Veterinary Medical Journal-Giza (ISSN 1110-1423)
Faculty of Veterinary Medicine,Cairo University
Accredited from national authority for Quality Assurance and Accreditation
Giza, 12211, Egypt

Studies on Pasteurellosis in birds

Mona, S.Abdelrahman.Jakeen, A.K.El-Jakee**Sherif, A. Marouf**and Youssef, I. Youssef**

*Dept. of Poult. Dis., **Dept. of Microbiol., .Faculty of Veterinary medicine, Cairo University, Egypt.

Abstract

Two hundred pooled samples including (liver, heart and spleen) were collected from examined 454 dead birds that were suspected to be infected with fowl cholera. These birds were collected from 132 poultry farms from different governorates. Eight isolates could be biochemically identified as *Pasteurella* spp.Using PCR, all isolates were identified as *Pasteurella multocida (P.multocida)* capsular type A with overall incidence of 1.76%. The incidence of avian pasteurellosis in Sharkya (4%) was higher than that in Qalioubia (1.38%). The incidence of isolation of *P.multocida* from examined chickens was 2.2 (8 out of 360 chickens) while it could not be isolated from duck and turkey samples. *Pasteurella multocida* could be isolated only from layers chickens.

Key words: Pasteurella multocida in birds, PCR capsular typing, fowl cholera, Avian pasteurellosis, Histopathological changes of *Pasteurella* .

Introduction

Avian pasteurellosis has been reported as an important disease in domestic poultry for more than 200 years that causes devasting economic losses to poultry industry worldwide (Aye *et al., 2001).P. multocida* is a Gram negative bacterium infects a wide range of birds causing fowl cholera in poultry (Glisson *et al.,* 2003) which is generally caused by serotype A: 1, A: 3 or A: 4.

Diagnosis of fowl cholera is based on clinical signs, pathological findings and isolation and identification of *P.multocida* (Rimler and Glisson, 1997).

Conventional methods of characterizing isolates of *P. multocida* are often time consuming and don't type all strains. Recently, DNA-based identification and typing systems are emerging as reliable alternatives, providing rapid identification of pathogens. (Blackall and Miflin 2000).

The polymerase chain reaction (PCR) has the potential to detect low numbers of a target organism in heavily contaminated samples. Several PCR tests have been described for detection or identification of *P.multocida* species (Kasten *et al., 1997;* Townsend *et al., 1998 and* Miflin and Blackall, *2001).*

The present study aimed to investigate the prevalence of *P.multocida* among chicken, duck and turkey samples in Egypt and the pathogenicity of the isolated organism in 12 week old layers.

Material and methods

Samples

A total of 200 freshly dead birds of different ages suspected to be infected with fowl cholera were collected from 132 different poultry farms (120 chicken farms, 7 duck farms and 5 turkey farms) from different localities at Qalubia, Sharkya, Minofia , Assuitand Gharbya Governorates, Egypt. (table-1 and 2).

Heart, liver and spleen were pooled from each bird. Heart blood smears, tissue impression smears from liver were prepared and stained with Leishman stain .Heart blood and tissues were subjected to bacteriological examination for isolation of *P .multocida.*

Bacterial isolation and identification

The heart blood and tissue samples were inoculated into brain heart broth (Oxoid) and incubated at 37°C for 18 hrs. Then subcultured on blood agar, MacConkey agar (Quinn *et al.,* 1994) and DAS media

(DAS 1958) and incubated at 37°C for 24 hrs for growth of *P.multocida*. The suspected colonies were subjected to biochemical tests for identification of *P. multocida* (Cruicshank *et al.*, 1975; Quinn et *al.*, 1994 and Holt *et al.*, 1994).

Pathogenicity in mice

Pure isolates were tested for pathogenicity to white mice. According to Balakrishnan. and Parimol (2012) as following 0.2ml of brain heart broth culture (108 C.F.U /ml) were inoculated intraperitoneally in mice and observed for 48 hr. Dead mice were subjected to post mortem examination and reisolation of the inoculated organism. Dead mice without signs and lesions were proved to their positive mortality.

Polymerase chain reaction (PCR) for identification of *P.multocida*

DNA was extracted from the overnight culture of *Pasteurella* isolates using QIAamp DNA Mini Kit Catalogue no.51304. *P.multocida* polymerase reaction (PM-PCR) was carried out using species specific primers KMT ISP6 and KMT 177 designed by Townsend *et al.*, (1998) to amplify KMT1 gene .The analysis of PCR product was carried out in 1.5 % agarose stained with ethidium bromide (10mg/ml) .100bp DNA ladder and appropriate controls were incorporated to rule out false positive and false negative results. The gel was viewed under UV transillumination.

