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# THE INCIDENCE AND PATHOGENICITY OF AEROMONAS HYDROPHILA IN FARM DUCKS IN ASSIUT GOVERNORATE

(With 5 Tables and 2 Figures)

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## تواجد وضراوة ميكروب الأيروموناس هيدروفيلا بمزارع البط بمحافظة أسيوط

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أجريت هذه الدراسة لمعرفة مدى تواجد ميكروب الأيروموناس هيدروفيلا فى مزارع البط الذى يربى ويذبح للإستهلاك الآدمى فى محافظة أسيوط. أجريت الدراسة على ١٧٠ عينة أشتملت على ٥٠ عينة تعانى من الإسهال والضعف العام و ٢٠ حالة سليمة ظاهريا ، وقد تم عزل الميكروب من المسحات المجمعية بنسبة ٣٠ ، ١٥% وكذلك أخذت مسحات من الأمعاء والكبد والرئة من البط المذبوح للاستهلاك الآدمى، وتم العزل بنسبة ١٢، ١٠، ٥% وكانت الأعمار التى تمت دراستها تتراوح من ٢-١٥ أسبوع وكانت نتائج الفحص البكتريولوجى تشير إلى عزل ٤٥ عتره (٢٦,٥%) من الميكروب من جميع الحالات. عند حقن الميكروب فى أجنة بيض الدجاج عن طريق غشاء الكوريو ألانتويس ظهر النفوق من اليوم الخامس بعد الحقن وكانت الآفات التشريحية على شكل إحتقان وتغيرات موضعية فى الأحشاء الداخلية، احتقان كيس المح. وعندما تمت عدوى الميكروب فى بط عمر ٤ أيام عن طريق كيس المح ومجموعة عن طريق الفم ، ومجموعة أخرى تحت الجلد. وجد أن الميكروب ضارى فى المجموعة الأولى والثانية حيث بلغت نسبة النفوق ٣٠-٢٠% وقد سجلت الأعراض الأكلينيكية والآفات التشريحية وتم عزل الميكروب مرة أخرى من الأعضاء الداخلية والأمعاء من البط النافق والمصاب. هذا وقد تم عمل إختبار الحساسية للميكروب المعزول وكان شديد الحساسية لكل من الجنتاميسين وحمض النلدكسك والكاناميسين بينما كان مقاوم لكل من الأمبسلين والبنسلين وسلفات الكولستين. وتم مناقشة الأهمية الصحية لعزل الميكروب من البط وكيفية الوقاية لحماية صحة الإنسان.

## SUMMARY

In the present study successful isolation of Aeromonas hydrophila (A. hyd.) was carried out on duck farms and slaughtered shops at Assiut Governorate. 170 samples were examined bacteriologically for determination of the occurrence and frequency of A. hyd. Diarrhea and

emaciation were observed in 50 out of 170 while 20 were apparently healthy at percentage of 30, 15%. Coloacal swabs were obtained from slaughtered ducks in addition to samples from intestinal tract, liver and lung at percentage of 12, 10% and 5%. The result of bacteriological examination revealed that total isolates were 45 at percentage of (26.5%). Inoculation of A. hyd. in chicken embryo through chorio-allantoic membrane (CAM) rout. inoculated embryos die 5 days post-inoculation (P.I.). Died embryos showed sever congestion of internal organs and yolk sac and curling of embryos. Yolk sac, oral and subcutaneous infection in 4 days old duckling revealed deaths of 30, 20% of birds, respectively during observation period in the first and second group but no mortality in the third group. The clinical observation and the post-mortem lesions of experimentally infected birds were recorded. Reisolation of infecting organism from internal organs and intestinal tract of dead and sacrified slaughtered birds at the end of observation period were conducted. The in vitro susceptibility of A. hydrophila isolates to a variety of antibiotics revealed that highest number of isolates were sensitive to Gentamycin Nalidxic acid and Kanamycin while it was resistant to Ampicillin, Penicillin and Colistin-sulphate. The public health significance and the economic losses of A. hydrophila were discussed as well as suggestions for their avoidance.

