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**“ Innovation of Technology and Bureaucracy towards
Good Governance to Improve the Nation’s
Competitiveness”**

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Proceedings

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Foreword: Assalamualaikum warrahmatullah wabarakatuh Greetings for all of you Excellency Prof. Dr. Hj. Sri Iswati, S.E, M.Si. Excellency Dr. Soekarwo S.H, M.H Excellency Dr. Ir. H. Sambari Halim Radianto, S.T, M.Si The Keynote speakers, invited speakers, presenters and participants of the conference Ladies and gentlemen Alhamdulillahirabbil 'alamin, we are here to The Second International Conference Postgraduate School with the theme Innovation of Technology and Bureaucracy Towards Good Governance to improve the Nation's Competitiveness. Universitas Airlangga is delighted to welcome you all in this two day conference. Ladies and gentlemen A university role is one of many pillars to build a nation that has great civilization, government system, human resources, natural resources, and other potentials It is our roles as academics and as a part of the university in maximizing the potentials to generally develop our nation or to specifically increase our nation's **(More)**

Volumes:

Vol. 1 - 978-989-758-348-3

Papers

Authors

Show papers**Moderating Effect of Employability on the Relationship Between Job Insecurity and Motivation** P. 5 - 9

Aditya Nugroho , Windijarto and Dewi Retno Suminar

DOI:10.5220/0007536600050009

The Empowerment of Micro And Small Industry of Wood Lathe By Department of Industry and Commerce - Case Study At Tanggung Village, Blitar, Indonesia P. 10 - 20

Laily Nur Hasanah

DOI:10.5220/0007536700100020

The Development Competence of Educational Staff Division in the Faculty of Veterinary Medicine Universitas Airlangga Through Training and Education Method P. 21 - 27

Ec Mardiana

DOI:10.5220/0007536800210027

Revitalization Of Religious Tourism Sidoarjo P. 28 - 35

Mohammad Mochtar M

DOI:10.5220/0007536900280035

Competency-Based: System Model Development Strategy to Increase Self-Performance P. 36 - 40

Amaliyah Amaliyah

DOI:10.5220/0007537000360040

The Analysis of Effective Recruitment towards Organizational Performance and Competitiveness in Rwanda P. 41 - 44

Obed Nahayo

DOI:10.5220/0007537100410044

The Influence Of Work Motivation And Organizational Culture On Improving Employee Performance In Financial Division At Companies In Energy Sector P. 45 - 50

Dita Bagus Hendra K , Windijarto and Pinky Saptandari

DOI:10.5220/0007537200450050

Additional Employee Income and Performance of Public Sector in East Java, Indonesia P. 51 - 59

Windijarto , Falih Suaedi , Indrianawati Usman and Rooswanti Putri

DOI:10.5220/0007537300510059

Efficiency Performance of Dompot Dhuafa Zakat Institutions: Using P. 60 - 64**Data Envelopment Analysis Approach**

Ninda Ardiani and Sri Herianingrum

DOI:10.5220/0007537400600064

The Impact of Government Bureaucracy System Change towards P. 65 - 69
Staff Anxiety

Moses Glorino Rumambo Pandin and Sri Rahayuningsih

DOI:10.5220/0007537500650069

Islamic Social Entrepreneur Organization of Zakat Management in P. 70 - 75
Empowerment of Socio-Economic Mustahiq in IndonesiaTika Widiastuti , M. Nafik Hadi Ryandhono , Irham Zaki ,
Imron Mawardi , Wisudanto and Taqiyah Dinda Insani

DOI:10.5220/0007537600700075

Islamic Banking (iB) Logo: In Santri Perception to Use Islamic P. 76 - 80
Banking Products in Indonesia

Tate Agape Bawana , Imron Mawardi and Sri Herianingrum

DOI:10.5220/0007537700760080

Islamic Family Budgeting P. 81 - 84

Elsha Sophia and Muhamad Nafik Hadi Ryandono

DOI:10.5220/0007537800810084

Restructuring of Non-Performing Financing of Murabaha Contracts P. 85 - 88
in Sharia BanksRachmi Cahya Amalia , Dina Fitriisa Septiarini , Sri Herianingrum and
Achsania Hendratmi

DOI:10.5220/0007537900850088

Analysis of Revenue Sharing and Liquidity for Total Profit Sharing P. 89 - 92
Financing with BI Rate as a Moderation in Indonesia Sharia
Commercial Bank and Sharia Bussiness Unit

Farhadi Arifiansyah , Ari Prasetyo and Gancar Candra Premananto

DOI:10.5220/0007538000890092

Concept of Scarcity in the Islamic Economic Perspective P. 93 - 96Hanif Fadhillah , Muhammad Nafik , Ririn Ratnasari , Tika Widiastuti
and Sri Herianingrum

DOI:10.5220/0007538100930096

Volunteer Management in Event Industry GIIAS SURABAYA AUTO P. 97 - 100
SHOW 2017

Afifah Rahmania , Windijarto and Indrianawati

DOI:10.5220/0007538200970100

The Influence of Compensation and Job Insecurity on Achievement P. 101 - 104
Motivation on Contract Employees at The Gresik Gas Cogeneration
Plant Project

Baswara Pawitra and Muhammad Tahajjudi Ghifary

DOI:10.5220/0007538301010104

Determinant of Saving in Islamic Bank: Case Study in Indonesia P. 105 - 110

Taufik Nugroho , M Faishol Luthfi and Sri Herianingrum

DOI:10.5220/0007538401050110

The Maslahah Principle against Sharia Financial Technology in Indonesia P. 111 - 120

Fiska Silvia Raden Roro and Abd Shomad

DOI:10.5220/0007538501110120

E -Commerce On Tourism Village Development P. 121 - 127

Aflit Nuryulia Praswati , Farid Wajdi and Muzakar Isa

DOI:10.5220/0007538601210127

The Role of Servant Leadership for Economic Development P. 128 - 132

Rachmadita Grandiz L , Fira Nurafini and Ririn Tri Ratnasari

DOI:10.5220/0007538701280132

The Influence of Job Security, Organizational Support, and Psychological Contract on Job Satisfaction in Organization X P. 133 - 136

Maindra Fauziannisa , Badri Munir Sukoco and Dewi Retno Suminar

DOI:10.5220/0007538801330136

Relationship between the Application of Good Islamic Business Governance and Voluntary Disclosure on Islamic Bank in Indonesia P. 137 - 141

Moh Arifin , Imron Mawardi , Muhammad Nafik Hadi Ryandono ,
Sri Iswati and Bahrina Almas

DOI:10.5220/0007538901370141

The Influence of Non Performing Financing (NPF) and Return on Assets (ROA) Against Linkage Multifinance at Sharia Commercial Bank in Indonesia P. 142 - 146

Safarinda Imani , Risma Ayu Kinanti , Achsanika Hendratmi ,
Tjiptohadi Sawarjuwono , Sri Herianingrum and Sri Iswati

DOI:10.5220/0007539001420146

Revitalization of Mosque Funds Management for Economic Empowerment P. 147 - 150

Ullya Nindyaningtyas , Ririn Tri Ratnasari and Sri Herianingrum

DOI:10.5220/0007539101470150

The Relation of Neurotic Personality and Impulsive Buying toward Adolescent in Tarakan P. 151 - 154

Friska Indryani Sitorus and Paulus Eddy Suhartanto

DOI:10.5220/0007539201510154

Assessing "Preparing to Excel in Emergency Response (PEER) Project" in Organizational Capacity Response Quality Improvement P. 155 - 158