Pathogenicity of the isolated *P. multocida* in chickens

Freshly prepared culture from *P. multocida* strain (108cfu/0.5ml) (Petersen *et al.*, 2001) was inoculated intratracheally into 12 week old layer type chickens (within 30s). The experimental birds were kept under observation for 14 days for clinical signs and/or mortality. At the end of observation all survived birds were sacrified for lesions, re-isolation and/or histopathological examination.

Results and Discussion

P. multocida has been consistently found in the upper respiratory tract, spleen, lungs, blood and liver of infected birds (Rhoades, 1964; Hunter and Wobeser, 1980).All isolates were non hemolytic and had bipolarity.

The results of the present study revealed the isolation of 8 (2.2%). *P. multocida* isolates out of 200 samples, collected from freshly dead chickens, ducks and turkeys from different localities at Qalioubia, Sharkya, Minofia, Assuit and Gharbya provinces in Egypt.

The presence of such organisms in these bird species reflects the distribution of the disease (Avian cholera) in these governorates. The isolation of *P. multocida* from poultry population in Egypt was reported earlier by Abd El-Dayem(1990);Ibrahim (1991): Gergis et *al.* (1992);Bebars(2000); Hassan *et al.* (2001);El-Shamy (2008) and Hekal (2009). The low isolation percentages in this study may be due to the fact that most of our samples were not taken from birds in the acute stage of the infection which agrees with the findings of Mraz et al., (1980), who found a higher prevalence of *P.multocida* in convalescent chicken flocks than in disease free flocks, or could be attributed to the uncontrolled use of antibiotics in nearly all farms.

For isolation of *P.multocida* from freshly dead birds on DAS media (DAS 1958), blood agar, brain heart infusion agar and MacConkey agar. (Carter, 1967 and Cruickshank et al., 1975).

Table (1): The prevalence of avian Pasteurellosis in different localities in Egypt.

Governorates	No. of examined farms	No .of examined suspected birds	*P.multocida* positive isolates	
Qalioubia	75	288	4	1.38%
Sharkeya	34	100	4	4%
Minofia	14	42	0	0%
Assuit	4	11	0	0%
Gharbeya	5	13	0	0%
Total	132	454	8	1.76%

Table (2):The prevalence of avian Pasteurellosis according to different avian species.

Species	No. of examined farms	No .of examined birds	Incidence of avian pasteurellosis in different species	
Chicken	120	360	8	2.22%
Duck	7	21	0	0%
Turkey	5	9	0	0%
Total	132	454	8	1.76%

The mice pathogenicity test is often used to detect *P.multocida* in samples contaminated with other microorganisms (Quan et *al.*, 1986).White mice injected I/P with *Pasteurella multocida* strain succumbed within 24hrs (Kasten 1997). However, virulence for mice has been reported to be variable (Curtis et al., 1980). 8 strains isolated from chickens were highly pathogenic for mice .all mice died within 18-24 hours of inoculation. These results agreed with the findings of Jaya Kumar, (1998) and Balakrishan and Parimol (2012) who recorded that *Pasteurella multocida* isolated from cases of fowl cholera were highly virulent for mice.

P.multocida species specific polymerase specific PCR (PM-PCR) assay developed by Townsend et *al.*, (1998) was used in this study to identify *P.multocida* isolates by amplifying the gene encoded by clone KMT1 of *P. multocida*. The primer pair KMT1SP6-KMT1T7 amplified a product of 455 ~bp from all tested isolates. (fig 1)

Fig (1): PCR amplified products of *P. multocida* isolates (Lane L: 100bp DNA ladder, Lane POS control positive, Lane Neg control negative and lane 1-8 the isolates.)