***Key words:*** *Aeromonas hydrophila, Ducks*

## INTRODUCTION

Aeromonas hydrophila (A. hyd.) infections is widely spread among different ages of ducks causing heavy economical losses. Such losses attributed to mass mortalities and decrease of the growth rate in addition to high costs of medication and control of infection (Ghittino, 1976). A. hyd. has been recognized as a primary pathogen in ducks (Aguirre *et al.*, 1992; Fan De *et al.*, 1997 and Ke Min *et al.*, 1998). El-Gohary and Amal, 2002 who reported that ducks were highly susceptible to A. hyd. causing anorexia, dyspnoea, emaciation, greenish-white diarrhea and mortality rate 25%.

A. hyd. is a facultatively anaerobic Gram negative motile rod shaped bacteria classified in the members of the vibrionacae. It had been reported in many countries in the world and isolated from a wide range of mammals, surface water and sewage (Schubert *et al.*, 1972 and Hazen *et al.*, 1978), in fish and shell-fish (Rippey and Cabelli, 1979;

Schaperclaus *et al.*, 1992 and Woo and Bruno, 1999) and birds (Glünder and Siegmann, 1989). In recent years the organism has received a renewed interest as a human pathogen due to the transmission of the organism from infected birds to human causing several diseases such as diarrhea, osteomylitis (Lopez *et al.*, 1968), meningitis (Qadri *et al.*, 1976), cellulites, endocarditis and ear infections (Koneman *et al.*, 1994). Bacterial agent is transmitted by water, intestinal discharge, parasitic and fungal infection and carriers which play an important role in transmission of the infection (Egusa, 1978; Sugita *et al.*, 1994; Dumontet *et al.*, 1996; Noga, 1996; Aoki, 1999 and Ahmed and Shoreit, 2001). Furthermore, isolation of A. hyd. in avian species was recorded by several studies that documented 20 isolation from 15 species of 200, free living and companion birds (Shan *et al.*, 1984), 10 isolations from 45 raptors (Needham *et al.*, 1979). 8 isolates of A. hydr. were recovered from 141 live birds and 14 isolates from 240 cases submitted for post-mortem examination (Shan *et al.*, 1984). A. hyd. was isolated from dead canaries (Panigraphy *et al.*, 1981), young poults (Gerlach and Bitzer, 1971), chicken (Frieker and Tompsett, 1989 and Sarimehmetoglu and Kuplu, 2001). Rabbit (Okewole *et al.*, 1989; Efuntoye, 1995 and Abdel-Gwad and Abdel-Rahman, 2004). A. hyd. was recovered from ducks with salpingitis and air sacculitis (Aguirre *et al.*, 1992; Bisgaard, 1995; Fan De *et al.*, 1997; Ke Min *et al.*, 1998 and El-Gohary and Amal, 2002) who isolated A. hyd. from duck farms 2-7 weeks of age and recorded that ducks were susceptible to infection with a. hydrophila. Some investigators revealed that A. hyd. can cause localized and systemic infections in poultry alone or in combination with other organisms (Shan and Gifford, 1985 and Glünder and Siegmann, 1989).

The pathogenicity of A. hyd. in experimental birds was studied. On chick, Shan *et al.* (1984) found that 2-4 day-old-chicks died within 48 hours of inoculation, while, Gerlach and Bitzer (1971) recorded that turkey poults were susceptible to exposure and mortality rate reached 80-100%. In Quail mortality rate ranged from 12-80% with severe diarrhea followed by deaths (Shan and Gifford, 1985). In Duck A. hyd. could be isolated from the internal organs of 5 commercial duck farms such as liver, intestine, spleen, kidney heart, lung, brain and pancrease (El-Gohary and Amal, 2002). The aim of the present investigation was carried out to record the occurrence and pathogenicity of the A. hydrophila in duck farms and the in-vitro sensitivity test of the strains isolates against different antibiotics.