Muhammad Kaimuddin , Setya Haksama and

Samsriyaningsih Handayani

DOI:10.5220/0007539301550158

Cryptocurrency Position in Islamic Financial System: A Case Study of Bitcoin P. 159 - 163

Nur Rizqi Febriandika and Raditya Sukmana

DOI:10.5220/0007539401590163

Implementation of the Principle of Unity in Business Activities Company - A Case Study on "X" Travel Indonesia P. 164 - 170

Abdullah Ahadish Shamad Muis , Tika Widiastuti , Puji

Sucia Sukmaningrum and Imron Mawardi

DOI:10.5220/0007539501640170

The Efficiency of Distribution of Zakat Fund at LAZNAS in Indonesia P. 171 - 175

Novi Nurul Aini , Ari Prasetyo and Tika Widiastuti

DOI:10.5220/0007539601710175

Effect of Financing of Revenue Sharing on Return on Assets of Sharia Commercial Banks in Indonesia P. 176 - 180

Faris Kurnia Hakim , Mauizhotul Hasanah and Sri Herianingrum

DOI:10.5220/0007539701760180

Implementation of Spiritual Capital in Islamic Perspective to Business Performance P. 181 - 185

Ziauddin Sardar , Muhammad Nafik Hadi Ryandono and Ririn Tri

Ratnasari

DOI:10.5220/0007539801810185

Analysis of Public Expenditure Strategy on Social Fund Sector for Poverty Reduction of Indonesia Islamic Economic Perspective P. 186 - 191

Fatimatuzzahro and Sri Herianingrum

DOI:10.5220/0007539901860191

The Effects of Islamic Marketing Mix on Consumer Satisfaction and Consumer Loyalty P. 192 - 200

Muhammad Syauqi , Ririn Tri Ratnasari and Sri Herianingrum

DOI:10.5220/0007540001920200

Analysis the Effect of Third Party Funds, Non Performing Financing, Capital Adequacy Ratio, and Bank Indonesia Sharia Certificates toward Financing - Case Studies of Indonesia Islamic Banking Period 2010-2015 P. 201 - 209

Novelinda Nurul Firdaus , Sri Iswati and Amalia Rizki

DOI:10.5220/0007540102010209

SWOT Analysis on Interest Development of Cash Waqf of Moslem Productive Age Entrepreneurs in Surabaya P. 210 - 213

Fitryani and Atina Shofawati

DOI:10.5220/0007540202100213

The Insurance Agreement in the Urgency of Microcredit on Banking P. 214 - 219

Hilda Yunita Sabrie , Prawitra Thalib , Faizal Kurniawan and Yuniarti

DOI:10.5220/0007540302140219

Analysis of the Effect of Public E-Health Service Quality on the Creation of Public Value - Case Study: PMR Hospital Surabaya City P. 220 - 226

Ria Rizki Wardani and Erma Suryani

DOI:10.5220/0007540402200226

Device of Lymphedema Pump Therapy Based on Pressure Pump: Design and Development P. 231 - 235

Suryani Astuti , Deny Arifianto , Dewa Supartha , Alfian Putra and

Tri Prijo

DOI:10.5220/0007540502310235

Characteristics of Composite Scaffold Chitosan-Chondroitin Sulphate / Hydroxyapatite as the Candidate of Bone Graft P. 236 - 243

Aminatun , Tia Rahayu Wijayanti , Dolfi Varton and

Prihartini Widiyanti

DOI:10.5220/0007540602360243

Expression of Caspase-3 in the Liver and Spleen Rattus norvegicus Which Infects Bacteria Klebsiella pneumonia and Klebsiella pneumoniae Extended Spectrum Beta Lactamase P. 244 - 248

I Gede Andika Sukarya , Willy Sandhika and Agung Dwi Wahyu

Widodo

DOI:10.5220/0007540702440248

Differences of Caspase-3 Expression in the Spleen and Liver of Sepsis Models in Rats Infected with Escherichia coli ESBL and Klebsiella pneumoniae Carbapenemase P. 249 - 252

Lisa Savitri , Willy Sandhika and Agung Dwi Wahyu Widodo

DOI:10.5220/0007540802490252

Differences Expression of Caspase-3 In Hepar and Spleen of Rattus norvegicus Infected with Methicillin Resistant Staphylococcus aureus and Enterococcus faecalis P. 253 - 257

Sri Wahyuni , Yoes Prijatna Dachlan and Agung Dwi Wahyu Widodo

DOI:10.5220/0007540902530257

Differences of Caspase-3 Expression in Liver and Spleen of Rattus norvegicus Infected with Streptococcus pyogenes and Acinetobacter baumannii P. 258 - 262

Nurul Amalia , Agung Dwi Wahyu Widodo and Willy Sandhika

DOI:10.5220/0007541002580262

Differences in Caspase-3 Expressions of Liver Organs and Spleen in Animals Model Rattus Norvegicus Infected with Candida Albicans P. 263 - 268

and Candida Non Albican Fungus

Yanuar Kristanto , Willy Sandhika and Agung Dwi Wahyu Widodo

DOI:10.5220/0007541102630268

Different Expression of Caspase-3 in the Spleen and Liver of Rattus norvegicus Infected with Enterobacter cloacae and Proteus mirabilis as a Septic Model P. 269 - 273

Rina Purwati , Yoes Prijatna Dachlan and Agung Dwi Wahyu Widodo

DOI:10.5220/0007541202690273

Dental Cement Physical and Mechanical Properties by In Vivo Approach P. 274 - 280

Prihartini Widiyanti , Siswanto and Idha Sa'Ada

DOI:10.5220/0007541302740280

Cytokine Profile Analysis of IL - 1 β and TNF-a in Diabetic Patients Infected by C.albicans P. 281 - 284

Regina Purnama Dewi Iskandar and Retno Pudji Rahayu

DOI:10.5220/0007541402810284

Effect of Extrinsic Motivation on Adversity Quotient in Patients With HIV & AIDS P. 285 - 291

Yulia Dewi Puspitawati , Misutarno and Nursalam

DOI:10.5220/0007541502850291

Isolation and Detection of Escherichia Coli Trimethoprim Resistance Gene from Layer Chickens in East Java Province, Indonesia by Polymerase Chain Reaction P. 292 - 300Emy Koestanti Sabdoningrum , Sri Hidanah , Sri Chusniati ,
Wiwik Misaco , Retno Sri Wahyuni and Laras Retno Kinasih Harianto

DOI:10.5220/0007541602920300

Mandibular Image Segmentation on the CT-Scan of the head using the Active Contour Method P. 301 - 305

Amillia Kartika Sari , Riries Rulaningtyas and Khusnul Ain

DOI:10.5220/0007541703010305

The Effect of Macrophage Dose to Secretion Interleukin 6 (IL-6) on Model of Tuberculosis Granuloma In Vitro P. 306 - 311

Imam Agus Faizal , Agung Dwi Wahyu Widodo and Jusak Nugraha

DOI:10.5220/0007541803060311

70% Ethylated and Non-Ethylated Swab: A Trace Evidence Recovery Method and Usefulness in Spectacles Forensic Evidence P. 312 - 316Simon Martin Manyanza Nzilibili , Muh. Abduh Dwi Putra and
Ahmad Yudianto

DOI:10.5220/0007541903120316

The Analysis of Victim Wound Quality and Quantity in Assuming The Perpetrator's Psychological Condition to Determine The P. 317 - 320

