Stunting syndrome associated with Avian Leukosis Virus subgroup J and Chicken Infectious Anaemia in broiler flocks in Egypt

Stunting Syndrom assoziiert mit dem aviären Leukosevirus der Subgruppe J und dem Virus der infektiösen Anämie in Broilerherden in Ägypten

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Dedicated to Prof. Dr. Erhard Franz Kaleta, on behalf of his 65th birthday

Introduction

Avian leukosis viruses (ALVs) are retroviruses associated with tumours involving, primarily, the haemopoietic cells in birds (FADLY and PAYNE, 2003). They are classified on the basis of viral envelope properties into different subgroups A to E and the most recently identified subgroup J (ALV-J). Studies on the cell tropism of strain HPRS-103, the prototype strain of ALV-J isolated in the United Kingdom, have shown that, in contrast to ALV of subgroup A, it has a tropism for cells of the myelomonocytic series, but low tropism for bursal cells, consistent with its induction of myeloid, and not lymphoid leukosis (ARSHAD et al., 1997). ALV-J were isolated from broiler breeder and commercial broiler flocks experiencing a relatively high incidence of myeloid leukemia (ML). ALV-J-induced ML was diagnosed in affected flocks at 4 weeks of age or older (FADLY and SMITH, 1999). ALV-J infection is considered a serious cause of tumour, mortality and other production problems in meat-type chickens.

Meat type chickens with ALV-positive meconium weighed 3.5% and 2.3% less than chicks with ALV-negative meconium at weeks 4 and 7 of age respectively (CRITTENDEN et al., 1983). Furthermore, ALV-J infected broiler chickens were stunted and weighed less than uninfected chickens in the absence of other poultry pathogens (STEDMAN and BROWN, 1999). Experimental inoculation of 10-day-old embryos (Layer-type) with one of several subgroups A, B, C, D, and F ALV strains resulted in drastic stunting of hatched chicks as early as 20 days of age (Carter and SMITH, 1984). ALV-J positive eggs were with few exceptions in the smaller range, whereas both large and small eggs tested positive for antibody (SPENCER et al., 2000).

Chicken infectious anaemia (CIA) is caused by a circovirus, which produces aplastic anaemia in chicks. Chicken infectious anaemia virus (CIAV) was isolated unexpectedly from commercial chickens in Japan in 1974 (YUASA et al., 1979). CIA has a worldwide distribution based on virus isolation and serology. Infection with CIAV has been confirmed as a cause of disease in chicken flocks between 2–4 weeks of age. In these flocks, growth was retarded and mortality was generally between 10%–20% but occasionally it reached 60% (SCHAT, 2003).

McNULTY et al. (1991) reported that subclinical CIA infection has a substantial, statistically significant effect on commercial broiler performance and profitability.

The target cells of CIAV infection are haemocytoblasts in the bone marrow and cortical thymocytes in the thymus. The disease is characterized by severe anemia, subcutaneous and intramuscular haemorrhage, destruction of the erythroblastoid cells in bone marrow, generalized lymphoid atrophy with a concomitant immune suppression, reduced body weight and increased mortality (BLOW and SCHAT, 1997).

In Egypt, several reports documented the presence of both viruses in commercial flocks (ALLY, 2000; 2001). Recently, we noticed increased incidence of stunting, poor performance and increased mortality conditions in several broiler flocks.

This study describes the association of ALV-J and CIAV with field cases of stunting broiler flocks in Egypt.

Materials and Methods

Case history

Stunted birds from 18 broiler flocks, ranging in age from 21–42 days old, were submitted to Animal Health Research Institute (AHRI), Giza, Egypt in 2001. The percentage of stunted birds ranged from 30–40%. The condition was usually followed by respiratory manifestation and increased mortality.

The parents were proved to be naturally infected with ALV-J and CIAV. The stunted chicks were vaccinated against Infectious bursal disease (IBD), Newcastle disease (ND) and Infectious bronchitis (IB) and were fed a starter ration containing an anticoccidial compound.

From every affected flock 10 dead and 10 diseased chicks were examined for gross lesions. Blood and tissue samples were collected for further examinations.

Histopathological examinations

Tissues from liver, spleen, heart, kidney, and proventriculus from dead and diseased birds were fixed in 10% buffered formaline and then embedded in liquid paraffin. Histological sections of 5 µm thickness were stained with haematoxylin and eosin (H&E) and examined for microscopic lesions.