# MATERIALS and METHODS

**(1) Collection of samples:**

Fifty samples were collected from diarrhotic and emaciated ducks and twenty from apparently healthy ducks. The samples obtained from animal production farm, Faculty of Agriculture, Assiut University from different ages (2-15 weeks old). One hundred samples were obtained from local slaughter shops in Assiut Governorate which mainly characterized by pericarditis, salpingitis, pneumonia, congested enlarged liver, and catarrhal exudates on the mucosal surface of the intestine. Intestinal tract, liver and lung were subjected for isolation of A. hydrophila. Coloacal swabs from living birds were applied for A. hydrophila isolation.

**(2) Isolation of A. hydrophila:**

Coloacal swabs, liver, intestinal and liver tissues from ducks were inoculated into tripticase soy broth with Ampicillin (10 µg/mL) was added to media and incubated at 28°C for 24 hour. The primary isolation was obtained by culturing the broth on Rimler-Shotts medium and incubated at 28°C for 18-24 hours. Suspected colonies were picked up and streaked onto the surface of starch Ampicillin agar, at 28°C for 24 hour. Suspected colonies were transferred onto tripticase Soy agar plates and nutrient agar for further identification. This technique recommended by Shotts and Rimler (1973); Shotts and Bullock (1975); Glünder and Siegman (1989) and Bisgaard (1995).

**(3) Identification of A. hydrophila**

The isolated bacteria were identified by culture morphology, gram stain and several biochemical reactions according to Finegold and Martin (1982), Popoff (1984), Palumbo *et al.* (1985), Glünder and Siegman (1989) and Bisgaard (1995). The colonies that showed typical reaction in TSI, positive for cytochrom oxidase test, catalase test and Asculine hydrolysis, oxidation and fermentation of gas from glucose were confirmed as A. hydrophila.

**(4) Pathogenicity of A. hdyrophila:**

**a- Embryonated chicken eggs:**

Thirty fertile chicken eggs were obtained from animal production farm, Faculty of Agriculture, Assiut University. The eggs were incubated in egg incubater. At 11-day of embryo age. The fertile eggs were classified into 3 equal groups (10 eggs in each).

The first 2 groups were inoculated via chorio allantoic membrane by $9x10^5$, $9x10^4$ broth culture of A. hyd. isolates. The last group were kept non-infected as control. Eggs were incubated at 38°C in humidity with frequent turning and daily candling. Dead embryos were examined (Yadov and Verma, 1998 and Kutkat *et al.*, 2001).

**b-Experimental infection of 4-day old ducklings with A. hydrophila:**

Sixty seven, day-old duckling obtained from private farms at Assiut Governorate. Three random birds were slaughtered and subjected to bacteriological examination to check their freedom from A. hyd. At 4 days of age the remaining 64 birds were divided into 4 groups, group 1, 2 and 3 consisted of 20 birds each and group 4 of 4 birds. Ducklings of groups 1, 2 and 3 were inoculated by the yolk sac, oral and subcutaneous routes respectively, with 0.1 mL of A. hyd. isolate suspension in saline containing $4.3x10^8$ organisms per duck, while group 4 was kept as uninfected control. Birds of all groups were kept isolated in separate cages and pens for 8 days observation period with daily examination for clinical signs and morality rate. Faecal swabs were taken daily for bacteriological examination. At the end of observation period all ducks were recorded as well as trials for reisolation of infecting organism from liver, kidney, lungs and intestinal were recorded.

**(5) Antibiotic sensitivity test:**

Mueller-Hinton agar was used for the disk diffusion test to produce large and clear zone of inhibition according to Finegold and Martin (1982). A total of 10 chemotherapeutic agents were used (Ampicillin (10 μg), Gentamicin (10μg), Tetracycline (30 μg), Nalidixic acid (30 μg), Streptomycin (10μg), Cephoxitin (30μg), Kanamicin (30 μg), Penicillin (10μg), Erythromycin (15 μg), Colistin-sulphate (10μg). The degree of sensitivity was determined according to Oxoid Manual (1982) and Koneman *et al.* (1983).