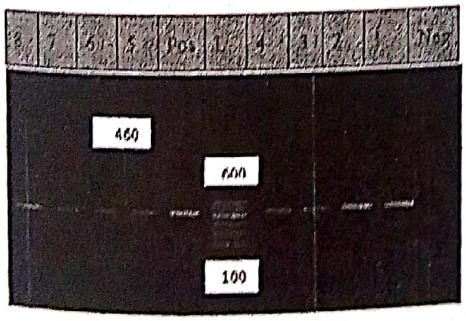
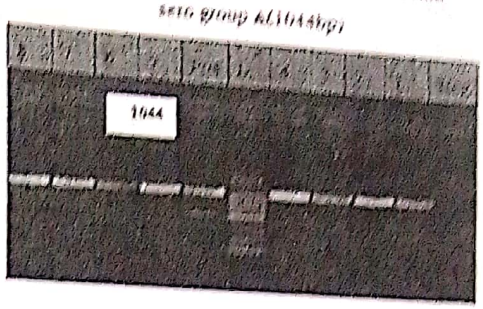


Fig (2): PCR amplified products of *P. multocida* sero group A(1044bp)



Purushothaman et *al.*,(2008) detected *P. multocida* by the use of PCR technique. A nucleic acid based diagnostic test has been found to be more sensitive and reliable than the conventional method .The main advantages of the nucleic acid based tests are that they reduce the time consumption and allow detection of the organism's genome even if it is in minute quantities, thus increasing sensitivity and specificity of the test (Innis *et al.*, 1990).PCR is one such test that can be used for the identification of organisms at any level, viz: strain, species, genus or all members of a domain, just by using a specific primer sequence. (Bhimani et al., 2014).

Within this investigation, *P. multocida* isolates were characterized serologically by the capsule serogroups with molecular serotyping. Carter and his colleagues (Carter and Rappay 1963 and Carter and Chengappa 1981) identified 5 serotypes (A, B, D, E and F) apparently on the basis of differences in the capsular substances.These results agreed with those reported by Karmy*et al.* (1983), Abd El-Motelib and Salem (1986),Akeila *et al.* (1986) and Kuczkowski*et al.* (2006). Also Shivachandra*et al.* (2006) isolated 72 strains of *P. multocida* from chickens, ducks, turkeys, geese and quails typed them in "A" and "D" serougroups. .All isolates were *P. multocida* serogroup A (Fig. 4).*P. multocida* isolates obtained from chickens mostly belong to serotype A and cause avian pasteurellosis , with incidences of high mortality and morbidity in infected farms, causing significant economic losses all over the world (Rhoades and Rimler, 1989).

Townsend *et al.*, (2001) and Mohamed and Moeman (2012) reported that PCR and multiplex PCR for capsular type detection were found to be a rapid and sensitive

method.The pathogenicity of the isolated *P.multocida* strains was tested in 12 week old chickens. An inoculation dose of approximately 2x108C.F.U./0.5ml of the isolates *P. multocida* was used. The post mortem lesions were recorded .Moderate clinical signs and no mortalities could be observed in experimental chickens infected with the isolated organisms although the same inoculated killed mice within 24 hrs.

Mariana and Hirst (2000) isolated five Pasteurella organisms pathogenic for mice.

Glisson et al(2003) reported that pathogenicity or virulence of *P.multocida* in relation to fowl cholera is complex and variable depending on the strain, host species and variations within the strain or the host and condition of contact between the two. Mature chickens are more susceptible than young ones and turkeys are much more susceptible

than chickens to infection with *P.multocida* (Heddleston, 1962).

Intratracheal inoculation of approximately 104C.F.U. of the strain isolated from an outbreak of fowl cholera in wild birds including eiders , cormorants, oyster – catchers and gulls, was highly virulent for turkeys (100% mortality), Partridges (91% mortality) and pheasants (38% mortality) , while chickens were found to be much more resistant (no mortality).The finding is in accordance with previous observations showing that turkeys of all ages were highly susceptible to *P.multocida* infections, while chickens under 16 weeks of age were resistant (Rimler and Glisson 1997) .Six – to fourteen weeks-old chickens, however have subsequently been found susceptible to intra tracheal inoculation with *P multocida* (Scott etal ., 1999 and Wilkie et al ., 2000).

The routes by which *P.multocida* gains entry to the body during outbreaks of avian cholera are presently unknown, but there is a prevailing belief that *P.multocida* is a respiratory pathogen (Simensen and Olson 1980 and Gustafson et al., 1998) .For this reason , an intra-tracheal challenge model was used in the present investigation .The inoculation dose of approximately

104c.f.u./0.5ml is considered to be a low dose (Matsumoto et al ., 1991)

Intratracheal inoculation of chickens aged six-to fourteen weeks old showed that 107 to 108 C.F.U.were required to achieve greater than 75% mortality (Scott et al., 1999). A universally accepted model using a standardized i.t. dose in chickens and other avian species of the same age would be useful for comparative studies of virulence Mbuthia *et al*., (2008) studied the clinical signs after experimental infection of chickens with *P. multocida* in relation to age group.They found that no birds died during the experiments, although all chickens except two (16 weeks old) expressed clinically signs of fowl cholera at some points during 14 day observation.