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Virological examinations

ALV-J isolation: C/E (resistant to infection with endogenous ALV) line 0 chicken embryo fibroblast (CEF) and C/AE (resistant to infection with subgroups A and E ALV) alv6 CEF were kindly supplied by Dr. A. Fadly (Avian Disease and Oncology Laboratory, ADOL, East Lansing, USA). Plasma samples were tested as described by FADLY and WITTER (1989). Briefly, samples were simultaneously inoculated on C/E line 0 CEF and C/AE alv6 CEF. 9 days later, cell lysates were tested for the presence of ALV group-specific antigen (p27) by commercial ELISA Kit (IDEXX, Main, USA).

CIAV isolation. MDCC-MSB-1 cell line derived from Marek's disease lymphoma was used as previously described by YUASA et al. (1983). MSB-1 cell culture containing 2 x 10^5 cells/ml was inoculated with liver homogenate and buffy coat in RPMI 1640 growth medium (Gibco). Isolation of the virus was confirmed by indirect immunofluorescent test as described by BALOW et al. (1985) and also by applying polymerase chain reaction (PCR) for detection of CIAV-DNA.

PCR for detection of CIAV-DNA

CIAV-DNA was detected from livers of suspected chicks and from infected MSB-1 cells using the primers described by THAM and STANISLAWEK (1992) which amplify a fragment of 583 base pairs (bp).

DNA was extracted from the livers of suspected chicks and from infected MSB-1 cells using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendation. In addition, DNA extracted from MSB1 cells inoculated with Roelofs strain was used as a positive control and DNA from un-inoculated MSB1 cells was used as negative control.

The DNA concentration was determined fluorometri­cally (VersaFlour, BioRad, Munich, Germany). DNA amplification was carried out using the “Ready To Go-PCR” Beads (Amersham Bioscience, Freiburg, Germany) in a total volume of 25 µl containing 50 ng DNA extracted from infected MSB-1 cells or 1 µg DNA extracted from liver respectively and 50 pmol of each primer. The PCR conditions were as follows: after 1 minute initial denaturation at 95 °C, 30 cycles consisting of 1 minute denaturation at 95 °C, 1 minute annealing at 56 °C and 1 minute elongation at 72 °C. The final extension was for 5 minutes at 72 °C. PCR products were separated on a 1% SeaKem agarose gel (Cambrex Bio Science, USA) together with a 100-bp ladder (Biolabs, Germany) and stained with ethidium bromide.

Further virological examinations

Reticuloendotheliosis virus (REV) isolation: Cell lysate of inoculated C/E were tested for the presence of REV by ELISA using monoclonal antibody kindly supplied by Dr Lee (ADOL) as described by FADLY and WITTER (1989).

Detection of Infectious bursal disease Virus (IBD) antigen: Agar gel diffusion test was conducted from the bursa of Fabricius of stunted chicks using polyclonal antibodies as described by ROSENBERGER et al. (1998).

Bacteriological examinations

Samples from liver, heart blood, spleen and lung were streaked on blood and McConkey agar and incubated at 37 °C for 48 hrs under aerobic conditions. Pure cultures were used for further identification using API-Systems (Bio Mérieux, France).

Serological examinations

Serum samples (15–20 sample per flock) were tested for antibody against ALV-J and REV using commercial antibody ELISA (IDEXX laboratories, Inc., Maine, USA). Also antibody against CIAV was tested using ELISA (KPL, Maryland, USA).

Broiler performance

A comparison was conducted between two broiler flocks namely, A & B. Both flocks were derived from one parent flock at different times during the production. The parent flock had been tested at 1st day of age and proved to be congenitally infected with the ALV-J. During the production (about 35 weeks old), the parent flock was naturally infected with CIAV.

Broiler flock (A) was examined while the parent flock was only infected with ALV-J. On the other hand, Flock B was examined when the parent flock was dually infected with ALV-J and CIAV. Each flock consisted of 10000 birds and were raised under field condition for 7 weeks. At weekly intervals, 100 birds were randomly collected from both flocks and production parameters (weight gain and feed conversion rates “FCR”) were determined. Also, cumulative mortality was recorded. The data were analysed for statistical significance using T student test (P < 0.05).

Results

Post mortem examination of the affected chicks showed emaciation, the carcasses were pale with subcutaneous and ecchymotic haemorrhages throughout the skeletal muscles. Femoral bone marrow was yellowish and fatty. The intestine was pale, dilated contained undigested food. There were small nodules in the liver, kidney, heart, and lung. The spleens were variable in size with discrete necrotic foci. In all the examined chicks, the bursa of Fabricius was small and the thymus glands were severely atrophied. The walls of the proventriculus were thickened and many had petechial haemorrhages on the mucous lining at the distal end.