Perpetrator's Legal Liability

Ignatius N.S. , Mieke Sylvia Margaretha and Ahmad Yudianto

DOI:10.5220/0007542003170320

Decidual Killer Immunoglobulin-Like Receptor (KIR)2DL1 Expression and the Onset of Preeclampsia, Birth Weight and Placental Weight in Early and Late Onset Preeclampsia

P. 321 - 324

Khairunnisa Abd Rauf , Erry Gumilar Dachlan and Ariyanto Harsono

DOI:10.5220/0007542103210324

Correlation of Parasite Density with Plasma Level of TNF- α and IL-10 in Patients Infected by Plasmodium Vivax in East Sumba District, East Nusa Tenggara Province

P. 325 - 328

Frieti Vega Nela , Heny Arwati and Yoes Prijatna Dachlan

DOI:10.5220/0007542203250328

The Effects of Environmental Temperature Exposure of Blood Spatter Towards Protein and Agglutinations Level in ABO Blood Typing

P. 329 - 332

Amanovitasari D. , Kahar K. , Prasetya R.A. , Lestari T.P. and Yudianto A.

DOI:10.5220/0007542303290332

Bite Mark Analysis with "Stamping" Method in Child Abuse Case - A Case Report

P. 333 - 336

Dina Karimah Putri , Mieke Sylvia MAR and Ahmad Yudianto

DOI:10.5220/0007542403330336

Effect of Meniran Extract (Phyllanthus niruri Linn.) to Alternate Antibiotic Growth Promoter (AGP) on Egg Quality and Economics Analysis of Layers Infected by Escherichia coli

P. 337 - 342

H. Sri , S.R. Rivienda , A.A. Ika , K. Emy , S. Koesnoto and F.L. Dika

DOI:10.5220/0007542503370342

Effect of Tumor Necrosis Factor Alpha (Tnf-A) And Interleukin-10 (Il-10) Levels of Aggressive Periodontitis In Rats (Rattus Norvegicus) Induced by Aggregatibacter Actinomycetemcomitans

P. 343 - 350

Dwi Leni Yuliana , Yoes Prijatna Dachlan and Heny Arwati

DOI:10.5220/0007542603430350

The Differences of the Result of Copper Test Using UV-Vis Spectrophotometry with Neocuproine Complexing Agent and AAS

P. 351 - 355

Indah Sari , Rima Merdaliani , Pra Dian Mariadi and Rosnita Sebayang

DOI:10.5220/0007542703510355

The Effect Collagen to Granuloma Structure and Immune Response on Granuloma Tuberculosis In Vitro Models

P. 356 - 360

Ira Pengesti , Agung Dwi Wahyu Widodo and Jusak Nugraha

DOI:10.5220/0007542803560360

Laten Blood Detection Test Sensitivity of Lizard (*Varanus salvator*) P. 361 - 364**Using Leuco Malachite Green (LMG) with Different Dilution**

Farah Aidah Nureza , Bilqisthi Ari Putra , Nadia Yohana and

Djoko Legowo

DOI:10.5220/0007542903610364

Quality and Sensitivity of Presumptive Blood Test of Chicken (*Gallus gallus domesticus*) Blood Using Leuco Malachite Green (LMG) P. 365 - 367

Nadia Yohana , Bilqisthi Ari Putra , Farah Aidah Nureza and

Djoko Legowo

DOI:10.5220/0007543003650367

The Difference of Chloride Levels from Blood Serum Centrifuge at 3662 RPM Speed for 10 Minutes and 20 Minutes P. 368 - 370

Rima Merdaliani , Indah Sari and Maria Nuraeni

DOI:10.5220/0007543103680370

Resistance Test of *Vibrio* Sp from Milkfish (*Chanos chanos*) in Fishpond Jabon Sidoarjo to Heavy Metals and Antibiotics P. 371 - 375

Pratikah Verdianti , Fitriyati Mukhlisoh , Tuty Putri and

Ayu Puspitasari

DOI:10.5220/0007543203710375

Spatial Analysis of the correlation between Topographic Wetness Index with Annual Parasites Incidence Malaria in South Central Timor District 2017 – Indonesia P. 376 - 380

Nelson , Iwan Dwi Laksono and Setya Haksama

DOI:10.5220/0007543303760380

The Resistance Profile Of Bacteria *Salmonella* Sp Towards Antibacterial Ethanol Extract Of Bidara Leaves (*Ziziphus Mauritiana*) P. 381 - 385

Fitriyati Mukhlisoh , Pratikah Verdianti and Dwi Krihariyani

DOI:10.5220/0007543403810385

The Effect of Macrophage Addition to Interleukin 10 (IL-10) in Tuberculosis Granuloma Model In Vitro P. 386 - 391

Dini Puspodewi , Agung Dwi Wahyu W and Jusak Nugraha

DOI:10.5220/0007543503860391

Effect of Macrophage Addition and Incubation Time to Interferon Gamma (IFN-g) Levels on Tuberculosis Granulomas In Vitro Models P. 392 - 397

Yusuf Eko Nugroho , Agung Dwi Wahyu Widodo and Jusak Nugraha

DOI:10.5220/0007543603920397

Analysis of Family Spiritual Factors with Resilience Theoretical Approach in Overcoming Problem of Family Stigma Endemic Leprosy at Community Health Service (Puskesmas) in Jombang Regency of East Java Indonesia P. 398 - 402

Nasrudin , Ah Yusuf , Rachmat Hargono and Tjipto Suwandi

DOI:10.5220/0007543703980402

The Consequences of Using Different Storage Temperature of 5°C and 10°C in The Identification of Blood Group ABO from Dental Pulp Samples P. 403 - 406

Riki Kristanto , Yanuar Kristanto and Slamet Soetanto

DOI:10.5220/0007543804030406

Potential of Black Seed Oil (*Nigella Sativa*) as Immunomodulator in Mice (*Mus Musculus*) Infected with *Salmonella Typhimurium* P. 407 - 411

Eka Yunita Wulandari , Dwi Krihariyani and Suliati

DOI:10.5220/0007543904070411

Correlation between Parasite Density with TNF- α and IL-10 in *Plasmodium Falciparum* Infected Patients in East Sumba District, East Nusa Tenggara Province P. 412 - 417

Akhmad Mubarok , Heny Arwati and Yoes Prijatna Dahlan

DOI:10.5220/0007544004120417

Correlation between Parasite Density with Plasma Levels of TNF- α and IL-10 in Malaria Mix Infection in East Sumba District, East Nusa Tenggara Province P. 418 - 421

Meka Faizal Farabi , Heni Arwati and Yoes Prijatna Dachlan

DOI:10.5220/0007544104180421

The Effect of Simulation Method for Flood Disaster Preparedness towards Nursing Students Faculty of Health Sciences Unipdu, Jombang, East Java, Indonesia P. 422 - 425

Sufendi Hariyanto , Kurniawati and Christrijigo Waloejo

DOI:10.5220/0007544204220425

Pathology and Abnormalities of the Teeth as a Biographical Profile in Uncovering the Identity of Unknown Skeletal Remains P. 426 - 430

Eko Prastyo , Ledy Ana Zulfatunnadiroh , Rachmadita Yoga Pratiwi and Myrtati Dyah Artaria

DOI:10.5220/0007544304260430

Bone Damage in the Antemortem, Perimortem, and Postmortem Periods: Looking for Traumatic Indications on Bones for Assessing the Quality of Life of Skeletal Remains P. 431 - 435

