

RINGKASAN
ISOLASI DAN PENGUKURAN BERAT MOLEKUL PROTEIN
OMP KUMAN *SALMONELLA PULLORUM* ISOLAT
LAPANGAN

(Ratih Ratnasari, Hasutji Endah Narumi, Susilohadi Widjajanto. 2005., 13 halaman)

Penyakit pullorum (berak kapur, pullorum disease) merupakan salah satu penyakit bakterial pada ayam yang bersifat akut pada anak ayam umur 1 sampai 10 hari dan berakibat fatal. Sedangkan pada ayam dara dan dewasa dapat bersifat khronis dan sebagai *carrier* (pembawa penyakit). Sampai saat ini uji pullorum (*Rapid Whole Blood Test*) merupakan deteksi dini untuk mendapatkan reaktor pullorum. Pencegahan penyakit hanyalah dengan manajemen yang baik. Pernah dicoba pencegahan dengan menggunakan bakterin yang berasal dari kuman *Salmonella pullorum* yang diinaktifkan, namun hasilnya kurang memuaskan. Pemberian bakterin pada ayam ternyata menghasilkan titer antibodi yang rendah dan daya proteksinya juga rendah.

Penelitian ini dilakukan dalam 4 tahap yaitu : (1) isolasi, identifikasi dan pemurnian kuman *Salmonella pullorum*, (2) isolasi OMP kuman *Salmonella pullorum*, (3) pengukuran kadar OMP dan (4) pengukuran berat molekul OMP.

Pada tahap awal yaitu isolasi, identifikasi dan pemurnian kuman *Salmonella pullorum*

yang berasal dari sampel tinja ayam penderita yang dijual dipasar dengan tanda klinis

berak putih. Uji laboratories dengan pewarnaan, kultur dan uji biokimiawi sehingga diperoleh kuman *Salmonella pullorum* yang murni. Kuman *Salmonella pullorum* dipecah membran luarnya dengan alat Ultra sonikator dengan modifikasi 3 menit jalan, 2 menit istirahat dan diulang sebanyak 3 kali dengan frekuensi 20 KHZ. Hasil ultra sonikasi dilanjutkan dengan isolasi Outer Membrane Protein (OMP) dengan metode Matsuyama disertai modifikasi penggunaan ultra sentrifugasi 40.000 rpm/20 menit pada suhu 4⁰C dan diulang sebanyak 3 kali. Hasil yang diperoleh adalah protein solubel dan dilanjutkan dengan pengukuran kadar protein. Kemudian dilanjutkan dengan pengukuran berat molekul OMP dengan metode SDS-PAGE.

Hasil isolasi, identifikasi dari sampel tinja ayam penderita pullorum diperoleh kuman *Salmonella pullorum* murni. Kadar protein (OMP) solubel yang diperoleh adalah 1973,4 ug/ml sebanyak 20 ml. Penghitungan berat molekul sample diperoleh : 40 k Da, 29 k Da, 25 k Da, 19 k Da, 9 k Da, 4 k Da dan 1 k Da. Menurut pendapat Wolfgang, *et al.* (1992) menyatakan bahwa berat molekul kuman Gram negative yang bersifat antigenik berkisar antara 30 sampai 45 k Da, sedangkan berat molekul protein *E. coli* sekitar 35 k Da.

 Dibiayai oleh Ditbinlitabmas Ditjen Dikti, Depdiknas.

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SUMMARY
ISOLATION AND MEASUREMENT MOLECULE WEIGHT
OUTER MEMBRANE PROTEIN (OMP) OF *SALMONELLA*
***PULLORUM* FIELD ISOLATE**

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Pullorum disease use to be called *Bacillary white diarrhoea disease* and it is one of contagious bacterial disease in poultry. The disease is an acute and it is highly fatal to young chicks during the first few day of life. In the adult there may be also be chronic cases and it is changeable as carrier.

Rapid Whole Blood Test (pullorum test) have been use for many years and can detect carrier with great accuracy. This test have been enormously successful since they enable immediate removal of the carrier from a poultry flock. The prevention of the birds against pullorum disease only with a good management. Unfortunately, the giving of bacterin that contain inactivated *Salmonella pullorum* for prevention has no satisfaction. It is not only producing low antibody titre, but also low protection.

This research was done in 4 steps : (1) isolation, identification and purification of *Salmonella pullorum* field isolate, (2) isolation of *Salmonella pullorum* Outer Membrane Protein (OMP), (3) measurement of OMP and (4) measurement weight molecule of OMP.

Salmonella pullorum had been isolated, identified and purified from feces of chick that suffering Bacillary white diarrhea. The laboratory examination for example staining, culturing and biochemical test had been done to find the pure of *Salmonella pullorum* field isolate.

The Outer Membrane Protein (OMP) of pure *Salmonella pullorum* field isolate was broken by ultra sonication. The modification of ultra sonication was 3 minutes on and 2 minutes off, with 20 KHZ frequency. It was repeatedly for 3 times.

After the outer membrane of *Salmonella pullorum* had been broken it was continued to isolate the Outer Membrane Protein (OMP) by Matsuyama method. This method was modified by using centrifugation (40.000 rpm in 20 minutes) at 4^o Celcius and it had been repeatedly for 3 times. The result was the soluble OMP and then that soluble OMP was measured. Finally the molecule weight of OMP was measured by SDS-PAGE.

The results showed that isolation, identification and purification from feces of chick was pure *Salmonella pullorum*. The soluble OMP was found 20 ml (1973,4 ug /ml). The molecule weight were showed by the band on SDS-PAGE : 40 k Da, 29 k Da, 25 k Da, 19 k Da, 9 k Da, 4 k Da and 1 k Da. The measurement of molecule

weight used standard marker (2,4,6,14,21,30 and 46 k Da). According to Wolfgang *et al.* (1992), the molecule of antigenically Gram negative bacteria was 20 to 45 k Da, whereas molecule weight of *E.coli* protein was around 35 k Da.