Histopathological examination of liver, spleen, heart, kidney, and proventriculus revealed the presence of immature myelocytes with characteristic eosinophilic cytoplasmic granules. The proventricular lesions were hyperplasia and necrosis of the mucosa, myelocytic and lymphocytic infiltration in the submucosa, and the proventricular glands are enlarged accompanied by lymphocytic infiltration. In the bursa of Fabricius, loss of lymphocytes from the lymphoid follicles caused shrinkage of the bursal plicae. There was marked atrophy of the thymus glands, with losses of most of the lymphocytes in the cortex. No evidence of Reo virus infection were noticed.

ALV-J was isolated from 8 of 9 tested flocks as shown by positive p27 antigen ELISA results (Table 1). All tested flocks were negative for REV isolation.

CIAVs were isolated from 4 of 5 tested flocks and induced cytopathic effects in MDCC-MSB1cells. Bright staining granules of fluorescence were observed in the nu-
ceti after staining with the CIAV-antibody. The results were confirmed using PCR (Fig. 1). In addition, DNA extracted from livers of diseased chicks yielded positive results in 11 out of 12 tested flocks. Infectious bursal disease antigen could not be detected in the bursa of Fabricius of any of the chicks examined.

Routine bacterial cultures of organs from examined chicks revealed a variety of organisms. There was a predominance of Escherichia coli.

All 18 serologically tested stunted flocks had antibodies to CIAV. Antibodies to ALV-J virus were detected only in 3 of 9 tested flocks (Table 1). In all flocks antibodies to REV could not be detected.

Results of average body weight, food conversion ratio and cumulative mortality of the two broiler flocks (A & B) are shown in Tables 2 and 3. The lowest average weight was recorded for the ALV-J and CIAV infected group. Comparison of the FCR between the 2 flocks revealed no significant differences (P > 0.05). The mortality rates of the 2 infected flocks were significantly higher than the standard. The highest mortality was recorded in the ALV-J and CIAV infected flock B.

### Discussion

Stunting syndrome was first reported in Netherlands in 1978 (Kouwenhoven et al., 1978). However, the pathogenesis and aetiology of stunting syndrome is still poorly understood. In many field cases the infectious nature of the stunting syndrome has been based on field observation and laboratory reproduction of the syndrome with intestinal contents from affected birds. Reovirus, Calicivirus, Coronavirus like particles and Toga like viruses have been demonstrated in intestinal content from stunted birds. However, the disease has not been completely reproduced with any of these viruses after they have been purified (Montgomery et al., 1997).

A previous study on broilers derived from breeder flocks infected with ALV-J, revealed significant decrease in body weight (Goodwin et al., 2000). Similar observations were also recorded in Egypt (Al Y and Hassan, 2004). In the present investigation parent flocks were exposed to natural infections with ALV-J and CIAV that may indicate the vertical transmission route of infection to the offspring.

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### Table 1. Results of laboratory investigations of 18 broiler flocks

<table>
<thead>
<tr>
<th>Flocks*</th>
<th>Age (days)</th>
<th>Isolation</th>
<th>PCR for CIAV</th>
<th>Serology</th>
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<tr>
<td></td>
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<td>ALV</td>
<td>CIAV</td>
<td>ALV</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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</table>

* All tested flocks had myelocytic infiltration in visceral organs
** ND = Not done

### Figure 1. Results of CIAV-PCR

Lane 1: extracted DNA of non infected SPF liver; lanes 2 to 5: extracted DNA from liver of clinical cases; lane 6: PCR reaction without DNA template (negative control); lane 7: extracted DNA from infected MSB1 cells (positive control); lane 8: extracted DNA from non infected MSB1 cells; lanes 9–10: extracted DNA from infected MSB1 cells (2nd passage): M: 100 bp DNA Ladder

### Table 2. Average weight (g) and food conversion ratio (FCR) of ALVJ infected flock (A) and ALVJ & CIAV infected flock (B) compared to the standard performance

<table>
<thead>
<tr>
<th>Age in weeks</th>
<th>Average body weight</th>
<th>Feed conversion rate</th>
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<tr>
<td></td>
<td>Standard</td>
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<tr>
<td>2</td>
<td>359</td>
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<td>675</td>
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<td>6</td>
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<td>1750</td>
</tr>
<tr>
<td>7</td>
<td>2612**</td>
<td>1800b</td>
</tr>
</tbody>
</table>

* Values followed by different lower case superscript (a, b, c) are significantly different (P < 0.05)

Archiv für Geflügelkunde 2/2004
ALV-J was isolated from broiler breeder and commercial broiler flocks experiencing ML at 4 weeks of age or older (FADLY and SMITH, 1999). Also, CIAV is known to be ubiquitous in many commercial flocks in Egypt (AMIN et al., 1998; SABRY et al., 1998; ALY, 2001). As shown in Table 1 both viruses were isolated with high incidence from stunted examined broiler flocks. ALV-J was isolated from 3 of 9 flocks, while CIAV was isolated and/or detected in 11 of 12 tested flocks. In addition to the virus isolation, serological and histopathological results confirmed the dual exposure of stunted birds to both viruses. Post-mortem and histopathological examinations showed that some stunted chicks had no gross lesions but myelocytic infiltration in visceral tissues. This result is in agreement with the observation reported by WEIKEL et al. (2000).