Ledy Ana Zulfatunnadiroh , Eko Prastyo , Reinaldy Octavianus

Yan Dimpudus and Myrtati Dyah Artaria

DOI:10.5220/0007544404310435

Correlation between C-Reactive Protein Concentrations and Erythrocyte Sedimentation Rate in Patients with Positive Widal Test P. 436 - 439

Citra Yolanda Sari , Budi Santosa and M. Evy Prastiyanto

DOI:10.5220/0007544504360439

The Effect of Seawater on the Quantity of Dental Pulped DNA in P. 440 - 445

Forensic Odontology Identification

Nazaratun Thaiyibah , Amalia Rozaiza Ightikhoma and

Ahmad Yudianto

DOI:10.5220/0007544604400445

The Effect of Different Washing Treatment against Protein Quantity and Agglutination Level in Bloodstain for Forensic Identification

P. 446 - 452

Ardyan Pradana Putra , Hartono Kahar and Ahmad Yudianto

DOI:10.5220/0007544704460452

The Impact of Landslide in Tangkil Village Banaran Ponorogo - Incidents of Post Traumatic Stress Disorder

P. 453 - 456

Siswanto Agung Wijaya , Mei Dia and Pramudana Ihsan

DOI:10.5220/0007544804530456

The Effect of Pakoasi (*Chromolaena odorata* L.) Leaf Extract in Curing Open Wound of Rabbit Skin (*Oryctolagus cuniculus*)

P. 457 - 461

F Ramdani , M Sriasih and A.S. Drajat

DOI:10.5220/0007544904570461

Determination of rate chloride (Cl-) with Argentometric Mohr on Drinking Water Refill Tidiness in the Village Purwodadi Districts Kras Regency Kediri

P. 462 - 464

Diah Ayu Nur Rochmawati and Mely Purnadianti

DOI:10.5220/0007545004620464

Optimization of Nano Coral-Based Synthesis Calcium Phosphate with Concentration Variation of Phosphoric Acid and Sintering Temperature

P. 465 - 468

Siswanto , Mayasari Hariyanto and Siti Nurmala

DOI:10.5220/0007545104650468

Pulse Sequence 3D WATS for Evaluation Of The Anterior Cruciate Ligament (ACL) nd Posterior Cruciate Ligament (PCL) on MRI Knee

P. 469 - 474

Muhaimin Muhaimin , Sensusiati A.D , Kartikasari A , Muqmiroh L

and Latifah R

DOI:10.5220/0007545204690474

Legal Law on Optical Consumer in Maintaining Visual Health

P. 475 - 478

Dwi Rosdian Yuanita , Lilik Pujiastuti and Agung Sujatmiko

DOI:10.5220/0007545304750478

The Relation between Pregnant Women Characteristics and Their Perceptions towards Early Breastfeed Initiation (EBI) in Mataram City

P. 479 - 483

Wahdana Kusuma Sari , Ardyan Pradana Putra and Melinda

Restu Putra

DOI:10.5220/0007545404790483

Isolation and Identification of *Acanthus ilicifolius*-Associated Fungi

P. 484 - 488

and Investigation of Their Antibacterial Activity

Farras Daffa Imtiyaz , Ferisa Lestari Nugrahayu , Kawidian Putri

Bayu Alam , Insan Shiddiq and Hermin Pancasakti Kusuma Sari

DOI:10.5220/0007545504840488

Waist-Hip Ratio of Boys and Girls based on Mother's Occupational Status at SMPN 22 Surabaya

P. 489 - 492

Rosida Clivara Sari Anjani and Myrtati D. Artaria

DOI:10.5220/0007545604890492

Electrocution Death: Exit Mark Injury Use in the Suggestion of Body Posture during Forensic Investigation

P. 493 - 498

Pudji Hardjanto , Simon Martin Manyanza Nzilibili and

Ahmad Yudianto

DOI:10.5220/0007545704930498

Earphone Swab-Derived 126bp and 143bp mtDNA at D-loop Region - Electrophoretic Band Reaction on Room Temperature

P. 499 - 503

Ahmad Yudianto and Simon Martin Manyanza Nzilibili

DOI:10.5220/0007545804990503

Low Cost Dual Frequency Impedance Analysis for Measuring Internal and External Cellular Fluid

P. 504 - 510

Khusnul Ain , R. Arif Wibowo , Soegianto Soelistono ,

Lailatul Muniroh , Tri Anggono and M. Rizky Yusdy

DOI:10.5220/0007545905040510

Android Application for Baby Immunization Schedule

P. 511 - 514

Erwin Sutanto , Chintya Mortisalma Romadhon , Fadilla

Rahmania Kamil and Iqbal Maulana Rahman

DOI:10.5220/0007546005110514

Modeling of Infant Mortality Rate in East Java Province Using Mixed Geographically Weighted Regression Approach for Improving Quality of Health Services

P. 515 - 519

Ninda Ayu Puspitasari , Nadia Murbani , Nopriyanti , Sartika Aprilia

and Nur Chamidah

DOI:10.5220/0007546105150519

Signal Transducers and Activators Transcription (STAT) 5b Protein as A Candidate of Growth Promoter in Broiler Chicken

P. 525 - 527

Anwar Ma'ruf , Ratna Damayanti and Nove Hidajati

DOI:10.5220/0007546205250527

The Potential of Protein Ghrelin as Material for Eenergy Balance Setting for Feed Efficency in Broiler Chicken

P. 528 - 531

Nova Hidayati , Anwar Ma'ruf and Ratna Damayanti

DOI:10.5220/0007546305280531

The Test of Saccharomyces sp. Potential Filtrate to Inhibit The Growth of Aspergillus flavus FNCC6109 Broiler Chicken

P. 532 - 536

Concentrate Feed Model

Putu Angga Wiradana , Ida Bagus Gede Darmayasa and Ngurah

Intan Wiratmin

DOI:10.5220/0007546405320536

The Potential of Antigenic Protein of *Sarcoptes scabiei* as a Serological Diagnostic Candidate for Scabies in Goats

P. 537 - 540

Nunuk Dyah Retno Lastuti , Dony Chrismanto , Poedji Hastutiek and

Agus Sunarso

DOI:10.5220/0007546505370540

Identification and Determination of Total Flavonoids in Ethanol Extract of Old and Young Angsana Leaves (*Pterocarpus indicus* Willd.) Using Visible Spectrophotometry

P. 541 - 544

M.A.H.F Fernanda , R.D. Andriani , Z. Estulenggani and G.G. Kusumo

DOI:10.5220/0007546605410544

Leukocyte Count and Differential Leukocyte Count of Carp (*Cyprinus carpio* Linn) after Infected by *Aeromonas salmonicida*

P. 545 - 549

Abdika Dwi Afyanti , M. Gandul Atik Yuliani and Didik Handijatno

DOI:10.5220/0007546705450549

Evaluation of Implementation of Occupational Health Safety Management System (OHSMS) in PT. X, Magetan, East Java

P. 550 - 555

Achmad Khoirur Rozi

DOI:10.5220/0007546805500555

The Effect of Tadoples (*Rana catesbeiana*) Serum on Total and Differential Leukocyte in Rats (*Rattus norvegicus*) That Have Been Induced With Dimethylbenz- α -anthracene as Animal MODEL of Skin Cancer