References

Abd El-Dayem, S. Nagah (1990): *Pasteurella* infection in poultry in Kaloubiaprovince. M.V.Sc. thesis, (Microbiology), Fac.Vet. Med. Zag. Univ. (Benha branch).

Abd El-Motelib, T.Y. and Salem, B. (1986): Characterization of *Pasteurella*strains isolated from ducks in the Newvalley. Assuit. Vet. Med. J., *16*:32-38

Akella, M.; Ezzat, M. and El-Nimr, M.M. (1986): Drug sensitivity of *Pasteurella multocida* isolated from carrier birds.Alex. J. Vet. Sci., *2* (1).

Aye, P.P., Angrick, E.J., Morishita, T.Y.and Harr, B.S., 2001: Prevalence and characteristics of Pasteurella multocida in commercial turkeys. Avian Dis.,45(1):182-190.

Blackall, P.J. and Miflin, J.K., (2000): Identification and typing of *Pasteurella multocida*: a review. Avian Pathol. 29: 271–287.

Baldrias, L.; Frost, A.G. and Boyle , D.(1988). Isolation of*Pasteurella*- like organisms from the tonsiller region of dogs and cats. J. of small Animal practice, 29,63-66.

Bebars A. S (2000): Characterization of *Pasteurella multocida* of duck origin by using protein profile analysis and DNA fingerprinting. M.V.Sc. thesis (Bacteriology, Immunology, Mycology) Fac.Vet.Med. Cairo Univ.

Balakrishnan.G and Parimol R. (2012): Isolation, Identification and Antibiogram of *Pasteurella multocida* isolates of avian origin .Tami: Lnadu J. V., Animal sci.8 (4) 199-202.

Bhimani M.P.; Roy, A ; Bhanderi , B.B. and Mathakiya, R.A. (2014) Isolation ,Identification and molecular characterization of *Pasteurella multocida* isolates obtained from emu (Dromaius novaehellandiae) in Gujarat state ,India. Veterinarski. ARHIV84 (4), 411-419.

Carter, G.R. (1967): Pasteurellosis : *Pasteurella multocida* and *Pasteurella hemolytica* . Adv.Vet.Sci.11, 321-379.

Carter,G.R. and Chengappa ,M.M.(1981) Recommendation for astandard system of designating serotypes of *Pasteurella multocida*. proc. Annu. Am. Assoc. Vet. Lab. Diag, 24: 37-41.

Carter, G.R. and Rappay, D.E. (1963): A haemagglutination test employing specific lipopolysaccharide for detection and measurement of *Pasteurella multocida* antibodies to *Pasteurella multocida*. Brit. Vet. J., *118*: 289-292.

Cruickshank R.; Duguid J.P.; Marmion B.P.and Swain R.H.A.(1975): Medical Microbiology. Vol. II 12th Ed. 522-35. Churchill. Livingstone, London and New York.

Curtis, P.E.;Ollerhead, G.E. and Ellis,C.E. (1980): Virulence and morphology of *Pasteurella multocida* of avian origin.Vet. Field Station, 107 (5): 105-108.

Curtis, P.E and Ollerhead, G.E. (1981): Investigation to determine whether healthy chickens and turkeys are oral carries of *Pasteurella multocida*.Vet. Rec., 108 (10) : 206- 207.

Das, M.S. (1958). Studies on *Pasteurella septica* (*Pasteurella multocida*). Observations on some biophysical characteristics. J Comp Pathol Ther. 68: 288– 294

El-Shamy, A. U. (2008): Studies on recent methods for diagnosis of fowl cholera in birds. Ph.D. Thesis (Poultry Diseases), Fac. Vet. Med. Zag. Univ.

Gergis, S. M. ; Ghanem, I.A.; El-Naenaeey E.Y. and Aly, N. M. (1992): Studies on *Pasteurella multocida* serovars in ducks and merits of autogenous vaccination .Zag. Vet.J.,20: 503-510.

Garlinghouse, L.E.; DiGiacomo, R.F. ; Van Hoosier, G.L. and Condon. J. (1971): Selective media for *Pasteurella multocida* and *Bordetella bronchiseptica*. J Lab Anim Sci. 31:39–42.