Investigations for other viral infections, revealed no evidence of REV, Reo and IBD infection in examined stunted chickens.

The results obtained in this study illustrated the impact of ALV-J and CIAV infections on the body weight gain and mortality. As shown in Table 2 the lowest average weight was recorded in chickens infected with either ALV-J or ALV-J and CIAV. Recently, STEADMAN and BROWN (1999) and GOODWIN et al., (2000) reported that congenital ALV-J infection is associated with significant weight suppression in broiler in the absence of other pathogens.

A study by ZAVALA et al. (2000) on the interaction between Marek's disease virus (MDV) and ALV-J in vivo showed that any groups receiving ALV-J alone or in combination with Marek's disease (MD) vaccine and/or very virulent MDV expressed the lowest body weights recorded in the experiment. Significant clinical signs in ALV-J vertically infected broilers at the end of the fattening period are a reduced body weight of 600 grams compared to 1800 grams in healthy birds (WEIKEL et al., 2000). Broilers with congenital ALV-J infection showed no lymphoid infiltrates but still developed severe stunting and hypothyroidism (BROWN et al., 2000).

Broilers Infected with CIAV show decreased body weight, and increased mortality, while feed conversion ratio is not affected (SCHAT, 2003). In the present study, it might be expected that congenital CIAV infection may play a possible role in stunting and exacerbate the adverse pathogenic effects of ALV-J.

In conclusion, results of this study illustrated that ALV-J and CIAV should be considered as possible factors that may contribute to stunting syndrome. Further research is needed to elucidate the interaction between the two viruses and the underlying mechanism(s) involved in the development of stunting syndrome. Also, preventive measures should be adopted in order to reduce the incidence of stunting cases in commercial broilers. Imported day-old grand parents and parent flocks should be tested to insure its freedom from ALV-J. The role of biosecurity should also be emphasized to reduce early horizontal spread of the virus. Also, breeder flocks should be vaccinated, if not naturally exposed to CIAV.

**Summary**

Birds from 18 broiler flocks suffering from stunting syndrome were examined. Stunted chicks were derived from parents naturally infected with avian leukosis virus subgroup J (ALV-J) and chicken infectious anaemia Virus (CIAV).

Größ und microscopic lesions were indicative of infection with ALV-J and CIAV. ALV-J was isolated from 8 of 9 tested flocks. CIAV was isolated from 4 of 5 tested flocks and DNA could be detected in the livers of 11 of 12 tested flocks. Investigations for other viral agents, revealed no evidence of REV, Reo and IBD infection in stunted chickens.

All 18 tested stunted flocks had antibodies to CIAV. Antibodies to ALV-J virus were detected only in 3 out of 9 tested flocks. In all flocks antibodies to REV could not be detected.

The performance of 2 broiler flocks derived from the same parent flock that was naturally infected with ALV-J (at day old) and with CIAV (at about 35 weeks old) was investigated.

The lowest average body weight and the highest mortality rates were recorded for the ALV-J and CIAV infected flock. These findings are suggestive of close association between ALV-J and CIAV infections with stunting conditions.

**Key words**

Stunting syndrome, Avian Leukosis Virus subgroup J, Chicken Infectious Anaemia, Broiler, Egypt

**Zusammenfassung**

Stunting Syndrom assoziiert mit dem aviairen Leukosevirus der Subgruppe J und dem Virus der infektiösen Anämie in Broilerherden in Ägypten

Mastbäuchen aus 18 Beständen, die unter dem so genannten Stunting Syndrom litten, wurden untersucht. Die Tiere stammen von Elterntieren, die eine natürliche Infektion mit dem Aviären Leukosevirus der Subgruppe J (ALV-J) und dem Virus der Infektionsanämie (CIAV) aufwiesen.


Die Auswertung dieser Befunde spricht für einen engen Zusammenhang zwischen einer ALV-J und CIAV Infektion und dem Auftreten des Stunting Syndroms.

Stichworte

Stunting Syndrom, Aviares Leukosevirus der Subgruppe J, Infektion Anämie, Broiler, Ägypten

Acknowledgment

This work was funded by the Middle East Regional Cooperation (MER), USA. The excellent technical assistance of Dr. A. El-Zaher and Dr. A. Arafa is appreciated.

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