P. 556 - 560

Nur Prabowo Dwi Cahyo , Prima Ayu Wibawati ,

Hardayay Primarizky , Ragil Angga Prastiya , Muhammad Thohawi

Elziyad Purnama and Ratna Damayanti

DOI:10.5220/0007546905560560

The Effect of Alpha-mangostin in Glucose Level, Cholesterol Level, and Diameter of the Islets of Langerhans of STZ-induced Diabetic Mice

P. 561 - 566

Saikhu Akhmad Husen , Salamun , Arif Nur Muhammad Ansori ,

Raden Joko Kuncoroningrat Susilo , Suhailah Hayaza and

Dwi Winarni

DOI:10.5220/0007547005610566

Amino Acid and Proximate Compositions of Cultured and Wild *Oreochromis niloticus* (Linnaeus 1758) from Makurdi-Nigeria

P. 567 - 570

A.S. Nege and E. T. Akange

DOI:10.5220/0007547105670570

Prevalence of Ancylostomiasis in Pet Cats from Banyuwangi City, East Java Province

P. 571 - 574

Aditya Yudhana , Ratih Novita Praja and Fifi Anik Suroiyah

DOI:10.5220/0007547205710574

Acupuncture Can Increase Spermatogenic Cells in Albino Rats (Rattus norvegicus) Exposed by Heatstroke

P. 575 - 577

Muhammad Thohawi Elziyad Purnama , Imas

Hapsari Rahmaningtyas , Ainun Septia Putri , Angela Swasti

Ivana Lee , Fachrun Nisa Tatimma and Herlina Masyitoh

DOI:10.5220/0007547305750577

Production of Rabbit Anti-Excretory/Secretory Product of Fasciola gigantica Lombok Isolate Antibody

P. 578 - 582

Made Sriasih , Sulaiman Ngongu Depamede , Djoko Kisworo and

Galuh Tresnani

DOI:10.5220/0007547405780582

Implementation of Fractional Logistic Growth Model in Describing Rooster Growth

P. 583 - 586

Windarto , Eridani and Utami Dyah Purwati

DOI:10.5220/0007547505830586

The Morphology of Contaminant Organism in Kappaphycus alvarezii Tissue Culture

P. 587 - 594

Ulfatus Zahroh and Apri Arisandi

DOI:10.5220/0007547605870594

The Perspective of Small-Scale Fisherman Toward the Seagrass Conservation Contributes to the Sustainability of Friendly Environmental: a Case Study in Coastal Waters of East Lombok

P. 595 - 598

Abdul Syukur , Mahrus and Syachruddin AR

DOI:10.5220/0007547705950598

The Perspective of Local Community's on Mangrove Resilience and Needs Conservation Along the Southern Coast of Lombok Island, Indonesia

P. 599 - 603

Agil Al Idrus , Abdul Syukur and Lalu Zulkifli

DOI:10.5220/0007547805990603

DEJUNGKIL (Bran, Corn, Meal) as Nutritional Formula for Free-Range Chicken (Gallus Domesticus) Egg Production Increase

P. 604 - 607

Palestin Binti Djazuli and Era Hari Mudji

DOI:10.5220/0007547906040607

The Level of Leukocytes, Eosinophils, Monocytes, and Lymphocytes in Mice (Mus musculus) on Post-Inoculation of Trypanosoma evansi

P. 608 - 610

Marek Yohana Kurniabudhi , Era Hari Mudji and Rini Fajarwati

DOI:10.5220/0007548006080610

The Effectiveness of Paris Agreement Regime in Control Global Warming in the Field of Food Security in Indonesia

P. 615 - 621

Suparto Wijoyo , Bagus Oktafian Abrianto , Wilda Prihatiningtyas

and Fitri Nur Amalia

DOI:10.5220/0007548106150621

A Simplification of Industrial Relation Dispute Solution to Improve the Nation's Competitiveness P. 622 - 626

Lanny Ramli

DOI:10.5220/0007548206220626

The Human Rights City Award: Sociolegal Study P. 627 - 631

Kurnia Indawati

DOI:10.5220/0007548306270631

The Policy of Central Borneo Provincial Government on Indigenous Peoples' Land Rights and Its Implications to Indonesia's Positive Laws P. 632 - 635

Ritwan Imanuel Tarigan

DOI:10.5220/0007548406320635

Law, Religious Freedom and National Development in Indonesia P. 636 - 640

Abednego Adinugroho

DOI:10.5220/0007548506360640

The Privacy Challenge in the 'Smart Era': A Study of the Implementation of e-Government in Surabaya P. 641 - 644

Masitoh Indriani and Ekawestri Prajwalita Widiati

DOI:10.5220/0007548606410644

Responsibility and Liability of Marine Pollution by Oil - Balikpapan Bay Pollution Case P. 645 - 648

Dina Sunyowati and Annisa Firdhausy

DOI:10.5220/0007548706450648

Distinction Principle in International Humanitarian Law Related to Civilian Objects and Military Objects P. 649 - 652

Eno Prasetiawan , Eka Widi Astuti , Helmi Amnihar and Febryan Topo

Ruru Artha

DOI:10.5220/0007548806490652

Criminal Profile of Pedophiles in East Java P. 653 - 659

Reno Fitria Sari and Margareta Margareta

DOI:10.5220/0007548906530659

Constitutional Court Decision Number 46 / Puu-Viii / 2010 Reviewed from the Perspective of Adat Law, Burgerlijk Wetboek (Bw) and Islamic Law P. 660 - 663

Hendra Eka Saputra , Irham Zaki and Muhammad Ubaidillah Al

Mustofa

DOI:10.5220/0007549006600663

The Challenges of Implementing Intelligence-led Policing in P. 664 - 667

Indonesia

Amira Paripurna and Sarwirini

DOI:10.5220/0007549106640667

Challenges of State Sovereignty and the Right of State to Self-defense: The Case of Cyber Attacks

P. 668 - 671

Joel Niyobuhungiro

DOI:10.5220/0007549206680671

The Problems of License Patent Agreement in Indonesia

P. 672 - 675

Agung Sujatmiko

DOI:10.5220/0007549306720675

Do Cities Really Want Human Rights? A Socio-legal Inquiry of Governance Performance in Decentralized Indonesia

P. 676 - 679

R. Herlambang Perdana Wiratraman , Ekawestri Prajwalita Widiati and Dwi Rahayu Kristianti

DOI:10.5220/0007549406760679

Collaborative Innovation in Maternal Mortality Rate (MMR) and Neonatal Mortality Rate (NMR) Handling Program in Sidoarjo

P. 680 - 684

Nurul Jamila Hariani and Falih Suaedi

DOI:10.5220/0007549506800684

The Development of Policy Instrument in the Application of the Law Number 1 of 1974 on Marriage as the Effort to Reduce Underage Marriage

P. 685 - 688

Lutfia Nurul Hidayati and Erna Setijaningrum

DOI:10.5220/0007549606850688

Service Quality Of M-Bonk Application In Sidoarjo District

P. 689 - 693

Berliana Mustika Rani , Allen Pranata Putra and Jitzhac Andrew Rafel Mandowen

DOI:10.5220/0007549706890693

Protection of Indonesian Seafarers in Overseas Employment Post Act Number 15 Year 2016

P. 694 - 697

Lina Hastuti , Dina Sunyowati , Enny Narwati and Nina Farah Adel

DOI:10.5220/0007549806940697

The Influence of Court Decision toward Overcapacity in Surabaya Detention Facility

P. 698 - 703

Suherman

DOI:10.5220/0007549906980703

The Move toward Dynamic Governance in Indonesian Public Service - Case Study of the Dynamic Capabilities of the Immigration Office Class I Surabaya Regions in Passport Service