Glisson ,J.R. Hofacre, C. L., and Christensen,J. P. (2003): Fowl cholera Diseases of poultry .13th Ed. B.W. Calnek ,Iowa State Univ. Press , Ames .IA page (807-823)

Gustafson, G.R.; Cooper, G.L.; Charlton, B.R.and Bickford, A.A. (1998): *Pasteurella multocida* infection involving cranial air spaces in white leghorn chickens . Avian Dis.42:413-417.

Hassan, A.H.;Hala , A.F and Fawzia,M. M (2001) Studies on *Pasteurella multocida* serotypes isolates from fowl cholera vaccinated chickens in Egypt. J. Egypt. Vet. Med.Assoc.61, 133-152.

Heddleston, K.L. (1962): Studies on pasteurellosis. V. Two immunogenictypes of *Pasteurella multocida* associated with fowl cholera. Avian Dis. 6:315–321.

Hekal I.A. (2009): Bacteriological and immunological studies on *Pasteurella multocida* in poultry .M.V.Sc.thesis (microbiology) F.V.M. Benha Univ., Egypt

Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994). Bergey,s Manual of determination Bacteriology 9th edn .196. Lippin co. Williams and Wilkins publication .Hagerstown, M.D. USA.

Hungerford, T.G.(1968). A clinical note on Avian cholera. The effect of age on the susceptibi lity of fowls. Australlian Vet. J., 44, 31-32.

Hunter, B., and Wobeser, G. (1980). Pathology of experimental avian cholera in mallard ducks. Avian Dis. 24:403–414.

Ibrahim, R.S. (1991): Some studies on avian pasteurellosis. M.V.Sc Thesis, (Poultry Diseases), Fac. Vet. Med., Assuit Univ.

Innis, M.A.;Gelfand, D.H.; Sninsky, J.J.; White, T.J.(1990): PCR protocols: A Guide to methods and application .Academic press Inc. san Diego, California.

Jaya Kumar, P.S. (1998) Mvsc thesis submitted to Kerala Agricultural university, Thrissur.

Karmy, S.A.; Samia A.; Fayed, A.A. and AbdEl-Ghani, M. (1983): Studies on *Pasteurella multocida* in domesticated animals and birds in Asswan Gvernorate.J. Egypt Vet. Med. Ass., *43*: 1-4.

Kuczkowski, M.; Krol, J.; Wieliczko, A.; Gawe, A.; Schmidt, J. and Mazurkiewicz, M. (2006): Prevalence of fowl cholera in poultry and characteristics of isolated*Pasteurella sp.* Strains MedycynaWeterynaryjna. PolskiegoTowarzystwaNaukWeterynaryjnych, Lublin Poland, *62*(5): 574-578.

Kasten, R.W.; Carpenter, T.E.; Snipes, K.P. and Hirsh, D.C.(1997) Detection of *Pasteurella multocida*. Specific DNA in turkey flocks by using of the polymerase chain reaction .Avian Dis.676-682.

Mariana, S. and Hirst, R.(2000) The immunogenicity and Pathogenicity of *P.multocida* isolated from poultry in Indonesia .Vet. Microbial.72.27-36.

Matsumoto, M.; Strain, J.G. and Engel, H.N.(1991) The fate of *Pasteurella multocida*

after intratracheal inoculation into turkeys .Poult. Sci. 70, 2259-2266

Mbuthia ,P.G.; Njagi,L.W.; Nyaga, P.N.; Bebora, L. C.; Minga, U. ; Kamundia, J. and Olsen, J. E.(2008) *Pasteurella multocida* in scavenging family chickens and ducks : Carrier status , age susceptibility and transmission between species, Avian pathol., 37:1, 51-57.

Miflin, J.K. and Blackall , P.J.(2001) : Development of a 23sRNA.based PCR assay for identification of *Pasteurell amultocida*let. Appl. Microbial., 33(3) 216-21.

Mohamed,W.A. and Moemen A. M. (2014): Molecular analysis of *Pasteurellamultocida* strains isolated from fowl cholera infection in backyard chickens Asian Pac J Trop Biomed. 4(1): 8–12.

Moore,M.K.; Cicnjakchubbs, L. and Gates , R.J.(1994) A new selective enrichment procedure for isolating P.M.from Avian Dis., 38,317-324.

Mraz,O.; Sisak, F. and Jelen , P.(1980): The *Pasteurella* carriers in farm and laboratory animals. Comparat. Immunol. Microbiol. and infectious dis., 2 ,437-445.