## RESULTS

**Table 1:** The frequency percentage of Aeromonas hydrophila isolated from duck samples.

| Samples | Number | Isolation | Total A. hyd. isolation | Rate of A. hyd. isolation |
|---|---|---|---|---|
| Diseased cases | 50 | Coloacal swabs | 15/60 | 30 |
| Apparently healthy | 20 | Coloacal swabs | 3/20 | 15 |
| Slaughter shops | 100 | Intestine | 12/100 | 12 |
| | | Liver | 10/100 | 10 |
| | | Lung | 5/00 | 5 |
| Total | 170 | | 45 | 26.47 |

**Table 2:** Biotyping of Aeromonas hydrophila isolated from ducks.

| Test | Result |
|---|---|
| Gram staining | Gram negative coccobacilli |
| Cytochrom oxidase | Positive |
| Catalase | Positive |
| TSI | Positive (yellow colour and black) |
| Asculine | Positive (dark brown to black) |
| Glucose | Positive (acid & gas) |

**Table 3:** Results of inoculation embryonated chicken eggs by different concentration of A. hydrophila.

| Groups | No. of Eggs | Inoculums | Dead Embryos Days P.I. | | | | | | | % | Reisolation of A. hyd. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| 1 | 10 | $9x10^5$ | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 30 | + |
| 2 | 10 | $9x10^4$ | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 10 | + |
| 3 | 10 | SS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 4:** Results of pathogenicity of A. hdyrophila in ducks.

| Group | Bird no. | Rout of inoculation | Dose | Daily death post infection | | | | | | | | Total no. of deaths | % | Reisolation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | | Fom various organs | | Fecal material |
| 1 | 10 | Yolk sac | $4.3x10^8$ | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 3 | 30 | 8/10 | 80% | +ve |
| 2 | 10 | Oral | $4.3x10^8$ | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 20 | 6/10 | 60% | +ve |
| 3 | 10 | Subcutaneous | $4.3x10^8$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2/10 | 20% | +ve |
| 4 | 5 | Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/10 | 0 | -ve |

**Table 5:** Antibiotic sensitivity test for Aeromonas hydrophila isolates.

| Antibiotic agent | | % Sensitive | | % Resistant | |
|---|---|---|---|---|---|
| Ampicillin | 10 µg | 0 | (0/20) | 100% | (20/20) |
| Gentamicin | 10 µg | 100-% | (20/20) | 0 | (0/20) |
| Tetracycline | 30 µg | 85% | (17/20) | 15% | (3/20) |
| Nalidixic acid | 30 µg | 100% | (20/20) | 0 | (0/20) |
| Streptomycin | 10 µg | 70% | (14/20) | 30% | (6/20) |
| Cephoxitin | 30 µg | 90% | (18/20) | 10% | (2/20) |
| Kanamicin | 30 µg | 95% | (19/20) | 5% | (1/20) |
| Penicillin | 10 µg | 0 | (0/20) | 100% | (20/20) |
| Erythromycin | 15 µg | 15 | (3/20) | 85% | (17/20) |
| Colistin-sulphae | 10 µg | 0 | (0/20) | 100% | (20/20) |

