model was set to 0.1. Statistical analysis procedures were carried out using the PC-SAS® (1988). Demographic data include: location of the farm, housing system, number of flocks, number of cycles per year, size of the flock, source of feed and source of water as well as history of disease problem of the farm were excluded from our study due to either incomplete information or similarities of these variables between surveyed farms.

Results

In case-control studies in EPSA, comparison was made between SHS and non-SHS birds in terms of presence of TRTV antibodies in broiler chickens' sera (Table 2). The study revealed strong association could be inferred between presence of TRTV antibodies and SHS (Chi^2 = 292.6; P value $\leq$ 0.001). In the comparison of affected and unaffected birds, there were a significantly greater proportion of seropositive birds on affected birds (100%) than unaffected ones (18.3%).

Table 3, represents the multiple logistic regression of possible variable associated with prevalence SHS in broiler chicken farms in EPSA. Of the 6 risk factors associated with SHS, farm hygiene (b = -0.585 ±0.059) and stocking density (b = 0.259 ±0.072) were contributed significantly with prevalence of SHS ($P < 0.05$) in open housing system whereas in closed housing system farm hygiene only (b = -0.712±0.204) contributed significantly with incidence of SHS ($P < 0.05$). Other variables did not significantly enhance presence of SHS ($P > 0.05$). Stepwise regression analysis (Table 4) estimated that; farm hygiene and stocking density contributed in prevalence of SHS in both open and closed housing system (R^2= 87.99 and 53.86%, respectively).

Discussion

SHS is a disease of the upper respiratory tract affecting broilers and broilers breeders. Barnes (2000) defined SHS as a result of inflammatory exudates beneath the skin that accumulate in response to bacteria, usually *E. coli*, following upper respiratory viral infection such as TRTV or IB. It has become a problem in many countries including Saudi Arabia in the last few years.

Case-control studies have not been widely used as field investigation techniques in veterinary medicine, despite their extensive use in human medicine, although their frequency has certainly increased in recent years. Their most frequent use has been for the retrospective analysis of hospital clinical data (Willeberg, 1975) and of slaughterhouse data (Aalund *et al.*, 1976; Willeberg, 1979). Recently the use has expanded to identify relationship between diseases (Dohoo and Martin, 1983) and in the study of complex multifactorial diseases (Pritchard *et al.*, 1983).

Table 1: List of management variables and their codes used to assess the risk of SHS in broiler farms in EPSA

Variable	Description and code of the variable		
Farming management	all-in-all out, coded=1;		multi-age, coded=2.
Stocking density	10-13 birds per m^2, coded=1;		14-18 birds per m^2, coded=2;
	19 birds and more per m^2, coded=3.		
Ventilation	adequate, coded=1;		inadequate, coded=2.
Labors experience	poor, coded=1;	fair, coded=2;	good, coded=3.
Farm's hygiene	poor, coded=1;	fair, coded=2;	good, coded=3;
	outstanding, coded= 4.		
Veterinary services	yes, coded=1;	no, coded=2.	

Table 2: Chi-squared (Chi^2) estimation between SHS and non-SHS broilers and pneumovirus antibodies in EPSA

TRTV Antibody	SHS	Non-SHS	Total
Positive	200*	31	231
Negative	0	169	169
Total	200	200	400

*Number of birds; Chi^2 = 292.641; Degree of freedom= 1; P-value= 0.000.

Table 3: Multiple logistic regression of possible variables[1] hypothesized to be associated with prevalence of SHS in broiler farms in EPSA

Dependent variables	Open system housing (n=31)				Closed system housing (n=16)			
	b	SD	t- ratio	P	b	SD	t- ratio	P
Farm management	0.074	0.093	0.08	0.434 NS	NA[2]	NA	NA	NA
Stocking density	0.259	0.072	3.59	0.001*	0.453	0.232	1.95	0.080 NS
Ventilation	0.150	0.113	1.33	0.197 NS	-0.418	0.255	-1.64	0.133 NS
Labor's experience	-0.108	0.071	1.52	0.142 NS	-0.356	0.235	-1.50	0.161 NS
Farm's hygiene	-0.585	0.059	-9.92	0.000**	-0.712	0.204	-3.50	0.006*
Veterinary service	-0.081	0.101	-0.80	0.431NS	-0.306	0.266	-1.15	0.277 NS
Constant (*a*)	3.210	0.365	8.79	0.000**	5.644	1.073	5.26	0.000**
s	0.2131				0.4825			
R-squared	89.9 %, R^2 (Adj) = 89.4%				70.0 %, R^2 (Adj) = 54.9%			