Petersen, K.D.; Christensen, J.P.; Permin, A. and Bisgaard, M. (2001): Avirulence of *P.multocida*subsp.multocida isolated from outbreaks of fowl cholera in wild birds for domestic poultry and game birds. Avian pathol., 30, 27-31.

Purushothaman, V.; Jayathangara,J.; Probhakar, T.G.andProbhakar,P.(2008) Incidence of Avian Pasteurellosis in wild geese in captivity .Tamil . Naduj. Vet.and Anim.Sci.4(5):195-197.

Quan, T.J; Tsuchiya, K.R. and Carter, L.G. (1986) : Recovery and identification of *Pasteurella multocida* from mammals and eas collected during Plaques investigation .J.Wildl.Dis.22,7-12.

Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter, G.R. (1994): *Pasteurella sp.* Clinical Vet. Microbiol., Wolfe Publishing, London. 258.

Rimler, R.B. and Glisson , J.R.(1997): Fowl Cholera. In Disaeses of Poultry.143-159.10th ed . By Calnek, Iowa State Univer. Press

Rhoades, K.R. (1964). The microscopic lesions of acute fowl cholera in mature chickens. Avian Dis. 8:658–665.

Rhoades , K.P. and Rimler, R.B. (1989) .*Pasteurella multocida* .Inc. Adlam and J.M. Rutter(Eds.) , *Pasteurella and Pasteurellosis* , 1st edn (37-73) .London : Academic Press.

Scott,P.C.; Markham,J.F. and Whithear, K.G.(1999) : Safety and efficiency of two live *Pasteurellamultocida*aro-A. mutant vaccines in chickens. Avian Dis., 45,83-88.

Shivachandra, S.B.; Kumar, A.A.; Gautam, R.; Vijendra P.; Sing Saxena, M.K. andSrivastava, S.K. (2006): Identification of avian strains of *Pasteurella multocida* in India by conventional and PCR assays. Vet. J., *172*: 561–564.

Simensen, E. and Olson, L.D. (1980): Aerosol transmission of*Pasteurella multocida* in turkeys. Avian Dis., 24, 1007-1010.

Toth.T.E.(2000): Non specific cellular defense of avian respiratory system. A review .Dev. Comp. Immunol. 24, 121-139.

Towesend, K.M.; Frost, A. J . ; LEE, C. W. ; Padimttriou,J.M. and Dawkins,H.J. S. (1998): Development of PCR assay for species and type-specific identification of *Pasteurella multocida* isolates.J. Clin. Microbiol.36, 1096-1100.

Townsend, K.M.; Hanh, T.X.; Boyle, D.O.; Wilkie, I.; Phan, T.T.; Wijewardana, T.G.; Trung, N.T. and Frost, A.J.(2001).PCR detection of analysis of *Pasteurella multocida* from the tonsils of slaughtered pigs in vietnam. Vet. Microbiol. 72:69-78.

Wilkie, I.W.; Grimes, S.E.; O'Boyle, D. and Frost, A.J. (2000): The virulence and protective efficacy for chickens of *Pasteurella multocida* administrated by different routes. Vet. Microbiol.*72*: 57-62.

دراسات على الباستيريلا فى الدجاج

الملخص

تم تجميع 200 عينة جمعوا من اعضاء (كبد وقلب وطحال) من 454 طائر ميت محتمل اصابتهم ببستريالا الطيور . تم اختبار العينات للعزل والزرع على اجار الدم والداس ميديا. تم الحصول على 8 معزولات وتم تصنيفهم بالتفاعلات الكيميائيه الحيويه عمل اختبار الضراوة فى الفئران ووراثيا بتفاعل انزيم البلمرة المتسلسل باستخدام الجين KMT1SP6 and KMT177 وهوجين خاص بالباستريالا مالتوسيدا .كشف التصنيف الجزيئي أن جميع العزلات تنتمى الى نوع ا .

ثمانى معزولات من الباستريالا مالتوسيدا تم تصنيفهم من 200 طائر ميت بمعدل كانت نسبة عزل باستريالا مالتوسيدا من دجاجة بياضة61.7 ٪ . وكانت اكبر نسبه عزل من الشرقيه 4% اكبر من القليوبيه1.38 ٪ .اكبر نسبة عزل من الدجاج 2.2 % . لم يتم التمكن من الحصول على معزولات للبستريالا مالتوسيدا من البط والديك الرومي والدجاج التسمين تحت ظروف عملنا فى هذا البحث.