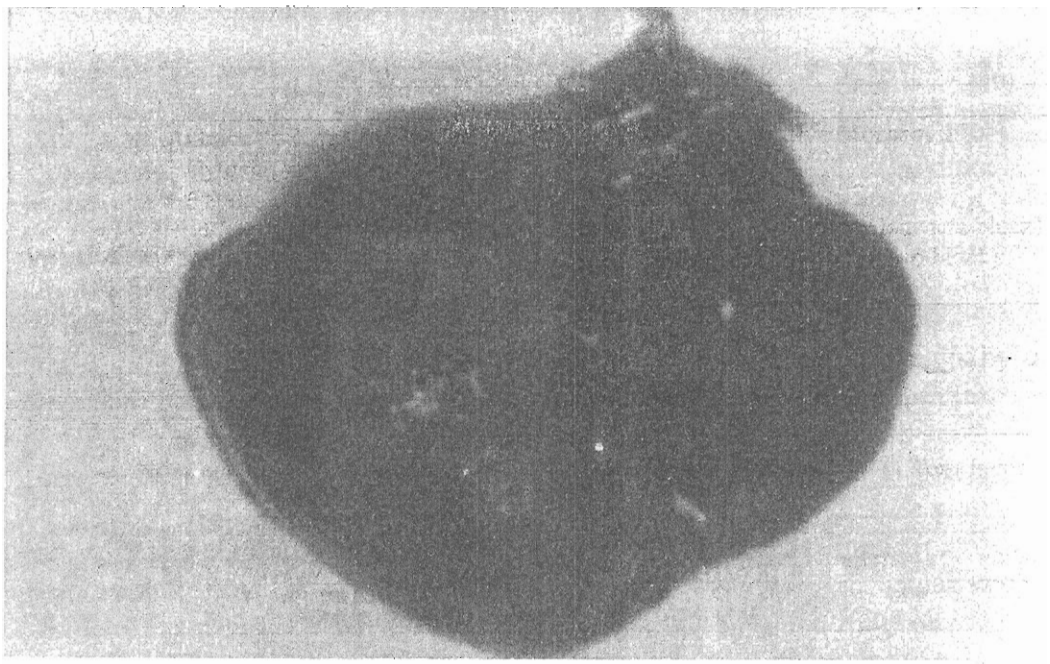

**Fig. 1:** Chicken embryo inoculated on cAM, died embryho showing sever cognition and curling

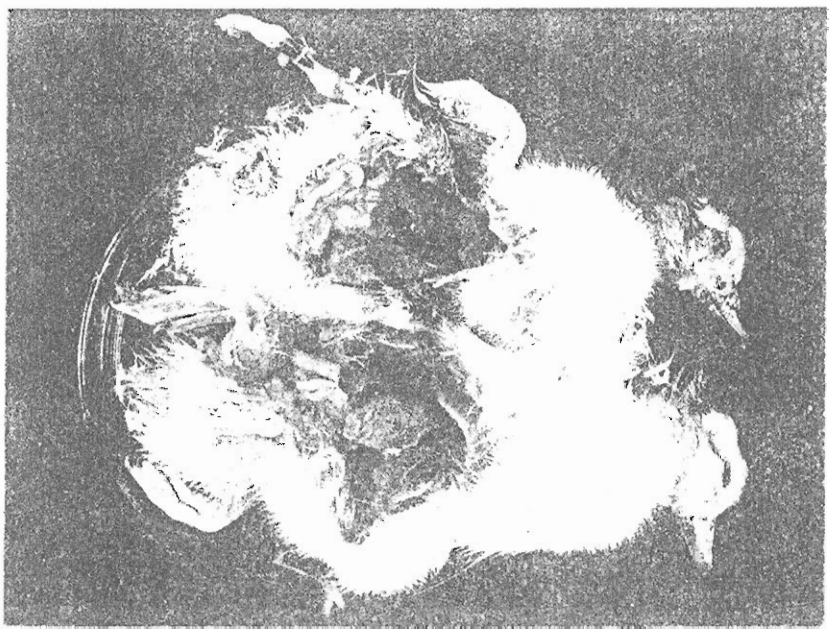

**Fig. 2:** Ducks yolk sac and orally infected with $4.3x10^8$ cfu/mL of broth culture of isolated A. hydrophila showing seve congestion of internal organs.

# DISCUSSION

It has been suggested that the production of ducks is often carried out under suboptimal conditions allowing the early establishment of the pathogens. Among the bacterial diseases encountered in domestic ducks. A. hydrophila infection has been well documented as a cause of considerable economic loss to the duck industry.

The results presented in Table (1) declared that 15 (30%) out of 50 coloacal swabs were done from dirrhroeitic duck samples and 3 (51%) out of 20 coloacal swabs from apparently healthy ducks from different ages investigated in this study. A. hydrophila were isolated from ducks in slaughter shops with percentage of 12 (12%), 10 (10%) and 5 (5%) out of 100 samples for each from intestinal tract, liver and lung respectively.

Higher isolation rate from coloacal swabs and intestine is reflected that A. hydrophila either infects birds by the oral route or had colonized the intestine as a part of the intestinal flora. However, isolation of A. hydrophila could be attributed to several factors especially diet, as the producers feed raw fish or poor cooked fish meal in the diet of ducks or watering with untreated drinking water from small nile tributaries.