P. 704 - 707

Endar Heryan Pajri and Sulikah Amorowati

DOI:10.5220/0007550007040707

The Potential Development of Trade on Services by Micro Small P. 708 - 711**Medium Enterprises in ASEAN**

Koesrianti Koesrianti and Tri Andjarwati

DOI:10.5220/0007550107080711

The Response of Association of Southeast Asian Nations (ASEAN) P. 712 - 715**towards the Issues of Rohingya Refugees from Myanmar**

Briyantoro Eko Yudianto

DOI:10.5220/0007550207120715

Transformational Leadership in Higher Education Institutions and P. 721 - 724**Employee Satisfaction Interventions**

Laily Fardillah , Budi Prasetyo and Rizca Yunike Putri

DOI:10.5220/0007550307210724

The Influence of Job Motivation and Job Environment Toward Job P. 725 - 731**Satisfaction of Employee in the Resources Directorate of****Universitas Airlangga**

Hendro Gunarto and Falih Suaedi

DOI:10.5220/0007550407250731

Professionalism and Skill Based on Performance: A Case Study of P. 732 - 739**Indonesia Navy**

Satria Wijaya and Falih Suaedi

DOI:10.5220/0007550507320739

Comparative Study of Job Motivation Between Librarian and P. 740 - 745**Administration Staff in Library of Universitas Airlangga**

Falih Suaedi and Dimas Agung Trisliatanto

DOI:10.5220/0007550607400745

Perception and Feasibility Study toward Mini Vocational School P. 746 - 752**under the Islamic Boarding School in East Java**

Bagus Oktafian Abrianto , Dimas Agung Trisliatanto and

Suparto Wijoyo

DOI:10.5220/0007550707460752

The Execution of the Collective Labour Agreement between the P. 753 - 757**Labour Union and the Management of "X" Hospital**

Sumarjoko and M. Hadi Subhan

DOI:10.5220/0007550807530757

The Effective Implementation Theory on Economic Development P. 758 - 764**Based on Part of District Region in Indonesia - A Study of****Government Developing Program in Soko Village, Glagah District,****Lamongan Regenc**

Noto Mashari Joyo and Philipus Keban

DOI:10.5220/0007550907580764

Community Development in CSR Programs Development of P. 765 - 768

Laguna Fishing Pond Tourism

Rachmad Ilham and Budi Setiawan

DOI:10.5220/0007551007650768

Job Motivation, Quality Work of Life and Organization Citizen Behavior toward Organizational Commitment

P. 769 - 773

Tiara Nurul Hidayah and Windijarto

DOI:10.5220/0007551107690773

The Values of Leadership Principles in Minangkabau Proverbs

P. 774 - 781

Misnal Munir

DOI:10.5220/0007551207740781

Poverty Culture of Community in Bengawan Solo River Bank in Surakarta, Indonesia

P. 782 - 785

Dita Agnes Dekasari , Ahmad Zuber and Yulius Slamet

DOI:10.5220/0007551307820785

Work Ethos and Religion Implementation in Supporting Business Existence in Metal Craftsmen in Cepogo Village of Boyolali Indonesia

P. 786 - 789

Susi Ningsih , Bagus Haryono and Argyo Demartoto

DOI:10.5220/0007551407860789

Competency Development of Traits and Motives in Case of Salt Farmer in Galis, Sub-district of Pamekasan Regency

P. 790 - 794

Sukron Ma'mun , Windijarto and Sutinah

DOI:10.5220/0007551507900794

The Effect of Education, Materialism, and Prestige on the Job Type Transformation at Young Generation

P. 795 - 800

Bagus Haryono and Susi Ningsih

DOI:10.5220/0007551607950800

Effectiveness of the "Jalin Matra" Material in Poverty Management - A Case Study of Turi Village, Jetis District, Ponorogo Regency, East Java Province, Indonesia

P. 801 - 804

Dwi Poernomo , Suparto Wijoyo and Abd. Shomad

DOI:10.5220/0007551708010804

Arrangement and Implementation of Pekanbaru City Spatial Control

P. 805 - 809

Dio Praseto Budi

DOI:10.5220/0007551808050809

The Politics of Law in Islamic Regional Regulation to Improve Regional Economy Competitiveness

P. 810 - 814

M.Haris Hidayatulloh , Ridan Muhtadi , Moh. Nafik H. R. ,
Sri Herianingrum , Sri Iswati , Irham Zaki and Tika Widiastuti

DOI:10.5220/0007551908100814

The Influence of Perceived Organizational Support and Leader P. 815 - 819**Member Exchange Against Cunter-Productive Work Behaviors on Civil Servants in Organization X**

Mochammad Zainuddin Arif , Pinky Septandari Endang Pratiwi and Dewi Retno Suminar

DOI:10.5220/0007552008150819

The Role of Government in Handling the Street Children Problem - A Study Case in Surabaya City, Indonesia P. 820 - 825

Bayu Prabhata Arinugraha

DOI:10.5220/0007552108200825

Freelancer's Personal Development - In the Industry of Meeting, Incentive, Convention, and Exhibition (MICE) P. 826 - 828

Afifah Rahmania , Windijarto and Dian Ekowati

DOI:10.5220/0007552208260828

Developing a Conceptual Model for Resilient Community Against Fire in a Densely Populated Settlement in Surabaya P. 829 - 833

Retno Indro Putri , Muhammad Zainuddin and Adjie Pamungkas

DOI:10.5220/0007552308290833

Coping Strategies for the Flood Disaster Practiced by the Pekauman Community in Sidoarjo Regency P. 834 - 838

Nurul Charisma , Hamidah and Moh. Adib

DOI:10.5220/0007552408340838

The Moderating Effect of Job Demands on Relationship Between Perceived Organizational Support and Psychological Capital to Work Engagement P. 839 - 845

Nurina Wulan Purnami and Seger Handoyo

DOI:10.5220/0007552508390845

Single Window Policy for Trade Licensing in Surabaya P. 846 - 848

Lanny Ramli , Luggas Radianto , Ayu Putri Azahrawany , Vidy

Fauziah Sampurno , Sandra Bagus M and Rintan Nur Indah

Sari Anwar

DOI:10.5220/0007552608460848

Relationship of Demographic Characteristics, Elderly Posyandu Membership, Organization Activities to Ability of Daily and Instrumental Elderly Activity P. 849 - 852

Yuly Sulistyorini , Nunik Puspitasari and Windhu Purnomo

DOI:10.5220/0007552708490852

Implementation of Permission Use of Space as an Instrument of Controlling the Space Utilization of High Control Zone of East Java P. 853 - 858

Festianty Karismandani , Suparto Wijoyo and Agus Sekarmadji

DOI:10.5220/0007552808530858

HIPPA - Himpunan Petani Pemakai Air (Water User Association- P. 859 - 865

WUAs) and Employment Opportunities in Rural Area - Case Study of a Village in Plumpang District, Tuban Regency

Rustinsyah Rustinsyah , Ratna Azis Prasetyo and Djoko Adi Prasetyo

DOI:10.5220/0007552908590865

Work Motivation, Job Satisfaction and it's Effect toward Organizational Commitment to Honorary Employees (Contract Employees) at PT X