[1]See text, [b]Regression coefficient, [SD] Standard deviation, [NS]Not significant, [2]Not Applicable
**Significant at level of P < 0.001, *Significant at level of P < 0.05

Table 4: Backward stepwise logistic regression analysis of possible putative risk factors associated with SHS in broiler in broiler chicken farms in EPSA

	Open system housing (n=31)		Closed system housing (n=16)	
Dependent variables	b	t- ratio	b	t- ratio
Farm's hygiene	-0.636**	-11.40	-0.680*	-3.40
Stocking density	0.264*	3.82	0.350NS	1.50
Constant (a)	3.642**		3.403**	
s	0.215		0.524	
R-squared	87.99%		53.86%	

[b]Regression coefficient, ** Significant at level of P < 0.001, * Significant at level of P < 0.05, [NS] Not significant

In this study, the control subjects were selected from the commercial broilers population at large, and not from a limited group of birds admitted to the Avian Clinic, which is an advantage over the more conventional case-control studies which rely on hospital or slaughterhouse data. Significant positive risk associations (Chi^2 = 292.6; P value $\leq$ 0.001) at the birds' comparison level were found between the presence of the disease and seropositive to TRTV (Table 2). Our results are in agreement with findings of Wyeth *et al.* (1987) who found high antibody titers for TRTV in broiler parent flocks known to be seronegative before the onset and with Hafez and Lohren, (1990) who reported chicken flocks free of SHS show positive antibody titers to TRTV.

It is difficult to state when and how the birds contracted infection by TRTV. Experimentally, antibodies against

TRTV were first detected in significant titres at day 14 post inoculation of the virus into turkey poults (Naylor *et al*., 1992). Generally, the presence of TRTV antibodies is a subsequence to either vaccination or exposure to infection. Two live TRTV-vaccines from France and United Kingdom were currently licensed. Application of the live vaccines in turkeys, broiler breeders and layers appears to give good protection with low antibody response but vaccination of commercial broiler chickens is not applicable in Saudi Arabia or elsewhere (Hafez, 1993) since some IB field or vaccine strains may be in certain circumstances inhibit the multiplication of TRTV vaccine strain in the upper respiratory tract (Gaudry, 1991).

The high antibody titre of TRTV in blood sera in this study, gave strong evidence of exposure of the flocks to TRTV infection. It is worth mentioning that TRTV had been detected from broiler chickens with SHS in Saudi Arabia by using PCR technique (D. Cavanaugh, personal communication) despite the absence of turkey industry in the area. Source and mode of infection of the virus in broiler chickens in ESPA still uncertain and the role of the virus in developing SHS is unknown.

Severity of the clinical signs in field outbreaks in EPSA led to the suggestion that certain organism acts as predisposing or exacerbating factors in SHS infection. In this study, many microorganisms including pathogenic serotypes of *E. coli* were isolated from the site of predilection of infected birds with SHS (Data not included). The severity of the disease, duration of the onset and mortality are extremely variable, they were influenced by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, and poor hygiene (Hafez, 1993).

For evaluating factors associated with prevalence of SHS, a cross sectional epidemiological study was carried out. A questionnaire was developed to collect data on risk factors hypothesized to associate with the likelihood of the occurrence of SHS in the broiler chicken farms in EPSA. A potential disadvantage of the technique was the use of a questionnaire format, which inevitably limited the depth of information obtained. However, this method was the only one available whereby the information required could be obtained, and its accuracy was enhanced by ensuring that data were obtained by conducting personal interviews on the farm. Multiple logistic regression was employed. The results clearly indicate strong association with hygiene measures so far analyzed in both open and closed housing system, at least at the depth permitted by the questionnaire and significant risk associations were also found with the heavy stocking density in open system (Table 3). Similar conclusion was reported elsewhere (Morely and Thomson, 1984; Naylor and Jones, 1993; Goodwin and Waltman, 1994).

In general, these findings demonstrate that TRTV was present in broiler chicken farms in the geographical area under study (EPSA) at least since 1999 and that TRTV might have played an important role in occurrence of SHS. Improvement of farm's condition especially ventilation; stocking density, litter condition and general hygiene could prevent the SHS.

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