Furthermore, the isolation of A. hydrophila from liver can be explained by infection via the blood stream, and from the lung indicates a systemic infection. Our findings is conformity with the observation of Aguirre *et al.* (1992), Glünder and Siegmann (1989), Shan *et al.* (1984) and El-Gohary and Amal (2002). 45 isolates (26.4%) were identified to be A. hydrophila that grew on RS media after 24 h. incubation at 28°C, these colonies were rounded, yellow to orange in colour and 2-3 mm in diameter, this agree with Shotts and Rimler (1973) and Hus *et al.* (1981) who noted that A. hydrophila were yellow colonies on RS media, while were white to pale pink, round and convex colonies on nutrient agar, Gram-negative, rod-shaped and facultative anaerobic.

A. hydrophila was the only identified aeromonad species during this study. All tested isolates gave typical reaction in TSI and positive for each of cytochrom oxidase, catalase test, Asculine hydrolysis and oxidation and fermentation reaction (Table 2). These results are similar to those reported by Finegold and Martin (1982), Popoff (1984) and Bisgaard (1995).

The data in (Table 3) revealed that, inoculation of A. hydrophila in chicken embryos via CAM causing deaths after 5 days post

inoculation with mortality rate ranged between 10-30%. Died embryos showed sever congestion of internal organs and curling of embryos and congestions of yolk sac (Fig. 1). Reduced of hatchability was noticed due to weakness of the embryos, so there is correlation between the level of infection by A. hydrophila and hatchability in groups 1-2. On the other hand, a high level of A. hydrophila revealed twice of embryo mortality if compared with other level. The hatched chicks were stunted in growth. The A. hydrophila organism were reisolated from dead embryo and hatched chicks. These results are similar to those reported by Yadov and Verma (1998) and Kutkat *et al.* (2001).

The experimental infection of 4-day-old ducklings with A. hydrophila isolate showed in Table (4) that the death rate of 30% (3/10) by yolk sac route, 20% (2/10) by oral route but no deaths in the third group which inoculated subcutaneously, these results agreement with Shan and Gifford (1985) and El-Gohary and Amal (2002). The clinical signs noticed were: loss of appetite, ruffed fure, depression, disinclination to move, inclination to separate in the corner of the cage followed by profuse watery diarrhea, emaciation and death. Post-mortem examination revealed congestion of internal organs, liver, spleen, heart, lung and sever interitis (Fig. 2). Reisolation of A. hydrophila post experimental exposure to the organism was successful and documented that A. hydrophila is a primary pathogen in ducks as concluded by Fan De *et al.* (1997), Glünder and Siegmann (1989) and KeMin *et al.* (1998).

In vitro sensitivity of 20 isolates A. hydrophila to a variety of antibiotics shown in Table (5), 100% of the A. hydrophila isolates sensitive to Gentamycin, Nalidixic acid and 95% to Kanamycin, 90% to Cephoxetin, 85% to Tetracycline, 70% Streptomycin and 15% to Erythromycin while all isolates of A. hydrophila were resistant to Ampicillin, Penicillin and Colistin sulphate, these results agree with Soliman (1988), Molero *et al.* (1989), Abou El-Gheit *et al.* (1995) and Abdel Gwad and Abdel-Rahman (2004) who found that all strains of A. hydrophila isolates to be sensitive to Gentamycin, Nalidixic acid and Kanamycin. On the other hand, all the isolates were resistant to Ampicillin, Penicillin and Colistin sulphate these findings agree to a certain extent with those reported by Khater *et al.* (1997), Sohair and Eman (2002) and Abd El-Gwad and Abd El-Rahman (2004), those reported that a great number of strains seemed to be resistance to Ampicillin, Penicillin and Colistin sulphate. The results of this study indicates that A. hydrophila occurred in a high frequency in intestine of

ducks and other organs, so there is a risk associated with consuming not sufficient cooked ducks or feeding ducks on raw fish. The risk can be avoided by only consuming thoroughly cooked ducks. In addition, good food handling practiced in the home reduce the risk of illness. Careful sanitary procedures as well as good personal hygiene and appropriate chemotherapy are of paramount importance.

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