Azizah Anshori , Fendy Suhariadi and Samian

DOI:10.5220/0007553008660869

Transport Management of Hazardous Materials and Toxic Waste

Khardian Eka Sulaksono , Suparto Wijoyo and Lilik Pudjiastuti

DOI:10.5220/0007553108700874

Intellectual Capital Model in SME's Tour and Travel in Surabaya

Sri Iswati , Agus Widodo M. , Hendarjanto , Azizah Anshori and

Dwi Swasana

DOI:10.5220/0007553208750878

Classification of Underdeveloped Regions in Indonesia Using Decision Tree Method

Bambang Widjanarko Otok , Rusdi Hidayat , Zumarsiyah Mahsyari , Siti Halimah Sa'diyah and Dimas Achmad Fadhila

DOI:10.5220/0007553308790883

Individualization through Observation of the Characteristic of Alveolar Bone of Edentulous Skulls

Reinaldy Octavianus Yan Dimpudus , Eko Prastyo and Myrtati

Dyah Artaria

DOI:10.5220/0007553408840887

The Development of Laku Lampah Community Existence as a Place of Historical Interested Members in Surakarta

Afika Fitria Permatasari , RB Soemanto and Supriyadi

DOI:10.5220/0007553508880892

Global Trade War: On the Efficiency of US Steel and Non-US Steel Companies

Yuan Ekananda Muhammad Adikara and Sri Herianingrum

DOI:10.5220/0007553608930897

Geographically Weighted Regression for Prediction of Underdeveloped Regions in East Java Province Based on Poverty Indicators

Rusdi Hidayat N , Bambang Widjanarko Otok , Zumarsiyah Mahsyari

, Siti Halimah Sa'diyah and Dimas Achmad Fadhila

DOI:10.5220/0007553708980907

Shariah Compliance of Rahn Contract Based in Fatwa National Islamic Council-Indonesian Council of Ulama (Dsn-Mui) at Pt. Bank

P. 866 - 869

P. 870 - 874

P. 875 - 878

P. 879 - 883

P. 884 - 887

P. 888 - 892

P. 893 - 897

P. 898 - 907

P. 908 - 912

Pembiayaan Rakyat Syariah (Bprs) Karya Mugi Sentosa

Annisa Aulia Yuniarti , Dina Septriani Fitriasia and Sri Herianingrum

DOI:10.5220/0007553809080912

**Staffs' Organizational Commitment at Archive and Library
Department of Bima District**

P. 913 - 917

Nur Anilawati

DOI:10.5220/0007553909130917

**Folklore as a Tool to Naturally Learn and Maintain Sasak Language
as Mother Tongue**

P. 918 - 926

Nuriadi

DOI:10.5220/0007554009180926

**Cultural Pattern of Children with Disabilities (Difabel) - Study of
Children with Special Needs (Tunagrahita) in Sekolah Luar Biasa
(SLB) Biromaru**

P. 927 - 930

Muh. Abduh Dwi Putra , Sulaiman Mamar and Rismawati

DOI:10.5220/0007554109270930

**Four Inter-ethnic Marriages of Two Chinese – Kaili Couples and
Two Arab – Kaili Couples - Study Hybrid Identity**

P. 931 - 934

Moh. Kurniadi Hendry Ekodiyanto , Muh Abduh Dwi Putra , Moh.

Fajar Agustian and Rosmawati

DOI:10.5220/0007554209310934

**Modeling of Poverty Rate in Indonesian Using Geographically
Weighted Logistic Regression for Supporting the Sustainable
Development Goals Program in 2030**

P. 935 - 938

Lailatus Syarifah , Putri Andriani , Nadhiyati Rizka , Retno

Dwi Puspitasari and Nur Chamidah

DOI:10.5220/0007554309350938

**Modeling of School Participation Rate for Senior High School in
Indonesia Using Mixed Geographically Weighted Regression Model**

P. 939 - 943

Febrina Kusumaningati , Jefri Utomo , Maya Zach , Ayu Puspitawati

and Nur Chamidah

DOI:10.5220/0007554409390943

**Application of Decision Support Systems for Selection of
Radiotherapy Methods Breast Cancer Patients**

P. 944 - 950

Rafli Filano and Boy Subirosa Sabarguna

DOI:10.5220/0007554509440950

**Intellectual Capital and Firm Performance - Evidence from
Indonesia**

P. 951 - 956

Budi Yuda Prawira and Rahmat Setiawan

DOI:10.5220/0007554609510956

Stress Factors and Impacts Toward Wardens in Surabaya Prison

P. 957 - 960

Anggi Isworo , Suryanto and Khanifatul Khusna

DOI:10.5220/0007554709570960



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Correlation of Parasite Density with Plasma Level of TNF- α and IL-10 in Patients Infected by Plasmodium Vivax in East Sumba District, East Nusa Tenggara Province

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Keywords: IL-10, Parasite Density, Plasmodium vivax, TNF- α .

Abstract: Introduction. Annual parasite incidence (API) in East Nusa Tenggara Province (NTT) 2015 per 1000 population is 7.04%. However, API in each Public Health Center (Puskesmas) Sumba Island remains high. High levels of pro-inflammatory cytokines in malaria infection, such as TNF- α is associated with severe pathology, whereas, anti-inflammatory cytokines such as IL-10 is associated with acute malaria. The objective of the study was to analyze correlation between parasite densities and plasma level of both cytokines in *P. vivax*-infected patients in East Sumba Regency East Nusa Tenggara Province. Methods. Parasite densities were calculated per 500 leucocytes on Giemsa-stained thick blood smears. The levels of TNF- α and IL-10 were measured by Enzyme-Linked Immunosorbent Assay (ELISA) method. Statistical analyses were done by Spearman test. Results. Correlation was observed significantly in parasite density and TNF- α $p = 0.032$ and parasite density and IL-10 $p = 0.000$. This result indicated that the stage of immunity in patients was not affected by the parasite density but clinical symptoms may have a greater role in increasing and decreasing the plasma level of cytokines. Conclusion. There was correlation between parasite densities and plasma level of TNF- α and IL-10 in *P. vivax* infected patients in the studied areas.

1 INTRODUCTION

Malaria incidence was still high in the eastern parts of Indonesia including Papua Province, West Papua, East Nusa Tenggara (NTT), Central Sulawesi and Maluku (Kemenkes, 2013). During 2015 the Annual parasite incidence (API) in East Nusa Tenggara Province (NTT) per 1000 population was 7.04%, the number of cases of positive malaria as high as 36,039 from 5,120,061 inhabitants. The API in each Public Health Center (Puskesmas) in Sumba Island remains high (Pusdatin, 2016).

Malaria has been known since 3,000 years ago and is caused by protozoa of the genus Plasmodium and transmitted by female Anopheles mosquitoes (Gunawan, 2000). There are 5 species of parasite causing malaria in humans, namely Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi (White et al., 2014).

Plasmodium vivax has a longer incubation time (12 days to several months), has a erythrocyte cycle

42-48 hours and produces fewer merozoites per schizon. It is generally known that *P. vivax* requires a duffy antigen as a receptor needed to invade host erythrocytes. In humans who do not have this antigen, they will become resistant to the infection (Andrade et al., 2010).

An immune response to malaria leads to parasite elimination or persistent responses are mediated by cytokines that cause immunopathology. In malarial infection high levels of pro-inflammatory cytokines, such as Tumor Necrosis Factor (TNF), Interferon Gamma (IFN- γ) and Interleukin-6 (IL-6) are associated with severe pathology whereas cytokines, anti-inflammatory agents such as Transforming Growth Factor Beta (TGF- β) and IL-10, are associated with acute malaria. IL-10 cytokines have an important role as immuno-regulators from infections caused by Plasmodium, by neutralizing the effects of cytokines produced by Th1 and CD8+ cells, which are responsible for immunopathology associated with excess cytokine production (Medina et al., 2011).

Activated macrophages release pro-inflammatory cytokines such as TNF- α , IL-1 and IL-6 (Tsokonas et al., 2002). The release of TNF- α apart from activated macrophages can also be directly induced by the malaria parasite and its dissolved antigen such as malaria pigment (haemozoin) and Glycosylphosphatidylinositol (GPI). TNF- α indirectly inhibits parasites by increasing phagocyte activity of monocytes (Wipasa et al., 2002; Korbel et al., 2004).

CD4+ T cells are classified into 2 major subsets according to the cytokine production pattern. Th1 produces IL-12, IFN- γ , and TNF- α . While Th2 produces IL-4, IL-5, IL-6, IL-10. In general, Th1 cells are responsible for cell-mediated immunity (CMI). The cytokine activates macrophages and other cells to produce mediators releasing inflammatory cytokines. Th2 cells regulate humoral immune by helping B cells to produce antibodies. Th2 cells promote the production of immunoglobulins. Both Th1 and Th2 cells are involved in protective immunity against malaria in the pre-erythrocytic stages, and the balance of cytokine production by both Th is the determining factor of the disease (Wipasa et al., 2002).

2 MATERIAL AND METHODS

2.1 Subject of Research

The blood samples were collected from East Sumba residents by active case detection in the villages with high API value. Passive case detection was done by collecting blood samples from *P.vivax*-infected patients who came to Puskesmas seeking medication. Blood samples were taken from those who meet inclusion criteria which is *P.vivax* positive by rapid diagnostic test (RDT) and microscopic examination followed by the signed informed consent.

2.2 Screening

A screening test is used to determine the inclusion criteria described above using 2 methods: RDT and microscopic tests. All samples were examined using a microscopic. RDT is used when sampling is active in the village because there is no microscope to support microscopic examination. Microscopic examination is still performed after the RDT results show a positive *P.vivax*.

2.3 Enzyme-Linked Immunosorbent Assay (ELISA)

Blood collection from vein cubitis patients with *P.vivax* malaria three milliliters (ml), inserted blood into the heparin tube, centrifuge for 15 minutes at a speed of 3000 rpm, the plasma is taken and transferred into ependorf tube using micropipette.

The measurements of TNF- α and IL10 levels use the Enzyme-Linked Immunosorbent Assay (ELISA) in accordance with manufacturer protocols, with all samples running in a single assay. The ELISA was performed and analyzed by a single operator, and standard curves were derived from cytokine standards.

3 RESULT AND DISCUSSION

3.1 Parasite Density

P.vivax positive samples that have been collected are smeared in thick drops and examined using a 1000x magnification microscope. The formula for calculating parasite density is as follows:

$$\text{Parasite density} = \frac{\sum \text{Parasite} \times 8000}{500}$$

The following is a microphotography picture of thick blood smear *P.vivax*:

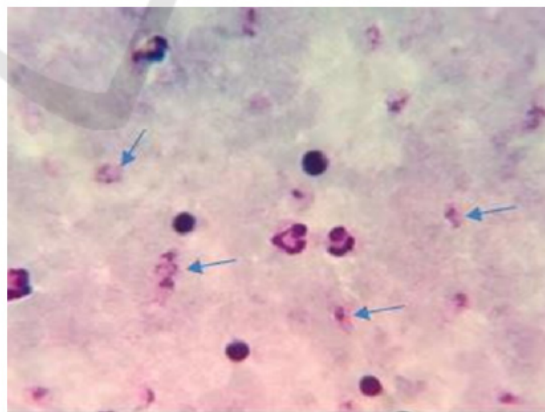


Figure 1: Microphotography *P.vivax* thick blood smear

Parasite density of samples diagnosed positive *P.vivax* as follows:

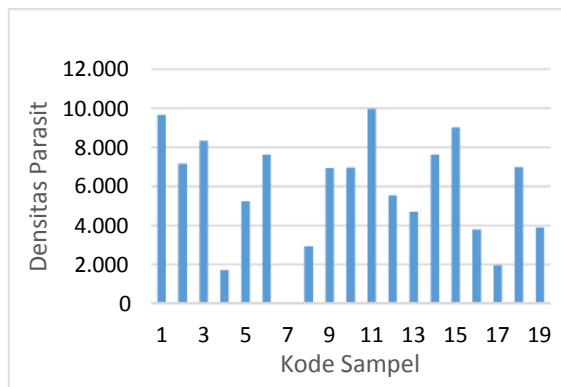


Figure 2: Parasite density P.vivax

3.2 Category of TNF- α and IL-10

Measurement of TNF- α and IL10 levels using ELISA can be categorized according to the following:

Table 1: Category of TNF- α levels

TNF- α (pg/ml)	Category	Frequency	%
0 – 100	Low	9	47,37
101 – 500	Intermediate	7	36,84
> 500	High	3	15,79

TNF- α level was categorized as 3 types that is low, intermediate, high. The percentage of TNF- α low is 0-100 is 47.37%, intermediate 101-500 is 36.84% and high > 500 is 15.79%.

Table 2: Category of IL-10 levels

IL-10 (pg/ml)	Category	Frequency	%
0 – 10	Low	11	57,89
11 – 50	Intermediate	7	36,84
> 50	High	1	5,26

TNF- α level was categorized as 3 types that is low, intermediate, high. Low IL-10 percentage is 0-10 is 57,89%, intermediate 11-50 is 36,84% and high > 50 is 5,26%.

3.3 Category of TNF- α and IL-10

Kolgorov-Smirnov Test was used to find out the normality of data. When the data is evenly distributed, then Pearson test was used to analyze the correlation between parasite density with TNF- α and IL-10. If the data is distributed unevenly, Spearman test was used. The correlation is significant if $p < 0.05$ is obtained. The results showed that significant

correlation was observed significantly parasite density and TNF- α $p = 0.032$ and parasite density and IL-10 $p = 0.000$.

3.4 Discussion

Infections caused by P.vivax have long been regarded as benign, especially when compared with infections caused by P.falciparum, but vivax malaria causes more severe disease than P.falciparum infection (Borges et al., 2013).

TNF- α is a pro-inflammatory cytokine that is the cause of fever (Hietbink et al., 2006). At high levels TNF- α can cause severe tissue damage (Couper et al., 2008). At the optimum level TNF- α can kill parasites directly, provide protection and lead to malaria recovery. Low levels of TNF- α can inhibit the growth of parasites in the stadium in the blood by activating the cellular immune system (Raza et al., 2013).

IL-10 is the main anti-inflammatory cytokine in the natural immune response and adaptive inflammatory response through the process of inactivation of macrophages and T cells (Doodoo et al., 2002). High level of IL-10 will prevent the development of severe malaria anemia (Weatherall et al., 2002). The occurrence of severe anemia is associated with a decrease in the concentration of IL-10 in the circulation and increases the ratio of TNF- α and IL-10. This condition contributes to the reversible suppression of bone marrow activity that occurs in malaria patients (Malaguarnera, 2002).

4 CONCLUSIONS

There was correlation between parasite densities and plasma level of TNF- α and IL-10 in P. vivax infected patients in the studied areas. This result indicated that the stage of immunity in patients was not affected by the parasite density but clinical symptoms may have more role in increasing and decreasing the plasma level of cytokines.

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